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Preparation and Evaluation of One Stage and Two Stage Polyherbal Hair Dye Formulations

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Abstract:

With a growing awareness of harmful effects of synthetic dyes on human health and environment, there is an extensive demand for sustainable alternatives. Herbal hair dyes offer a range of benefits, including the absence of synthetic chemicals such as ammonia, parabens, and resorcinol, which are commonly found in conventional hair dyes. Instead, these dyes harness the coloring properties of plant based ingredients. Various plant species including henna, beetroot, amla, areca nuts, black berry, indigo were explored for their dyeing potential and color variations. The dyeing properties of 13 hair dye formulations in different combinations, single stage and two stage applications were studied. This work provides insights of the scientific principles in herbal hair dyeing, including extraction methods, formulation techniques, and color variations achievable with different plant-based ingredients. Additionally, considerations such as application methods, color longevity, and compatibility are studied to provide a strong support for the safe and effective use of herbal dyes. Two-stage applications containing henna gave better results as these Formulations offered darker colours. An added advantage of the polyherbal hair dye formulations developed is the synergistic effect of tannins, carotenoids and flavonoids that contribute to the colouring as well as protective effect on human hair and skin.

Key words: Herbal dyes, conventional dyes, dyeing potential, polyherbal, parabens, color longevity etc.

1. INTRODUCTION:

The colour of human hair is attributed to the pigment known as "Melanin" present in the follicles of hair. Greying of hair is an inevitable physiologic process in aging. It is caused by the loss of melanocytes, the melanin forming cells, from the hair follicles. (Yale et al.,2020; Kim et al.,1999) Three types of hair dyes are available, which differ in their mechanism of colouring. They include temporary, semi-permanent, and permanent hair dyes. Temporary hair dyes get washed away very quickly. Semi-permanent hair dyes retain colour up to 5 to 10 washings. Permanent hair dyes can show long-lasting chemical changes to the hair and the dye is maintained as long as hair grows out or falls out. In India, henna has been used traditionally for colouring palms and hairs. (Jyotshna et al., 2017) Amla, (Senug-Hee Jang et al.,2018) Bhringraj, (Rathi Bharat et al.,2012) Henna,(Aneesa et al., 2019) Hibiscus, (Imam Budi Putra et al.,2020) Jatamansi, (Hari Ranjit Naik and Vijay Jadhav, 2020) Reetha, Sariva,(Prasanna et al., 2019) Curry leaves(Muthulinggam Nishan and Partiban Subramanian, 2015)and Methi seeds are popularly known ayurvedic herbal drugs conventionally used as hair colourants and for hair growth.(Rashmi Saxena et al.,2018) Indigo, commonly known as an initial fabric dye, could be blended with henna to produce different light brown to black shades of hair dye.(Debendranath et al., 2020)Natural colouring agents obtained from plant sources are more economical, safe, and efficient with very few side effects.(Martins,2013) The purpose of the present investigation is to prepare and evaluate a polyherbal hair dye using a combination of selected medicinal plants.(Alekhya et al.,2021)The selected medicinal plants include dried leaves of *Lawsonia inermis* and *Indigofera tinctoria*, dried fruits of *Phyllanthus emblica*, dried *Areca catechu* nuts, the decoction of dried leaf powder of *Camellia sinensis*, fresh juice of *Beta vulgaris*.(Uttpal Anand et al.,2021)

2. MATERIALS

The leaves of henna, indigo, fruits of amla, peels of blackberry and betel nuts were collected from our institutional medicinal plant garden and nearby certified organic stores and subjected to shade drying. They were authenticated by the Department of Botany, P.B. Siddhartha College of Arts and Science, Vijayawada, Andhra Pradesh, a voucher specimen is preserved in our institution. They were powdered in a household mixer and used in formulating the dye. Basic ingredients used in the poly herbal hair dye are shown in Table 1 and Fig 1.

3. METHODS

3.1. Formulation of different combinations of hair dye:

Different Formulations were developed using the basic components mentioned in Table 1 & fig 1.

Some Formulations included modifying agents (FeCl₃& CuSO₄) in varying percentages. Some of them include moisturizing agents. One-stage application hair dye and two-stage application hair dyes (with and without henna) were also formulated. The details are given in table 2.

Table:1Basic composition of the hair dye

COMPONENT	QUANTITY
<i>Lawsonia inermis</i>	15g
<i>Indigofera tinctoria</i>	45g
<i>Phyllanthus emblica</i>	15g
<i>Areca catechu</i>	15g
<i>Beta vulgaris</i>	15ml
<i>Syzygium cumini</i>	15g



Fig1: Ingredients of Hair dye

Table 2: Experiment protocol:

FORMULA TION	Henna	Indigo	Amla	Betel nut	Beet root	Black Berry	Urea	Aloe vera	1% FeCl ₃	3% FeCl ₃	1% CuSo ₄	3% CuSo ₄
1	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	-
2	✓	✓	✓	✓	✓	✓	✓	-	✓	-	-	-
3	✓	✓	✓	✓	✓	✓	✓	-	-	✓	-	-
4	✓	✓	✓	✓	✓	✓	✓	-	-	-	✓	-
5	✓	✓	✓	✓	✓	✓	✓	-	-	-	-	✓
6	1 st stage	✓	-	✓	✓	✓	✓	-	-	-	-	-
	2 nd stage	-	✓	-	-	-	-	-	-	-	-	-
7	1 st stage	✓	-	✓	✓	✓	✓	-	-	-	✓	-
	2 nd stage	-	✓	-	-	-	-	-	-	-	-	-
8	1 st stage	✓	-	✓	✓	✓	✓	-	-	-	-	✓
	2 nd stage	-	✓	-	-	-	-	-	-	-	-	-
9	1 st stage	-	-	✓	✓	✓	✓	-	-	-	-	-
	2 nd stage	-	✓	-	-	-	-	-	-	-	-	-
10	1 st stage	-	-	✓	✓	✓	✓	-	-	-	-	-
	2 nd stage	-	✓	-	-	-	-	-	-	-	✓	-
11	1 st stage	-	-	✓	✓	✓	✓	-	-	-	-	-
	2 nd stage	-	✓	-	-	-	-	-	-	-	-	✓
12	✓	✓	✓	✓	✓	✓	✓	✓	-	-	-	-
13	✓	✓	✓	✓	✓	✓	-	-	-	-	-	-
14	Marketed chemical dye-1											
15	Marketed Organic herbal dye-2											

3.2. Evaluation of the Herbal Hair Dye

3.2.1. Patch test:

The hair dye formulation was applied as a small patch in an area of 1 sq.cm. behind the ear or on inner elbow and left for 24 hours. During this period the area was observed for redness, irritation, swelling if any. (Rashmi saxena et al., 2018)The test results are displayed in table3.

3.2.2. Physico-Chemical Evaluation:

pH, moisture content and ash value were determined to assess the physico-chemical parameters. (Ujwal et al., 2023) The results are given in table4.

3.2.3. Organoleptic Evaluation:

Organoleptic characters (Wallis, 2002) of the poly herbal hair dye like colour, odour, state were studied and recorded in table5.

3.2.4. Phytochemical Evaluation

The formulated poly herbal hair dye was subjected to phytochemical screening to identify phyto constituents such as carbohydrates, saponins, alkaloids, lipids, sterols etc following standard protocols (Karunasree et al.,2012; Kokate CK, 2007). The results are highlighted in table6.

3.2.5. Application of Formulations 1,2,3,4 &5:

Formulations 1,2,3,4&5 were prepared by mixing all the ingredients with sufficient quantity of water to make a smooth paste. They were carefully applied to the hair samples with a hairbrush. After application, the samples were left aside for 1 hour and washed with a mild shampoo and rinsed with water, and observed for their colouring. The above process of application and washing was repeated 8 times, results are given in fig2-4

3.2.6. Application of Formulations 6,7,8,9,10 &11:

First stage: All the ingredients of Formulations 6,7,8,9,10&11 excluding indigo powder, were made into a paste with sufficient quantity of water and were carefully applied to the hair with a hairbrush. The hair samples were left aside for 1 hour and washed with water and dried.

Second stage: Indigo paste was freshly prepared in warm water and applied to the coloured hair and left aside for 1 hour. The hair was washed with water, and observed. The results of dyeing are shown in fig5&6.

3.2.7. Application of Formulations 12,13,14&15:

All the ingredients of Formulations 12&13 were made into a paste with sufficient quantity of water and carefully applied to the hair with a hairbrush. The hair samples were left aside for 1 hour and washed with mild shampoo and rinsed with water and dried. The process was repeated for 15 times.

Marketed formulations (Formulations 13&14) were applied to the hair with a hairbrush. The hair samples were left aside for 1 hour and washed with mild shampoo and rinsed with water and dried. The process was repeated for 15 times. The results of dyeing are depicted in fig 7&8. The final colour developed after applying various formulations is shown in table 7.

4. RESULTS:

4.1. Patch test: All the prepared formulations were evaluated for skin sensitivity. There was no evidence of redness, irritation and swelling. These formulations are safe on human skin. The results are shown in table 3.

Table 3: Patch test

S. No	Parameter	Result
1	Redness	Negative
2	Irritation	Negative
3	Swelling	Negative

4.2. Physico-Chemical Evaluation

pH, loss on drying and ash value were determined in order to assess the stability, compatibility and amount of inorganic matter present in the formulation. (Wallis, 2002) The results are given in table 4

Table 4: Physico-chemical parameters

S. No	Parameter	Result
1	p ^H	6.9
2	Ash value	1.5
3	Loss on drying	0.15%

4.3. Organoleptic evaluation:

All components of the herbal dye were separately studied by organoleptic and morphological characters like colour, odour, texture and appearance and found to be as per the standards. (Tandon and Sharma, 2010) The organoleptic features of the prepared formulation are described in table 5.

Table 5: Organoleptic characters of the tested formulations

FORMULATION		Colour	Odor	Appearance	Texture
1		Greenish red	Characteristic	Thick paste	Creamy
2		Greenish brown	Characteristic	Thick paste	Creamy
3		Greenish black	Characteristic	Thick paste	Creamy
4		Greenish brown	Characteristic	Thick paste	Creamy
5		Greenish brown	Characteristic	Thick paste	Creamy
6	1 st stage	Greenish red	Characteristic	Thick paste	Creamy
	2 nd stage	Greenish blue	Characteristic	Thick paste	Creamy
7	1 st stage	Greenish brown	Characteristic	Thick paste	Creamy
	2 nd stage	Greenish blue	Characteristic	Thick paste	Creamy
8	1 st stage	Greenish brown	Characteristic	Thick paste	Creamy
	2 nd stage	Greenish blue	Characteristic	Thick paste	Creamy
9	1 st stage	Reddish brown	Characteristic	Thick paste	Creamy
	2 nd stage	Greenish blue	Characteristic	Thick paste	Creamy
10	1 st stage	Reddish brown	Characteristic	Thick paste	Creamy
	2 nd stage	Dark Greenish blue	Characteristic	Thick paste	Creamy
11	1 st stage	Reddish brown	Characteristic	Thick paste	Creamy
	2 nd stage	Dark Greenish blue	Characteristic	Thick paste	Creamy
12		Green	Characteristic	Thick paste	Creamy
13		Green	Characteristic	Thick paste	Creamy
14		Black	Strong and Characteristic	Thick paste	Creamy
15		Green	Strong and Characteristic	Thick paste	Creamy

Table 6: Phytochemical Screening

S. No	Test name	Result
1	Molisch test	+ve
2	Fehling's test	+ve
3	Dragendorff's test	+ve
4	Test for saponins	+ve
5	Volatile oil	-ve

+ve= present ; -ve = absent

Table7: Effects of Hair Dyeing

Formulation	Colour developed	No. of Washes
Formulation-1	Brown	upto10
Formulation-2	Dark brown	upto12
Formulation-3	Dark brown	upto12
Formulation-4	Dark brown	>15
Formulation-5	Dark brown	>15
Formulation-6	Orange changing to brownish black	>12
Formulation-7	Orange changing to greenish-black	>15
Formulation-8	Orange changing to dark greenish-black	>15
Formulation-9	Brown changing to Light purplish black	>12
Formulation-10	Brown changing to light purplish blue	>15
Formulation-11	Blue changing to dark bluish black	>15
Formulation-12	Orange-red	>15
Formulation-13	Light orange	>15
Formulation-14	Black	>15
Formulation-15	Greenish black	>15

4.4. Microscopic observation:

Microscopic features of human white hair, human white hair after application of various formulations, were observed under 40X magnification in a microscope and results are depicted in fig 9-12 .

5. DISCUSSION:

The intensity of colours produced, quality of hair colouring, and dye retention ability were compared between the prepared Formulations and marketed products by visual comparison as well as microscopic observations. It is evident from the observations that in Formulations 1-5, the intensity of colour increases with the number of applications, reaching maximum intensity by the 8th application. The prepared poly herbal hair dye formulations contain all the integrity of natural ingredients. Henna, is well known throughout the globe for its colouring properties and hence regarded as a universal hair dye. It is also beneficial in the removal of excess oil from the scalp and is a hair conditioner as well. (Saif, 2016; Sharma Neeru et al., 2020) Indigo treats and prevents premature graying, leads to new hair growth, and treats baldness, dandruff, and conditions like dry hair, prevents scalp infections, and offers soothing effect on the scalp. (Padam Meena et al., 2016) Amla conditions the scalp, promotes healthy hair growth, improves the tone of henna dyed hair, and also reduces dandruff, and treats head lice. (Ambika and Nikita, 2023) Betel nuts are used since several years in treating hair fall and for promoting healthy hair growth. It also cures itchiness, dandruff, and split ends. Beetroot prevents premature balding and hair loss, relieves from an itchy scalp, prevents dandruff, and also acts as a natural hair colour. (Sandra Dias et al., 2020) Blackberry paste promotes healthy hair growth. Urea moisturizes hair, alleviates itchy dry scalp, decreases dandruff, reduces inflammation, potentially stimulates hair growth, and promotes a healthier scalp. (Belhadj et al., 2017) Apart from acting as a hair dye, this formulation, because of the perfect blend of herbals, also acts as a hair growth promoter, conditioner, hair nourishing, and anti-dandruff agent as well. These experimental observations are supported by similar experiments reported by many other researchers. (Jae et al., 2017; Yushi et al., 2019) Natural pigments and colourants are scientifically proved to be non-toxic in short-term as well as long-term toxicity studies and are safe when compared to chemical based hair dyes. They do not show any adverse health effects even when used for prolonged durations. (Panthip et al., 2012)

The colour offered by Formulation 1 was brown. Formulations 2 & 3 produced dark brown colouration due to the presence of iron salts. After adding 1% & 3% ferric chloride, it was observed that there was an increase in the intensity of brown colour, and colour retaining property was also enhanced. Formulations 4 & 5 produced dark brown colouration. Copper sulfate is preferably added to the dye composition as a solution and is effective in quantities varying from trace amounts upto about 50 milligrams per Liter. The colour stability offered by formulations containing Copper Sulphate was remarkable as compared to formulations containing Ferric Chloride. It was observed that even after 15 washes, more than 50% of the dye was retained by the hair samples.

Formulations 6, 7 & 8 produced an orange shade after the first stage of application. In the second stage, when indigo was applied to the hair samples, the orange colour turned to brownish black, greenish-black, and dark greenish-black shades. It was also observed that even after 15 washes the dye was retained by the hair samples.

After 1st stage of application, Formulations 9, 10 & 11 produced a brown colour. Following the application of indigo paste in the second stage, it was observed that the hair colour turned to light purplish black in case of Formulations 9, light purplish-blue with Formulation 10 and dark bluish black which was almost equal to black colour in case of Formulation 11. It was also observed that even after 15 washes the hair samples retained the dye.

Formulation 12 containing aloe vera shows a soft and moisturized texture on the treated hair samples. Formulation 13 was prepared excluding Urea. Urea helps soften hair keratin to facilitate specifications (colouring, styling). Due to the absence of urea in this Formulation, the hair samples received faint colour. Whereas the formulations including urea showed more uniform dyeing. Formulation 14 containing marketed synthetic hair dye showed black colour. Formulation 15 containing marketed organic hair dye showed orange tint.

When compared to single application Formulations, repeated applications proved to be more efficient in imparting colour as well as its retention. Two stage applications produced colour shades almost equal to black colour.



Fig2: White hair sample before application of dye Formulations



Fig3:Hair samples after application of dye Formulation-1,2&3



After 1st application.



After 2nd application



After 3rd application.



After 4th application



After 5th application.



After 6th application



After 7th application.



After 8th application

Fig4: Hair samples after application of dye Formulation-1,4&5



Fig5: Hair samples after application of Formulations-6,7&8

FORMULATION -9	FORMULATION -10	FORMULATION -11
		



Fig6: Hair samples after application of Formulations-9,10&11



With aloe vera.



Without urea

Fig7: Hair samples after application of dye Formulations-12&13



Fig8: Hair samples after application of dye Formulation-14&15

Microscopic observations:

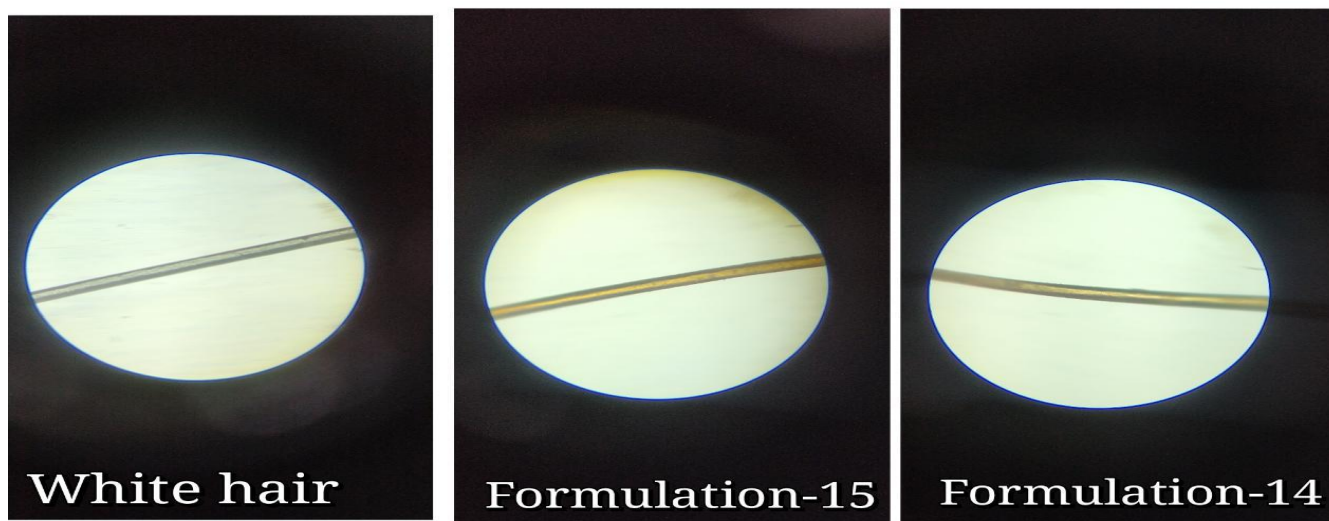


Fig9: Microscopic observation of white hair, Formulations-14&15

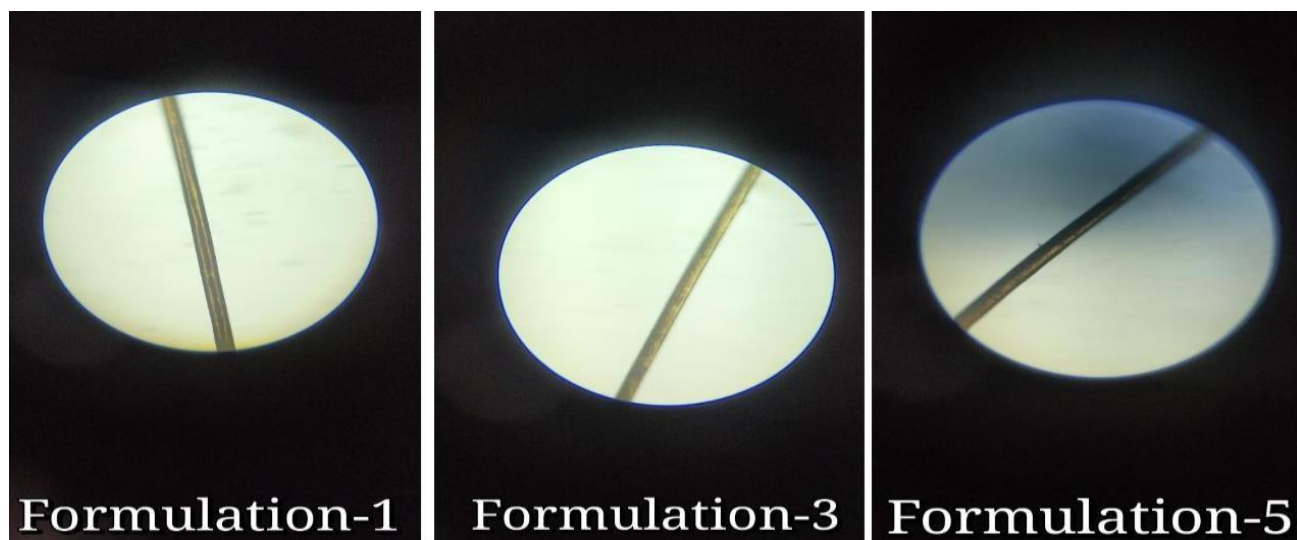


Fig10: Microscopic observation of Formulations-1,3&5

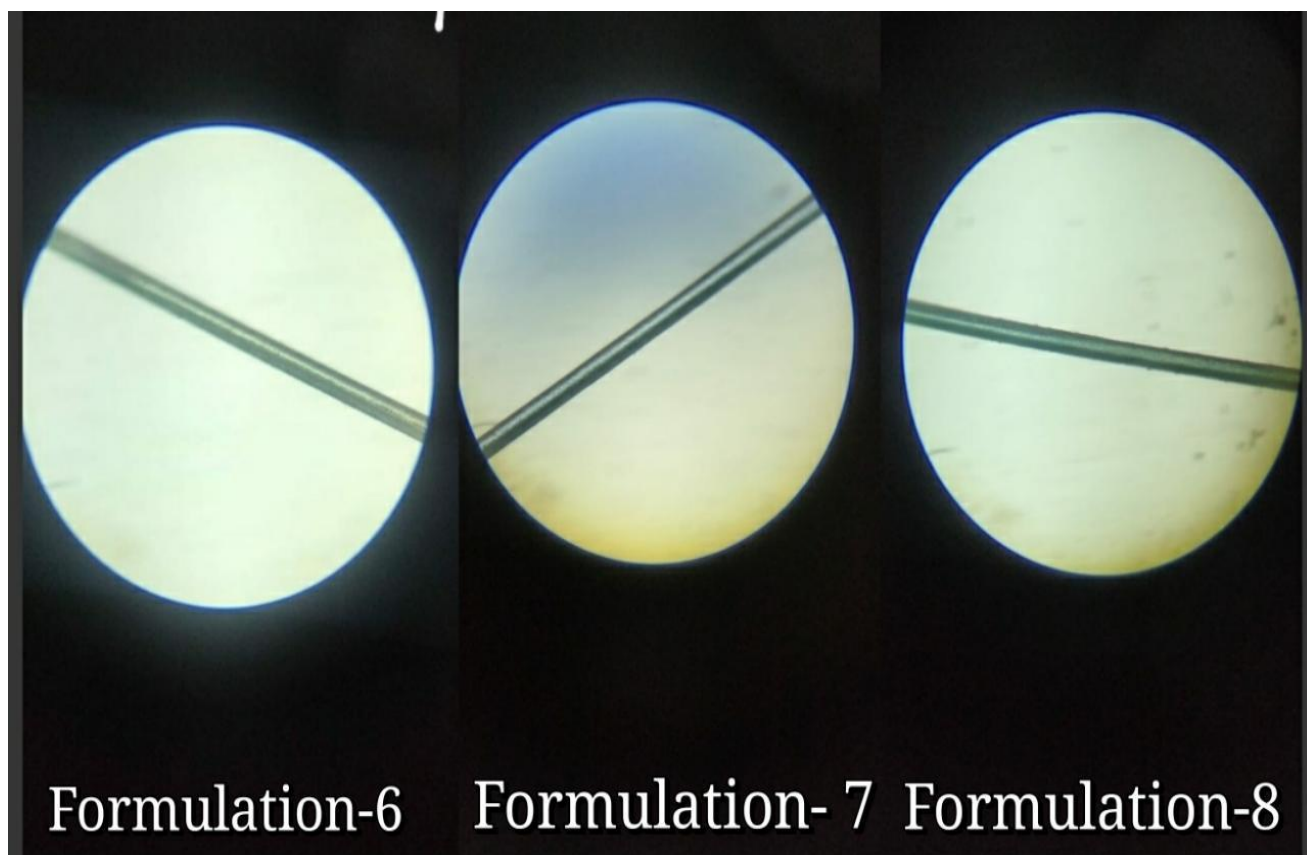


Fig11: Microscopic observation of Formulations-6,7&8

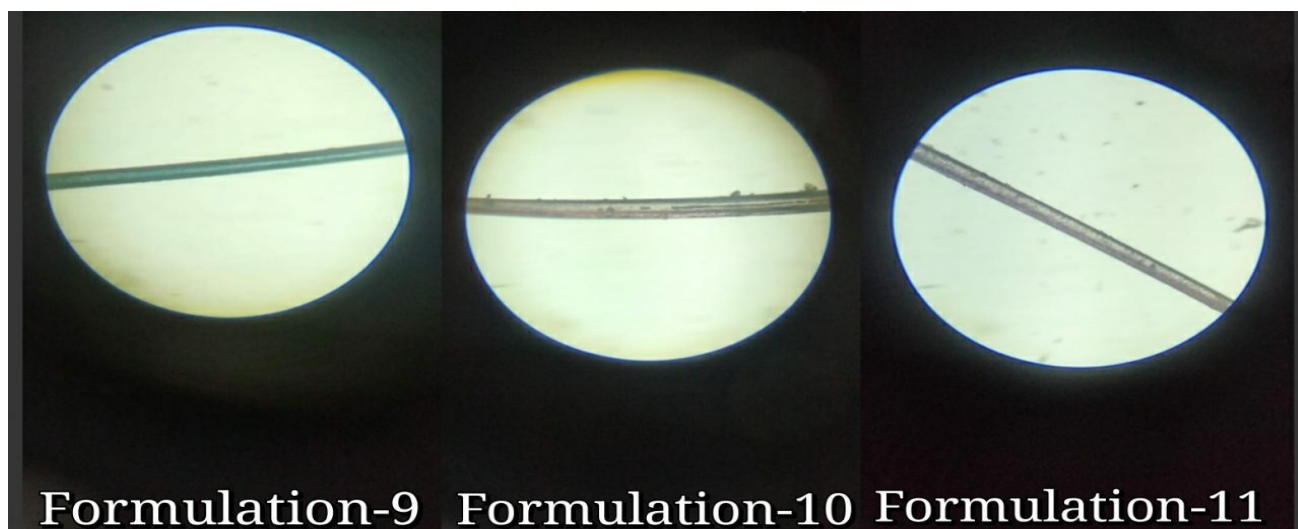


Fig12: Microscopic observation of Formulations-9,10&11

6. CONCLUSION:

The hair dye formulations containing FeCl_3 produced brownish shades and dye formulations containing CuSO_4 produced a greenish tint. It was also observed that Two-stage applications containing henna gave better results as these Formulations offered darker colours. The synergistic effect of tannins, carotenoids and flavonoids in the tested polyherbal hair dye formulation contribute to the colouring as well as protective effect on human hair and skin. The colouring activities of the herbal hair dye were similar with that of the commercially available synthetic and organic hair dye. At this juncture, there is an enormous need for natural dye formulations from herbal products.

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