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Formulation and Evaluation of Temporary Herbal Hair Colour Spray

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ABSTRACT
Conventional methods of hair dyeing involve use of chemicals that result in unpleasant untoward effects which include irritation, breakage of hair, skin discoloration and hair lead to cancer. Marketed hair colours containing crude plant powders require processing prior to use, which is inconvenient to the consumer and these products also have poor rinsability. Hence, there is a need to develop a formulation of temporary herbal hair spray with colour extract from plant source which is ready to use with good rinsability. Five different formulations were prepared with different concentration, and formulation was found to be effective. The concentration of 1%, 2%, 4%, 6% & 8%, were tried on hair and the 4% showed good result.
Keywords: *Herbal hair colour spray, Herbal drugs, Indigo, Amala, Heena, Black tea.*

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INTRODUCTION

Nowadays, herbal hair dyes are being most preferred. Today majority of the human beings (specially women's) are very careful about their beauty and hairs play an important role in this Herbal drug without any adverse effects are used for healthy hair. Nearly 70% of human beings above 50 years struggle with the problem of balding and graying of hair.[1] Natural dyes are the color derived from plant, animal, or insect matter without any chemical processing. In the past natural organic substances were mixed with metals such as copper and iron, to produce more lasting or richer shades. Many plants like *Lawsonia inermis, Acacia arabica, Eclipta alba, Aloe barbadensis,* etc. are used as main ingredients in hair care preparations mainly for colouring the hair [2]. Benefits of herbal hair dyes includes Reduces or eliminates dandruff, thickens hair, reduces shedding, cover greys, etc. Plant-based hair color is composed of plant pigments making it a 100% natural alternative. Synthetic hair colorants involve the use of chemicals like 1-3% phenylenediamine, ammonia, peroxide and coal tar dyes that can remove and replacing or covering the natural hair color. Inorganic salts like aluminium sulphate, copper sulphate, lead acetate and potassium dichromate 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[3].

Each strand of hair is made up of the medulla, cortex, and cuticle. The innermost region, the medulla, is not always present which is an open, unstructured region. The highly structural and organized cortex, or second of three layers of the hair, is the primary source of mechanical strength and water uptake. The diameter of human hair varies from 0.017 to 0.18 millimetres (0.00067 to 0.00709 in) [4].The root of the hair ends in an enlargement, the hair bulb, which is whiter in color and softer in texture than the shaft and is lodged in a follicular involution of the epidermis called the hair follicle.

Gray hair occurs when melanin production decreases or stops, while poliosis is white hair (and often the skin to which the hair is attached), typically in spots that never possessed melanin at all, or ceased for natural reasons, generally genetic, in the first years of life. Hair pigmentation is one of the most unique features in humans ranging from black, brown, and blonde to red. The color of human hair is due to pigment melanin produced by melanocytes which are neural crest derivatives. Human hair follicles contain two types of melanin as follows: eumelanin and pheomelanin[5][6]

1) *Eumelanin* is the dominant pigment in brown hair and black hair.
2) *Pheomelanin* is dominant in red hair.

Based on how long the colouring lasts, hair color can be divided into,

1. Permanent
2. Semi-permanent
3. Temporary

1. Permanent Hair Colouring:

Any hair color that penetrates deep into the hair shaft and seals color into the hair cortex, is called a permanent hair color. As the name says, this type of hair dye is permanent and is not removable by shampooing. Permanent hair dyes are called oxidation hair dyes, because of the oxidizing agent used for the color development. Permanent hair color penetrates deep into the hair shaft, remove the natural color pigment (melanin) from the hair and then deposit the new color. Color is infused into the hair shaft rather than just painted over it. The hair shaft sustains oxidative damage with permanent hair-dye use [7][8]

Semi-Permanent Hair Colouring:

It contains further less ammonia and peroxide or neither at all. Semi-permanent dyes coat the hair shaft cuticle in a similar fashion to temporary dyes while also partially infiltrating the cortex. The semi-permanent dyes are retained within the hair shaft by van der Waals forces. Semi-permanent dyes are removed in 4 to 12 shampoos. It does not use hydrogen peroxide to develop the hair color[7][8].

Temporary Hair Colouring:

Temporary dyes weakly adhere to the hair shaft cuticle via van der Waals forces. Temporary dyes are otherwise called as non-oxidative dyes because colouring process was carried out without any oxidizing agent. It does not contain ammonia, so hair shaft is not opened. Temporary colouring molecules have the high molecular weight and deposits on the hair surface only and not penetrate on the cortex. They just get absorbed on the hair surface only and easily wash out within one - two shampoo [7][8]

MATERIAL AND METHODS

Plant Profile

The following plant materials were collected from the Market.

Henna [9]

Plant – *Lawsoniainermis*

Family – Lythraceae

Synonym - Camphire, Henna Plant, Mehndi etc.

Fig. 1. Henna Plant

Chemical Constituents

- The leaves of this plant possess a red dye molecule called lawsone (2- Hydroxy – 1yl-naphthoquinone), which has the ability to bond with protein.
- It also consists of white resin, sugar, and tannin[9]
- In general, the available information regarding henna states that the dying principle is lawsone, 2-hydroxy-1,4-naphtoquinone, that is contained in dried leaves in concentration of 0.5-2%[10]

Uses

- Henna balances the pH of the scalp preventing premature hair fall and graying of hair.
- Colour obtained is relatively stable.
- Its antifungal and antimicrobial properties may be beneficial for the hair and scalp, particularly for premature graying and reducing dandruff [1-11]

Purpose for choosing Henna

- It is used in its natural form can make your hair thicker, stronger, silkier, and shinier (due to the presence of tannins in it)
- It even acts as a natural conditioner due to its nutrient-and-moisture-locking properties, which simultaneously build a protective layer on your hair[12]

Amla [13]

Plant – *Emblica officinalis*
Family – Euphorbiaceae
Synonym - Myrobalan, Indian gooseberry

Fig. 3. Amla plant with fruit

Chemical Constituents

- The fruit of Amla is rich in vitamin C (ascorbic acid) and contains several bioactive phytochemicals, of which majority are of polyphenols[1]
- Ascorbic acid content in fresh ***amla*** was 430.1 mg/100 g [14]

Uses

- It is used to promote hair growth [9]
- The herb is also used in shampoos, hair oils, and Hair dyes[6]

Purpose for choosing Amla

- The dye made up of amla will keep you free from hair infections that cause itchy scalp due to chemical action of synthetic dye.
- It also improves its efficacy in colouring the hair & prevent premature greying.
- Also has antioxidant property[15]

Indigo [16]
Plant – *Indigofera tinctoria*
Family – Fabaceae
Synonym- Anil, Darkviolet, Delft blue, indigo plant,
Dresden blue and steel blue.

Fig. 4. Indigo Plant

Chemical Constituents

- Natural blue pigments occur extensively throughout the plant kingdom, mainly in the form of anthocyanins (Anthos = flower, kyanos = blue).
- Anthocyanins are important members of the flavonoid group of chemicals [17]
- Amla is rich in vitamin C, and it also helps in stopping premature greying.

Uses

- It acts as a good conditioner.
- It promotes hair growth and prevent itching on the scalp and reduce the dandruff [16]

Purpose for choosing Indigo

- It helps treat baldness and restore hair volume.
- When henna and indigo are mixed together and apply on your hair it ensures that the products are 100% natural, organic, and free of any chemicals.
- The henna and indigo pastes when applied repeatedly i.e., 3 times or 4 times, applied repeatedly i.e., 3 times or 4 times, there was an increase in intensity of brown coloration [18]

Black Tea [19]

Biological Name- *Camellia sinensis*
Family– Theaceae
Synonym - English Breakfast Tea

Fig. 5. Black Tea Plant

Chemical Constituent

- It contains caffeine, gallic acid, theobromine, theogallin, rutin and several other polyphenols.
- Tannin content in black tea ranged from 11.76 to 15.14% with an average of 13.36%.10–12% phenolic acids [19]

Uses

- It is used for improving mental alertness as well as learning, memory, and information processing skills.
- It helps to increase hair growth, enhance hair color, and boost hair sheen.[20]

Purpose for choosing Black Tea

- It contains tannic acid, which effectively darkens your white hair.
- It also adds shine to your hair, making it an excellent remedy for reviving dull, lifeless hair[11]

Collection of Plant materials

All the powder herbal ingredients like- Amla (Phyllanthus Emblica), Indigo(Indigofera tinctoria), Henna(Lawsoniainermis) and Black Tea(Camellia sinensis) are purchased from Abbumiya Ayurvedic Shop(Shop no. 10 Pritam apartment Kashmir galli, indora chowk, Nagpur). Different solvents were collected from college laboratory.

Extraction Process

The extraction process was carried out by following techniques for different plant materials and the extractive value was mentioned in the table no. 2.

Henna

Alcohol Maceration Process: The alcoholic extract was prepared by mixing 25gm of henna powder with 250ml of 70% ethanol for 12 hours. This mixture was cooled and filtered by Buchner funnel using filter paper (Whatman No. 185).The solvent was dried and concentrated using rotary evaporator at 50^0C. The concentrated solvent was air-dried for one day[21].

Amla

Alcohol Maceration Process: The powder 20gm was placed with the whole was alcohol i.e., 360 ml in a closed vessel for 7 days. During this period shaking was done occasionally. Then mixture was filtered by Buchner funnel using filter paper (Whatman No. 185).The solvent was dried and concentrated using rotary evaporator at 50^0C.The concentrated solvent was air-dried for one day[22].

Indigo

Indigo powder (20gm) was extracted with ethanol (250ml) under reflux. Indigo powder leaves and ethanol was placed and refluxed for about 5 to 6 hours. Then mixture was filtered by Buchner funnel using filter paper (WattmanNo.185).The solvent was dried and concentrated using rotary evaporator at 50^0C.
The concentrated solvent was air-dried for one day[16.]

Black Tea

Black tea leaves were subjected to extraction with hot water at a temperature between 60°C and 130°C. The mixture was filtered by Buchner funnel using filter paper (Whatman No. 185). The solvent was dried and concentrated using Liebig-type condenser. The concentrated solvent was air-dried for one day [23] After extraction of herbal powder ingredients (Henna, Amla, and Indigo)solvent was dried and concentrated using rotary evaporator at 50^0C.
The solvent was poured into the petriplate for further process.

Black Tea solvent was dried and concentrated using Liebig-type condenser. The solvent was poured into the petriplate for further process .

Henna	Amla	Indigo	Black Tea
			

Fig. 6: Petri-plate containing sample

Phytochemical Screening of plant extracts

The qualitative phytochemical analysis of Henna, Amla, Indigo, and Black teapowder extract was performed by using different tests are given below and the results are presented in Table No.4. Phytochemical analysis was done to check the active constituents which gives colour to the hair spray.

Henna

Test For Protein [24][25]

a) Biuret test (General test): To 3 ml TS. add 4% NaOH and few drops of 1% $CuSO_4$, solution.
b) Millon's test (for proteins): Mix 3 ml T.S. with 5 ml Million's reagent. White ppt. Warm. ppt. turns brick red or the ppt. dissolves.
c) Xanthoprotein test (for protein containing tyrosine or tryptophan): Mix 3 ml. TS. with 1 ml conc. H_2SO_4. White ppt. is formed. Boil it. Precipitate tums yellow. Add NaOH.
d) Test for proteins containing sulphur: Mix 5 ml T.S. with 2 ml 40% NaOH and 2 drops 10% lead acetate solution. Boil mixture.
e) Precipitation test: The test solution gives white colloidal ppt. with following reagents:(a) absolute alcohol, (b) 5% $HgCl_2$, solution, (c) 5% $CuSO_4$, solution, (d) 5% lead acetate,(e) 5% ammonium sulphate.

Amla

Tests For Vitamin C (Ascorbic Acid),[24][26]

a) Dilute 1 ml of 2% w/v solution with 5 ml of H_2O and add 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of dilute NaOH solution. Add 0.6 ml of HCl dropwise and stir.
b) To 2 ml of a 2% w/v solution add 2 ml of H_2O, 0.1 g of $NaHCO_3$ and about 20 mg of $FeSO_4$, shake and allow to stand; a deep violet colour is produced. Add 5 ml of 1 H_2SO_4, the colour disappears.

Indigo

Test for Flavonoids [24][27]

a) Shinoda Test: To dry powder or extract, add 5 ml 95% ethanol/t-butyl alcohol, few drops conc. HCl and 0.5 g magnesium turnings. Orange, pink, red to purple colour appears (flavanols, dihydro derivatives and xanthene's).

Black Tea

Tests for Tannins & Phenolic Compounds[24][28]

To 2-3 ml of aqueous or alcoholic extract, add few drops of following reagents:
(a) 5% $FeCl_3$ solution.
(b) Lead acetate solution.
(c) Gelatine solution.
(d) Bromine water.
(e) Acetic acid solution.
(f) Dilute iodine solution.
(g) Dilute HNO_3

Thin Layer Chromatography

Pre coated silica gel plates were received from the college. The plate was marked 1 cm from the bottom and spots were made with the standard and samples. Then the plate was suspended lightly in the solvent and was allowed to run until it reaches a 3/4th position. Ninhydrin (3 % ninhydrin in 100 ml butanol containing 3 ml of Acetic acid) was used as the spraying agent and it was sprayed all over the plate and was allowed to dry. The coloured spots developed were noted and the Rf value was calculated by measuring the distance travelled by the solute and the solvent [29]

TLC of Henna

We have used different-different types of mobile phase like Toluene: Ethyl acetate: Glacial acetic acid in the proportion of 8:1:1 (v/v/v)was used respectively[30] and Chloroform: Methanol (17:3) (v/v) was used respectively[31] Toluene can be replaced by Chloroform. But TLC plate shows 2 spots by using mobile phase of Toluene: Ethyl Acetate: Formic acid of 22:16:2 (v/v) was used respectively [32]

TLC of Amla

We have used different types of Mobile phase like Ethyl Acetate: Acetic Acid Glacial: Formic Acid: Water 6:1:1:2 (v/v/v/v)was used respectively[33] and Toluene: Methanol: Water in the proportion of 5:3:2 (v/v/v)was used respectively. But using Toluene: Methanol in the proportion of 8:2 (v/v) was used respectively, gives result and 3 spots where identified.

TLC of Indigo

Chloroform: Hexane: Methanol in the proportion of 7:4:1 (v/v/v) was used respectively[34] and Ethyl acetate: Pyridine: Water in the proportion of 50:23:20 (v/v/v)was used respectively[35]. But using Toluene: Methanol in the proportion of 17:3 (v/v) respectively [31] Toluene can be replaced by Chloroform. This mobile phase gives result, and two spots were identified.

TLC of Black Tea

Acetone: Chloroform: Water in the proportion of 80:20:10 (v/v/v) was used respectively[35] Chloroform: Methanol: Water in the proportion of 65:35:10 (v/v/v) respectively [36], Toluene: Methanol in the proportion of 8:2 (v/v) respectively, Toluene and Methanol in the proportion of 7:3 (v/v) respectively. By Using this solvent as mobile phase then 2 spots were obtained upper layer of the mixed solvent system butan-1-ol, glacial acetic acid, water (4:1:5) as, the mobile phase.

Formulations

The different formulations were prepared on the trial-and-error basis form and among 25 batches we selected 5 formulations. Total 8 formulations were prepared and shown in Table 1.

Table 1: Formulation of Temporary hair colour formulation

Concentration	Henna	Amla	Indigo	Black Tea	Ethanol
1%	1.30gm	0.247gm	0.153gm	0.311gm	Sufficient Quantity
2%	2.618gm	0.494gm	0.306gm	0.622gm	Sufficient Quantity
4%	4.44gm	0.852gm	0.568gm	1.228gm	Sufficient Quantity
6%	7.84gm	1.482gm	0.918gm	1.866gm	Sufficient Quantity
8%	10.44gm	1.976gm	1.224gm	2.488gm	Sufficient Quantity

RESULTS & DISCUSSION

Extraction

The following are the extract value from Heena, Amla, Indigo, and Black Tea through extraction process which is shown in Table 2.

Table 2: Shows the Extraction value and different solvent

Sr. No.	Ingredients	Extraction	Final Extract
1.	Henna	200gm of henna powder was dissolved in 2,000ml of 70% ethanol.	27.38g
2.	Amla	Powder 200gm is placed with the whole of the alcohol 3,600ml in a closed vessel for 7 days.	5.34g
3.	Indigo	200gm of indigo – powder, on a dry mass basis, may be added to 1,200ml of ethanol.	11.02g
4.	Black Tea	The powder 200gm is mixed with hot distilled water 2,000ml in a closed vessel for 1 days.	7.62g

Phytochemical Tests

The results of the phytochemical screening of the investigated extracts of *Henna, Amla, Indigo, and Black Tea* showed the presence of protein, flavonoids, phenolic compounds, tannins, and vitamin C powder extracts as shown in Table No. 4.

Table 3: Phytochemical Test [24]

Sr. No.	Plant material	Test	Observations	Outcome
1.	**Henna**			
	Tests for Protein			
		a) Biuret Test	Violet or pink colour appears	**- -**
		b) Millon's Test	Red coloured solution	**- -**
		c) Xantho Protein Test	Precipitate turns yellow & after addition of NH_4OH precipitate turns orange	**+ +**
		e) Test for protein containing sulphur	Solution turns black or brownish	**- -**
		f) Precipitation	Solution gives white colloidal precipitate	**+ +**
2.	**Amla**			
		Tests for Vitamin C		
		a) Dilute 1 ml of 2% w/v solution with 5 ml of H_2O and add 1 drop of freshly prepared 5% w/v solution of sodium nitroprusside and 2 ml of dilute NaOH solution. Add 0.6 ml of HCl dropwise and stir, the yellow colour turns blue.	Blue colour	**+ +**
		b) To 2 ml of a 2% w/v solution add 2 ml of H_2O, 0.1 g of $NaHCO_3$ and about 20 mg of $FeSO_4$, shake and allow to stand; a deep violet colour is produced. Add 5 ml of 1 H_2SO_4, the colour disappears.	Violet to colourless	**+ +**
3.	**Indigo**			
		Test for Flavonoids		
		a) Shindo Test	Solution gives deep red colour	**+ +**
4.	**Black Tea**			
		Tests for Tannins &Phenolic Compounds		
		a) 5% $Fecl_3$ Solution	Deep blue-black colour	**+ +**
		b) Lead Acetate Solution	White precipitate	**+ +**
		c) Gelatine Solution	White precipitate	**- -**
		d) Bromine Water	Decolouration of bromine water	**+ +**
		e) Acetic Acid Solution	Red colour solution	**+ +**
		f) Dilute iodine solution	Transient red colour	**+ +**
		g) Dilute HNO_3	Reddish to yellow colour	**+ +**

[**Positive** (**++**) &**Negative** (**--**)]

Thin Layer Chromatography

The separation of the crude extract by TLC using different solvent given in Table 3. TLC plate is visualised under Visible light and long UV wave(365nm).

- The spots are small & clearly defined
- A Higher the Rf value indicates that the compound has travelled for up the plate & is less polar.
- A lower Rf value indicates that compound has not travelled far & is more polar.

Henna

Mobile Phase: Toluene: Ethyl Acetate: Formic Acid
5.5 : 4 : 0.5

Solvent Used: Small quantity of henna dissolved in Ethanol

Standard Rf Value of Henna = **0.87 ± 0.01**

Rf Value of A = **0.78**

Rf Value of B = **0.38**

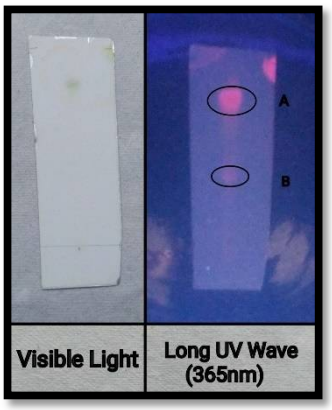

Fig 7. TLC of Henna

Amla

Mobile Phase: Toluene: Methanol
8 : 2
Solvent Used: Small quantity of amla dissolved in Ethanol
Standard Rf Value of Amla = **0.74 ± 0.01**
Rf Value of A = **0.81**
Rf Value of B = **0.75**
Rf Value of C = **0.61**

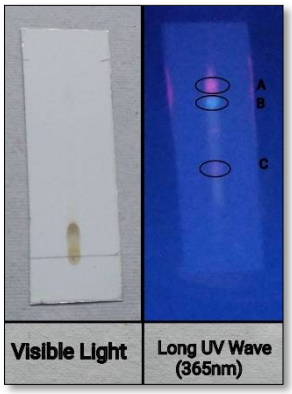

Fig. 8. TLC of Amla

Indigo

Mobile Phase: Toluene: Methanol
8 : 2
Solvent Used: Small quantity of indigo dissolved in Ethanol
Standard Rf Value of Indigo Dye = **0.57 ± 0.01**
Rf Value of A = **0.56**
Rf Value of B = **0.51**

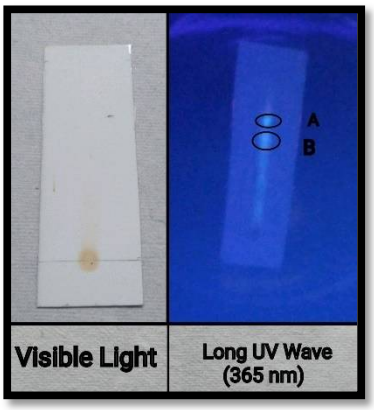

Fig. 9. TLC of Indigo

Black Tea

Mobile Phase: Upper layer of the mixed solvent system
Butan-1-ol: Glacial Acetic Acid: Water
8 : 2 : 10

Solvent Used: Small quantity of black tea dissolved in Methanol

Standard Rf Value of Black Tea

Tannic Acid = **0.48 ± 0.05**

Rf Value of A = **0.63**

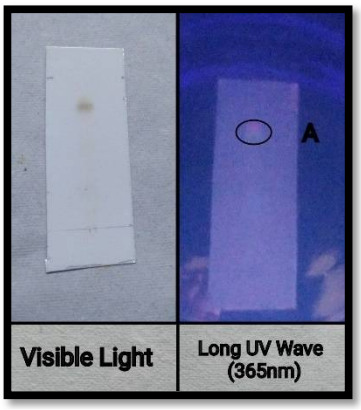

Fig. 10. TLC of Black Tea

EVALUATION

We have sprayed our formulation into the hair and observed that sample 3, 4, and 5 shows similar colour effect. So, we have selected 4 Sample as an optimum formulation. Shown in figure 11.

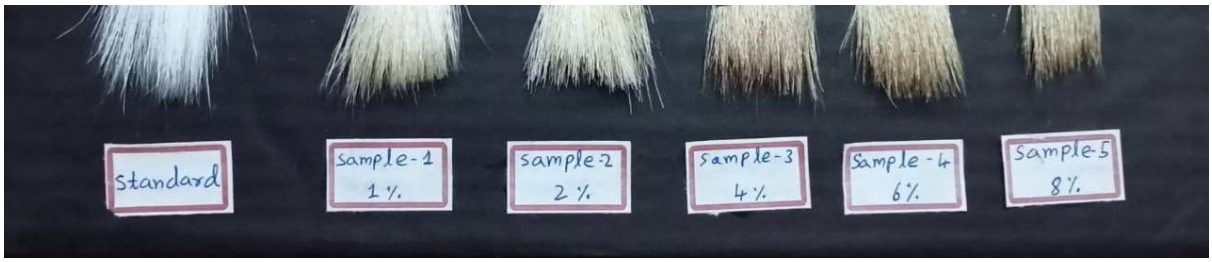

Fig. 11. Indicating colour over natural hair

The present study is directed to a herbal hair colour spray composition for dying human hair. Henna acting as a base powder act as a universal hair dye as it used for its colouring properties throughout the globe The sufficient amount of vitamin C in Amla helps to halt pre-mature greying. Black tea imparts perfect colour to the hair in combination with other herbs. It shows the presence of major phytoconstituents which act as a true nourisher for the scalp as well as hair. From the above observation it has been concluded that the formulation constituted with naturally occurring dried herbal ingredients, having minimal possibilities of the deterioration of the formulation, due to less moisture content. The formulation was kept for room temperature to observe the changes in its colour, order, texture, and appearance. The formulation was found to be stable. It can be easily stored and used by any person. Since it was a natural herbal based formulation, it was free from the ill effects of ammonia-based chemical dyes. This leads to an increase shelf life with stable ingredients.

CONCLUSION

The world is changing towards the use of safer and natural product with traditional usage. The present study evaluates the formulation of herbal hair Color spray comprising a mixture of powdered plant material having natural dye which was useful for colouring the hair. Efficacy data shows that all these active constitute have dying effect on natural hair. This formulation contains ethanol soluble herbal extracts which was highly environment friendly. In this research, we found effective properties of the herbal hair pack and further studies are needed to be performed to explore more useful benefits of herbal hair spray. So, with this we achieved our aim and objectives.

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