

**Formulation and evaluation of polyherbal formulation as hair colorant**

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\*Corresponding author e-mail: [velpharmamsu@gmail.com](mailto:velpharmamsu@gmail.com)**ABSTRACT**

Herbal cosmetics formulated by using various coloring ingredients as the base, in which one or more coloring ingredients are used to provide defined cosmetic benefits, are termed as "Herbal Cosmetics". The need of herbal medicines is increasing rapidly due to their lack of side effects. Herbal drugs constitute a major part in all the traditional systems of medicine. Hence, an attempt has been made to review the use of natural products obtained from plant sources to replace the synthetic dyes. The composition of natural hair dye consisting of amla, bhringraj, henna, mandara, indigo, ratanjot, sariva, curry leaves and methi seed powders are blended with urea and the mixture is soaked to obtain a dye. The plant samples have been standardized by physico-chemical parameters such as total ash, acid insoluble ash, water soluble ash and extractive value of different solvents. The formulated hair dye have been characterized by pH, microscopic study, effect of sunlight, effect of detergent, effect of three time coating and patch test. Result of the present studies shows that out of 17 hair colorant formulations four formulations, F2, IF2, BAF3 and KM2 have better activity. The studies have also shown that there is no hair damage or scalp irritation on dyeing the human hair. Hence this formulation proves to be a key alternative for modern synthetic hair dyes.

**Keywords:** Henna, Indigo, Ratanjot, Microscopic study, Patch test.**INTRODUCTION**

Hair is a filamentous biomaterial that grows from follicles found in the dermis. Hair is one of the defining characteristics of mammals<sup>1</sup>. The human body, apart from areas of glabrous skin, is covered in follicles which produce thick terminal and fine vellus hair. Hair fibers have a structure consisting of three layers, starting from the outside, the cuticle, which consists of several layers of flat, thin cells laid out overlapping one another as roof shingles. The cortex, which contains the keratin bundles in cell structures that remain roughly rod-like. The medulla, a disorganized and an open area at the fiber's center. Hair dye is one of the oldest known beauty preparations<sup>2</sup>, and was used by ancient cultures in many parts of the world. Ancient Egyptians, Greeks, Hebrews, Persians, Chinese, and early Hindu literature mention the use of hair coloring agents. Earlier, hair dyes were made from plants, metallic compounds, or a mixture of the two. Rock alum, quicklime, and wood ash were used for bleaching

hair in Roman times. Herbal preparations included mullein, birch bark, saffron, myrrh, and turmeric were used as hair dye. Many plant extracts were used for hair dye in Europe and Asia before the advent of modern dyes. Indigo, known primarily as a fabric dye, could be combined with henna to make light brown to black shades of hair dye. Most of the synthetic hair coloring agents rely on harsh chemicals like phenylene diamine, which have some side effects<sup>3</sup>. The demand of herbal medicines is increasing rapidly due to their lack of side effects. Amla, bhringraj, henna, Mandara, indigo, ratanjot, sariva, curry leaves and methi seeds are well-known ayurvedic herbal drugs traditionally used as hair colorant and for hair growth. To our best knowledge, there is no scientific report on the combination of these herbal drugs hair dye formulation. Hence, the present study has been designed to formulate and evaluate the polyherbal formulation of Amla, bhringraj, henna, Mandara, indigo, ratanjot, sariva, curry leaves and methi seeds as hair coloring agent.

## MATERIALS AND METHODS

**Collection of plant material:** The plant powders of henna, bhringraj, sariva and ratanjot were collected from Amsar Pharmaceutical, Colvale, Bardez, Goa. Dr. Jains product of mandara, amla and indigo were collected from Union pharmacy, Mapusa, Goa. Curry leaves and methi seeds were collected from Mapusa vegetable market, Goa.

**Collection of unpigmented hair:** The human hair was collected from barber shop in which white hair were separated and used for studies.

**Extraction of Plant Material:** About 100 g of the plant powder (henna, bhringraj, mandara and amla) was extracted separately with 500 ml of water (solvent), mixed well and kept for around 24 hours at room temperature with frequent shaking. After 24 hours the above extract was kept for a refluxing for around 3 hours. After refluxing, filtrations were carried out using non-absorbing cotton as the filter media. Then the filtrate was evaporated and the extract was collected and packed in a polythene packets so that no micro-organism get accumulated on it. The same plant material was extracted 3-4 times to extract all the water soluble phytoconstituents in the plant material.

**Physico-chemical Properties:** Different physico-chemical values<sup>4</sup> such as ash value that included total ash, acid insoluble ash, water soluble ash and extractive values of different solvents such as petroleum ether, chloroform, ethanol and water were also determined.

### Formulation Studies<sup>5-7</sup>

**Formulation I:** Henna, bhringraj, amla and mandara were extracted with water separately, water extract of the above plant material were mixed using the composition as per Table 2. After formulation the above paste were applied on white human hair and kept in evaporating dish for 3 hours and washed the hair using distilled water.

### Preliminary Preparation of Hair Colouring Agent

**Procedure:-** One gram of each henna, indigo, amla, sariva, ratanjot, bhringraj, hibiscus, curry leaves and methi seeds were taken separately in a china dish and then 200mg (20%) of urea was added in each sample. A smooth paste was made with water separately. The paste was kept aside for 1 hour for inhibition. The hair was kept in the above paste for 1 hour and then washed with water and observed for its coloring.

### Selection of Suitable Combination

**Formulation 1:** 2 grams of different ratio of plant materials such as henna, sariva, ratanjot, bhringraj with 400mg of urea were taken in a china dish (Table 3). A smooth paste was made with water separately. The paste was kept aside for 1 hour for inhibition. The hair was kept in the above paste for 1 hour and then washed with water and observed for its coloring.

**Formulation 2:** 4 g of different ratio of plant materials such as henna, indigo, bhringraj with 800mg of urea were taken in a china dish (Table 4). A smooth paste was made with water separately. The paste was kept aside for 1 hour for inhibition. The hair was kept in above the paste for 1 hour and then washed with water and observed for its coloring.

**Formulation 3:** 2.5 g of different ratio of plant materials such as henna, indigo, bhringraj, sariva, ratanjot with 500mg of urea were taken in a china dish (Table 5). A smooth paste was made with water separately. The paste was kept aside for 1 hour for inhibition. The hair was kept in the above paste for an hour and then washed with water and observed for its coloring.

**Formulation 4:** 2.0 g of different ratio of plant materials such as henna, amla, curry leaves and methi seeds with 400mg of urea were taken in a china dish (Table 6). A smooth paste was made with water separately. The paste was kept aside for 1 hour for inhibition. The hair was kept in the above paste for 4-5 hours and then washed with water and observed for its coloring.

**Effect of three times coating:** The best formulations such as F2, IF2, BAF3, KM2 were coated three times using the same procedure followed for single coating.

### Characterization of the hair colorant formulations<sup>8-10</sup>

**Microscopic studies:** Microscopic observation of colored hair was studied using compound microscope (Leica EC3).

**Test for pH:** 1 g of plant powder was weighed and 9 ml of water was added in a beaker, stirred well and the pH was found using pH meter. The results of pH were presented in Table.7

**Effect of Sunlight on colored human hair :** The colored human hair were pasted on a white paper sheet covered with transparent cellophane sheet and then kept in sunlight for 2 hours daily for 15 days at the interval of 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> days photographs were taken.

**Effect of Natural detergent on colored human hair:** A 10 % w/v aqueous solution of Reetha (*Sapindus mukorossi*) was prepared. The colored human hair were washed with Reetha aqueous solution for one

minute on alternate days and their photographs were taken on 1<sup>st</sup>, 4<sup>th</sup>, 7<sup>th</sup>, 9<sup>th</sup>, 12<sup>th</sup> and 15<sup>th</sup> days.

**Patch test:** A small quantity of paste was applied on the ear back. After 15 minutes this paste was removed and the area was washed carefully. There was no irritation/allergic reaction, if there was irritation/allergic reaction, the application of that formulation was avoided.

## RESULTS AND DISCUSSION

**Physico-chemical Properties:** The ash values are indicating the purity of drug. The extract values are useful to evaluate the chemical constituents present in the crude drug and to estimate specific constituent soluble in a particular solvent. It was observed that there was an increase in the extract values with the increasing polarity of the solvent. The higher extract value of water in all the plant powders such as Henna, indigo, ratanjot, amla, bhringraj, hibiscus, sariva, curry leaves and methi seeds may be due to the greater degree of polarity of water. The results are presented in Table.1

### Formulation studies

**Formulation 1:** After formulation (Table 2) the above paste were applied on white human hair and kept in evaporating dish for 3 hours and washed the hair using distilled water. There is no color change. This may be due to the active pigment from the plant material not being extracted by water.

**Formulation 1:** The different plant material and its composition in Formulation 1 were made as mentioned in Table 3. From the photographic examination, it was confirmed that out of four formulations, hair treated F2 formulation was showing a brown color that may be because of the higher henna concentration in F2 compared to other formulation.

**Formulation 2:** The different plant material and its composition in Formulation 2 were made as mentioned in Table 4. From the photographic examination, it was confirmed that out of four formulations, hair treated IF2 formulation was showing a purple black color that may be because of the higher Indigo concentration in IF2 compared to other formulation.

**Formulation 3:** The different plant material and its composition in Formulation 3 were made as mentioned in Table 5. From the photographic examination, it was confirmed that out of four formulations, hair treated BAF3 formulation was

showing a purple brown color that may be because of the higher Indigo, henna and bhringraj concentration in BAF3 compared to other formulation.

**Formulation 4:** The different plant material and its composition in Formulation 4 were made as mentioned in Table 6. From the photographic examination (Figure 1) it was confirmed that out of four formulations, hair treated KM2 formulation was showing a golden brown color that may be because of the higher henna and curry leaves concentration in KM2 compared to other formulation. The photographic representation of the coloring effect of best formulations, F2, IF2, BAF3, KM2 were presented in Figure.1

**Effect of three times coating:** The best formulation in each case such as F2, IF2, BAF3 and KM2 was selected for the three time coating of the same formulation in hair separately. From the Figure 1 and Figure 2. it was confirmed that F2 one time coated shows brown color converted to dark brown when coated three times, IF2 one time coated shows purple black color converted to dark black when coated three times, BAF3 one time coated shows purple brown converted to black when coated three times and KM2 one time coated shows brown converted to golden brown when coated three times. The photographic representation of the effect of three times coating were presented in Figure.2

### Characterization of the Hair Colorant Formulations

**Microscopic Study:** Microscopic studies were done only for the best formulation such as F2, IF2 and BAF3 (Figure 3). The microscopic studies of the human hair treated with best formulation F2, IF2, BAF3 shows that the three times coated human hair was more thicker than one time coated human hair. It shows that the effect of sunlight is lesser than the effect of detergent.

Standard marketed hair (Figure 4) dye have better coloring effect compared to that of the our formulated hair dye but continuous coating of synthetic hair dye damage the cortex of the hair, as was shown in the microscopic studies of the three times coated hair of standard-1 and standard-3. Another herbal marketed hair dye, standard-2 does not damage the cortex of the hair.

**pH:** pH is an important parameter in coloring agent. The same chemical compound may have different color on different pH. Amla and henna are basically acidic in nature and that may be the reason for the lower pH values in their formulations (Table 7). The formulations showed a higher thickness of dye coat

over the hair at pH 5-6, since this near to the normal pH of the skin and hair.

**Effect of Sunlight on colored human hair :** All the three best formulation F2, IF2, BAF3 from 1<sup>st</sup> day to 15<sup>th</sup> day (Figure 5) there is a continuous color decrease in the hair because of the exposure to the sunlight. It was clear that the sunlight decreases the color pigments present in the colored human hair.

**Effect of Natural detergent on colored human hair:** Natural detergent wash decreases the color pigments present in the colored human hair and this was shown in all the three best formulation F2, IF2, BAF3. It was found that from 1<sup>st</sup> wash to 15<sup>th</sup> wash (Figure 6) there is a continuous color decrease in the hair .

**Patch test:** The herbal hair dye samples were subjected to patch test since herbal hair dye should not produce any skin sensitization or irritation when applied on hair. All the herbal formulation does not show any irritation. All the formulations are completely free from irritation.

#### CONCLUSION

**Table 1: Physiochemical properties of Henna, Indigo, Ratanjot, Amla, Bhringraj, Hibiscus, Sariva, Curry leaves and Methi seeds**

Particulars	Henna Values (%)	Indigo Values (%)	Ratanjot Values (%)	Amla Values (%)	Bhringraj Values (%)	Hibiscus Values (%)	Sariva Values (%)	Curry leaves Values (%)	Methi seeds Values (%)
Total ash	5.04%	9.91%	1.28%	8.23%	21.99%	35.14%	7.02%	10.91%	3.55%
Acid insoluble ash	0.4%	0.6%	0.8%	1.82%	11.52%	31.2%	2.07%	1.165	0.48%
Water soluble ash	1.93%	2.15%	2.62%	2.33%	19.33%	31.68%	5.18%	5.52%	1.45%
Extractive value									
Pet ether	5.86%	5.86%	20.18%	4.66%	5.74%	0.88%	2.38%	6.92%	6.2%
chloroform	7.02%	24.26%	14.4%	5.96%	5.74%	8.54%	5.42%	10.32%	9.04%
ethanol	21.88%	35%	22.1%	19.62%	5.6%	10%	10.28%	15.58%	10.48%
water	28.94%	48.72%	61.96%	42.78%	20.74%	16.6%	20.34%	33.12%	31.16%

**Table 2: Formulation I**

HENNA	MANDARA	AMLA	BHRINGRAJ
1.0 gm	0.5 gm	0.5 gm	1.0 gm

Medicinal plants such as Henna, indigo, ratanjot, amla, bhringraj, hibiscus, sariva, curry leaves and methi seeds are alternative to synthetic hair dye to be used in hair color cosmetic because it gives the beautiful and long lasting hair color without causing hair damage and there is no harmful effect for hair and skin. From present study it has been concluded that the maximum colorant action of IF2, F2, and BAF3 were due to synergetic effect of each plants extract. As the results were found to be encouraging, these plants may be further explored for developing the suitable formulation and to determine the exact mechanism.

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**Table 3: Formulation.1**

	HENNA	BHRINGRAJ	SARIVA	RATANJOT
F1	0.5 gm	0.5 gm	0.5 gm	0.5 gm
F2	1.0 gm	0.5 gm	0.25 gm	0.25 gm
F3	0.75 gm	0.75 gm	0.25 gm	0.25 gm
F4	0.25 gm	1.0 gm	0.25 gm	0.5 gm

**Table 4: Formulation 2**

	HENNA	BHRINGRAJ	INDIGO
IF1	1.5 gm	1.0 gm	1.5 gm
IF2	0.5 gm	1.0 gm	2.5 gm
IF3	2.0 gm	1.0 gm	1.0 gm

**Table 5: Formulation 3**

	HENNA	BHRINGRAJ	SARIVA	RATANJOT	INDIGO
BAF1	0.5 gm	0.5 gm	0.5 gm	0.5 gm	0.5 gm
BAF2	0.5 gm	0.5 gm	0.25 gm	0.25 gm	1.0 gm
BAF3	0.75 gm	0.5 gm	0.25 gm	0.25 gm	0.75 gm
BAF4	1.0 gm	0.25 gm	0.5 gm	0.25 gm	0.5 gm

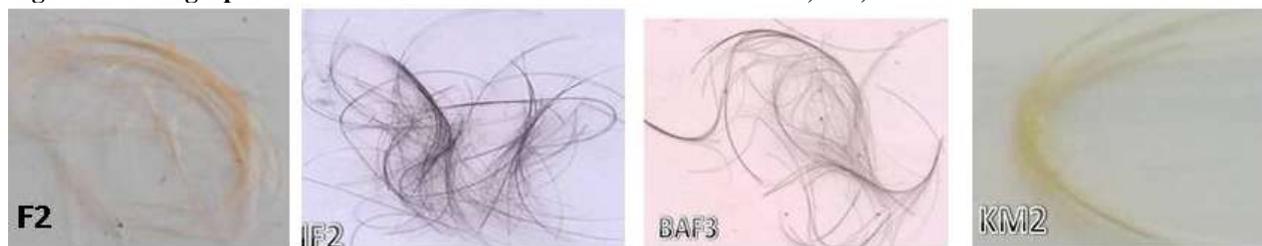
**Table 6: Formulation 4**

	HENNA	CURRY LEAVES	AMLA	METHI SEEDS
KM1	0.5 gm	0.5 gm	0.5 gm	0.5 gm
KM2	0.75 gm	0.75 gm	0.25 gm	0.25 gm
KM3	0.5 gm	0.5 gm	0.25 gm	0.75 gm
KM4	0.5 gm	0.25 gm	0.75 gm	0.5 gm

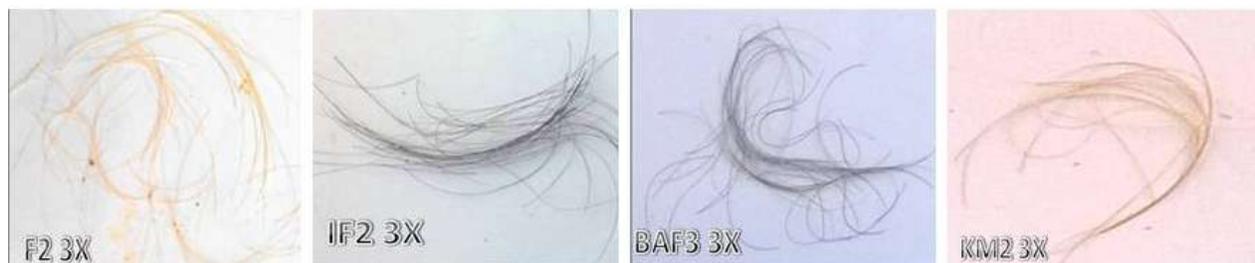
**Table 7: Effect of pH in the formulations.**

Formulation	Without urea (pH)	With urea (pH)
FI	5.9	5.8
F1	5.00	5.03
F2	4.79	4.79
F3	4.52	4.48
F4	5.24	5.25
BAF1	5.25	5.24
BAF2	5.79	5.80
BAF3	5.28	5.28
BAF4	5.61	5.60
IF1	5.44	5.44
IF2	5.88	5.89
IF3	4.81	4.82
KM1	3.21	3.23
KM2	2.98	2.99
KM3	3.55	3.56
KM4	2.83	2.85

**Figure 1: Photographs of human hair treated with best formulation F2, IF2, BAF3 and KM2**



**Figure 2: Photographs of human hair treated with three times of best formulation F2, IF2, BAF3, KM2.**

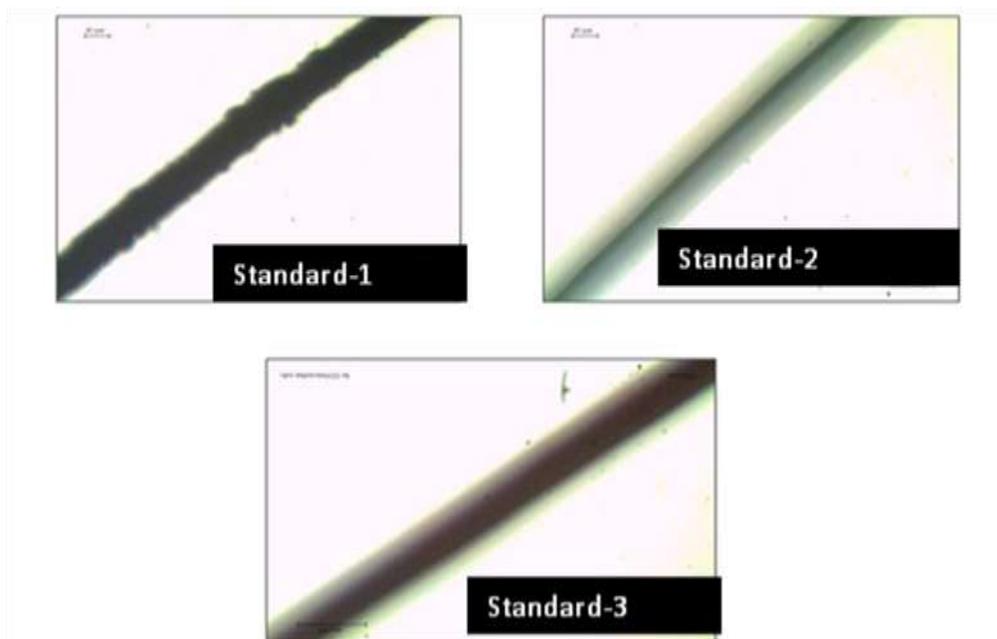


**Figure 3: Microscopic photos of human hair treated with IF2, F2 and BAF3 at different condition such as one time coating , three time coating, effect of sunlight after 20 days and effect of detergent after 20 days.**

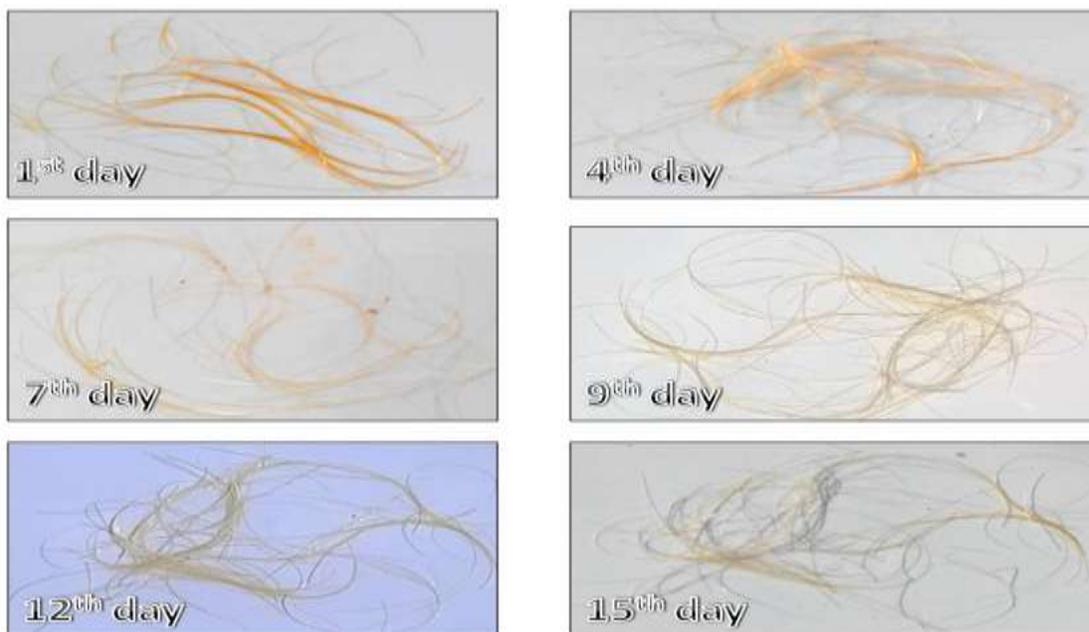


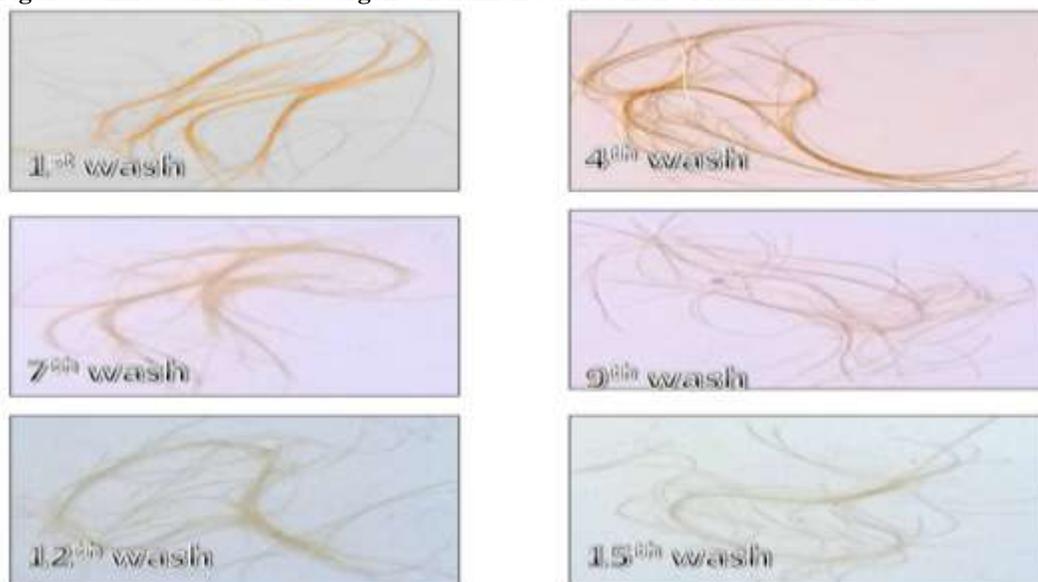
1X- one time coating, 3X-three time coating, Sunlight-effect of sunlight, detergent-effect of natural detergent wash.

**Figure 4: Microscopic photos of human hair treated with marketed hair dyes three times.**



**Figure 5: Effect of sunlight on F2 formulation.**



**Figure 6: Effect of natural detergent on human colored hair of formulation F2****REFERENCES**

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