Research Article

Evaluation of Organic Hair Dye Formulation by an Environment Friendly Process

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ABSTRACT

Graying of hair is a natural phenomenon attributable to ageing and frequent use of synthetic shampoos which has encouraged application of synthetic dyes with the increase in the usage of hazardous chemicals in the process of manufacturing. 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 organic hair dye consisting of *Lawsonia inermis, Hibiscus rosa* –sinenis (leaves and flowers), *Murraya Koenigii, Eclipta alba, Punica granatum, Emblica officinalis, Azadirachta indica, Ocimum Sanctum, Trigonella Foenum* powders are blended with aloevera gel along with Iron filings and soaking the mixture to obtain a dye. The plant samples have been tested for the presence of alkaloids, Quinones, Saponins and for polyphenolic compounds. A supportive analytical data has been obtained using EDXRF, FTIR and TLC. The samples have been tested for their antibacterial activity. Further this study is directed for coloring of hair, by applying each and every plant material and also the mixture of formulated dye. This has shown that there is no hair damage or scalp irritation because of dying the human hair. Hence this formulation proves to be a key alternative for the synthetic hair dye.

Keywords: *Aloe vera, Azadirachta indica, Eclipta alba, Emblica officinalis, Hibiscus rosa –sinenis, Lawsonia inermis, Punica granatum.*

INTRODUCTION

Although graying of hair is a natural phenomenon associated with ageing, there has been a significant occurrence of premature graying specially in women, attributable probably to stress and use of synthetic shampoos. Loss of colour in hair is due to varied reasons like genetic influence, effect of environmental factors, use of alcoholic preparation, etc.[1] Graying of hair results from an absence of pigment, it occurred to the scientists that hydrogen peroxide and catalase might play a critical role in the process. Every hair cell makes a little hydrogen peroxide, but over time the amount builds up. The European team discovered that this buildup ended up blocking the normal synthesis of melanin, the natural pigment in hair turns out, bleaches itself from the inside out. And by identifying the chemicals involved, researchers may be closer to understand that graying is influenced by stress. Gray hair at early age encourages frequent use of synthetic dyes to color the gray patches of hair.

Among the Egyptians, there were hairdressers and the art of dyeing hair with vegetable dyes was known already at that time. The first artificial dye was synthesized in the laboratory in 1856, and permanent hair colorants have been in commercial use for over 100 years.

Hair dyes can be divided into five categories, each with a specific composition and action mechanism: gradual hair coloring (using metallic dyes such as salts of lead, bismuth or silver), vegetable hair dyes (such as henna), temporary dyes (water-soluble dyes that withstand only one-time shampooing), semi-permanent dyes (which can withstand 4-5 times of shampooing) and permanent hair colors.

Permanent hair colors are the most popular hair dye products. They may be further divided into oxidation hair dyes and progressive hair dyes. Oxidation hair dye products consist of a solution of dye intermediates, e.g., p-phenylenediamine,[2] which form hair dyes on chemical reaction, and preformed dyes, e.g., 2-nitro-p-phenylenediamine, which already are dyes and added to achieve the intended shades, in an aqueous, ammoniacal vehicle containing soap, detergents and conditioning agents; and solution of hydrogen peroxide, usually 6%, in water or a cream lotion.

The ammoniacal dye solution and the hydrogen peroxide solution, often called the developer, are mixed shortly before application to the hair. The applied mixture causes the hair to swell and the dye intermediates (and preformed dyes) penetrate the hair shaft to some extent before they have fully reacted with each other and formed the hair dye.[3]

It is common knowledge that many of these synthetic dyes can induce dermatitis[4] and other related problems. This has prompted us to search for herbal dyeing materials of plant origin as alternatives. An attempt here has been made to review the use and suitability of these herbal products to replace the synthetic hair dyes.[5] Continuous usage of such compounds on natural hair causes multiple side effects such as skin irritation, erythrema, loss or damage of hair and also skin cancer.[6, 7]

The disadvantages of chemically derived dyes can only be overcome by nontoxic ingredients derived from herbal sources. Composition of herbal dyes and hair coloring mordants can be used to deliver a variety of colors to hair. However substantial improvement is needed in the areas of color saturation, color development, initial color

consistency, improved wash fastness, improved hair conditioning without causing hair damage and skin irritation.

MATERIALS AND METHODS

Plant sources

The following plant materials have been collected from the garden and authenticated by the botanist.[8,9] The materials have been dried in shade and powdered.

a) *Lawsonia inermis* (Henna) b) *Hibiscus rosa* –sinenis (leaves and flowers) c) *Murraya Koenigii* (Curry leaves) d) *Eclipta alba* (Guntagalagaraku) e) *Punica granatum* (Pomegranate fruit) f) *Emblica officinalis* (Amla) g) *Azadirachta indica* (Neem) h) *Ocimum Sanctum* (Tulasi) i) *Aloe vera* j) *Trigonella Foenum* (Fenugreek) k) Juglone (Walnuts)

Henna: The botanical name is *Lawsonia inermis* which is the only species of the genus *Lawsonia* and belongs to the family *Lythraceae*. The leaves of this plant posess a red dye molecule called lawsone (2- Hydroxy – 1yl- naphtha quinone), which has the ability to bond with protein. The other components like Lawsone 1, 4 – naphtha quinone; 2- methoxy- 3- methyl- 1,4 - naphtha quinone; flavonoids, coumarins and phenolic acids; 5-10% gallic acid and tannin.[10] Henna balances the pH of the scalp preventing premature hair fall and graying of hair.

Hibiscus: The botanical name is Hibiscus rosa –sinenis, Family: Malvaceae. Commonly known as rose mallow or Jamaica or Chinese rose. This flower is used for controlling dandruff. The leaves of hibiscus species exhibit antioxidant properties by producing flavonoids, anthocyanins and other phenolic compounds and are immune-modulating reducing the harmful effects of UV radiation. It can be used to rejuvenate the hair by conditioning.

Curry leaves: The botanical name is *Murraya Koenigii*. This is found to have many phenols, flavanols, amino acids and alkaloids which are known to have high antioxidant capacity.

Eclipta alba: (L.) Hassk. syn. E. prostrate (L.) L. Guntagalagaraku:

The botanical name is *false daisy*. It belongs to Asteraceae family. The herb is used to check hair loss and stimulate hair growth.[11] The presence of coumestans (derivative of coumarin), alkaloids, thiopenes, flavonoids, polyacetylenes, triterpenes and their glycosides in Eclipta alba qualifies it as a good source of dye.

Pomegranate fruit*:* The botanical name is *Punica granatum* or Punicaceae. This has tannins which make it good mordant and also as dye because of the presence of large quantities of antioxidants, secondary plant substances like poly-phenolens.

Amla*:* The botanical name is *Emblica officinalis* or *Phyllanthus emblica* L. (Emblic Myrobalan, Indian Goosberry) It has anti inflammatory, antibacterial and antioxidant properties that can help promote the growth of healthy, lustrous hair.

Neem: The botanical name is *Azadirachta indica* A. Juss. (Indian Lilac, Margosa Tree, Neem Tree) Terpenoids are present. It has well anti bacterial and insecticidal property.

Tulasi: The botanical name is *Ocimum Sanctum* (Basil).It belogs to Laminaceae family. It has anti viral and anti bacterial property.

Aloevera: The botanical name is *Gawar Patha*, It belongs to Liliaceae family. Aloevera gel is effective for scalp and can be used not only to treat hair loss, but to promote hair growth as well. Aloevera contains aloe emodin which promotes hair growth by stimulating hair follicle. It is also useful in treating the scalp from sun burn. It is used as a natural mordant. It is known for its emoluent effect.

Trigonella Foenum: The botanical name is *Fenu greek*. It is used both as an herb (the leaves) and as a spice (the seed). It is used as a conditioner for the hair

Juglone: The botanical name is Walnut (Husk). The plant species like Juglans cinerea, J. Regia. J. Nigra (butternut) are the sources for the class of naphthaquinone compound i.e 5-hydroxy-1,4-napthoquinone, an isomer of lawsone, including juglone and juglandin. It is generated by hydrolysis from the glycoside of its hydroquinone form. It is responsible for giving brown color to the dye.

Black Tea: The tannins present in tea will increase the color intensity.

Preparation of Plant extracts (Methanolic extract)

50 g of dried powdered samples were soaked in 125 ml of methanol for 16 hours in a rotatory shaker. Whatman No.1 filter paper was used to separate the extract of plants. The filtrates were used for further phytochemical analysis.[12]

Preliminary Phytochemical Screening of plant extracts

Phytochemical screening was performed for the analysis of different phytochemicals like carbohydrates, saponins, oils, fats, flavonoids, terpenoids, alkaloids *etc*., in methanolic extracts of plant samples. The screening was performed with some modifications from the method of Harborne et al.[13]

Test for Carbohydrates (Reducing Sugars) (Fehling's test)

The extracts were treated with 5.0 ml of Fehling's solution and kept in boiling water bath. The formation of yellow or red color precipitate indicates the presence of reducing sugars.

Test for Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water bath and filtered. The 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a suitable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously and

then the formation of emulsion was observed. This indicates the presence of Saponins.

Test for Terpenoids

A volume of 5 ml of the plant extract was mixed in 2 ml of chloroform and concentrated H_2SO_4 was added to form a layer. A reddish brown color of the interface shows the presence of terpenoids.

Test for Alkaloids

The plant extract was mixed with a few drops of acetic acid followed by Dragendroff's reagent and mixed well. An orange red precipitate formed indicates the presence of alkaloid.

Test for Flavonoids

5 ml of dilute ammonia solution was added to the aqueous filtrate of the plant extract followed by the addition of concentrated H_2SO_4. A yellow color observed in the extract indicates the presence of flavonoids.

Test for Tannins and Phenolic compound

About 0.5 g of the dried powdered sample was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride are added and observed for brownish green or a blue-black colour. A few drops of alcohol and ferric chloride solution were mixed with the plant extract. A blue green colour indicates the presence of phenol.

Test for Amino Acids and Proteins

To 1ml extract, 2 drops of freshly prepared 0.2% ninhydrin reagent was added and heated. Blue colour indicates the presence of proteins and amino acids.

Test for Quinones

A few drops of sodium hydroxide was mixed with the plant extract and shaken vigorously. A blue green or red color indicates the presence of quinones.

Analytical Methods

Spectroscopic Characterization

The raw dye and different isolates of the dye were subjected to spectroscopic techniques for the identification of components.

XRF elemental analysis of the dye

The sample was analyzed for trace element using energy dispersive X-ray fluorescence (EDXRF) spectrometry at the The International Advanced Research Centre for Powder Metallurgy and New Materials (ARCI) Sensitivity calibration using thick pure metal foils (Ti, Fe, Co, Ni, Cu, Zn, Zr, Nb, Mo, Sn, Ta, Pb) and stable analytical grade chemical compounds (K_2CO_3, $CaCO_3$, Ce_2O_3, WO_3, ThO_2 and U_3O_8.) The spectral data collected with the Maestro software were first converted to the standard AXIL format and then fitted with a model created from the qualitative information on the spectra using a nonlinear least square strategy of AXIL software package. Quantification of the concentration of detectable elements was made using a modified version of emission-transmission method.[14]

Infrared (IR) spectroscopy

Infrared spectroscopy (IR) is an analytical tool that can be used in the determination of chemical compound of a dye. It can also be used for the elucidation of the structure of both organic and inorganic components of the dye. It usually reveals the functional groups present in a sample[15] Infrared spectra was recorded on a Perkin – Elmer 1600 series-FTIR Spectrometer as KBr discs.

Thin Layer Chromatography (TLC)

Pre coated silica gel plates were obtained from the market. Toluene: Acetone: Formic acid (11:6:1 v/v) mixture was used as a solvent. 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 colored spots developed were noted and the R_f value was calculated by measuring the distance traveled by the solute and the solvent.

Open Patch Test

Sensitizing the potential of formulation is to be tested. Hence a small quantity has been applied on the fore arm[16] to check for any local reaction like irritation and erythrema within three hours of application.

Extraction and formulation of dye

Each raw material has been applied to the hair sample brought from a parlour to check the fastness and dying effect on it for 30 minutes.

Fresh leaves of aloevera were collected, washed thoroughly and the outer surface has been peeled off and inner mass was collected with the help of a scoop.

Finally 50 gms of *Lawsonia inermis*, 20 gms of *Hibiscus rosa* –sinenis (leaves and flowers), 20 gms of *Murraya Koenigii*, 20 gms of *Eclipta alba*, 20 gms of *Punica granatum*, 30 gms of *Emblica officinalis*, 5 gms of *Azadirachta indica*, 5 gms of *Ocimum Sanctum*, 5 gms of *Trigonella Foenum*, 20gms of *Juglone Hussk* powders were blended with 5 gms of aloevera gel along with 2 gms of Iron filings and soaking the mixture for 1 hour in water along with tea decoction to obtain a dye.

The soaked dye composition has been applied to hair to check color imparted to it.[17] When the formulated paste is applied on the hair shaft, the dye molecule penetrates inside the hair shaft and binds with the keratin fiber. When the light falls on the dye applied hair, the outer layer of the hair shaft shimmers / reflects the light in such a way to present the hair to be in brownish red color.

RESULTS AND DISCUSSION

EDXRF (Energy Dispersive X-ray Fluorescence measurement)

Table 1: Results of elemental analysis of sample *Lawsonia Inermis* by EDXRF techniques

Element Line	Net Counts	Weight %	Weight % Error	Atom %
C K	1177	12.85	+/- 0.32	20.85
O K	3207	46.90	+/- 0.64	57.13
Na K	205	0.90	+/- 0.15	0.76
Mg K	873	3.16	+/- 0.11	2.54
Si K	168	0.58	+/- 0.08	0.40
P K	836	3.08	+/- 0.13	1.94
Cl K	694	3.75	+/- 0.17	2.06
K K	3428	26.02	+/- 0.43	12.97
Ca K	290	2.75	+/- 0.27	1.34
Total		100.00		100.00

Table 2: Results of elemental analysis of sample *Ocimum Sanctum* by EDXRF techniques

Element Line	Net Counts	Weight %	Weight % Error	Atom %
C K	935	10.90	+/- 0.34	16.88
O K	5522	50.44	+/- 0.54	58.61
Na K	5115	17.53	+/- 0.25	14.18
Si K	138	0.40	+/- 0.07	0.26
P K	470	1.43	+/- 0.09	0.86
Cl K	1456	6.46	+/- 0.18	3.39
K K	1512	9.29	+/- 0.39	4.41
Ca K	360	2.69	+/- 0.22	1.25
Mo L	142	0.86	+/- 0.25	0.17
Total		100.00		100.00

Table 3: Results of elemental analysis of sample *Emblica officinalis* by EDXRF techniques

Element Line	Net Counts	Weight %	Weight % Error	Atom %
C K	347	6.12	+/- 0.32	10.89
O K	2229	43.63	+/- 0.70	58.28
Na K	295	1.70	+/- 0.13	1.58
Mg K	1348	6.49	+/- 0.15	5.71
Al K	137	0.64	+/- 0.10	0.50
Si K	907	4.26	+/- 0.15	3.24
P K	580	2.93	+/- 0.20	2.02
S K	170	1.01	+/- 0.14	0.67
Cl K	141	1.05	+/- 0.15	0.63
K K	2120	21.51	+/- 0.48	11.76
Ca K	586	7.38	+/- 0.74	3.94
Zr L	354	3.28	+/- 0.52	0.77
Total		100.00		100.00

The results of EDXRF analysis of the natural dyes are summarized in Tables 1, 2 & 3. Nearly ten elements were recorded and their concentrations range from major to ultra-trace levels. The major elements include C, Mg, P, Cl, K and Ca; minor elements are Na, Si, S, Al, Zr and Mo. These results reveal that the dye does not have toxicological potential due to the presence of trace amounts of some toxic elements such As, Pb, Cr, Co and Zn in the sample.

Infrared (IR) spectroscopic measurement

The IR spectrum of all samples show broad peaks in region of 3300 – 3600 Cm^{-1} could be assigned to OH stretching frequency of coordinated water. Absorption peaks at 1600 – 1750 Cm^{-1} have been assigned to the carbonyl C=O stretchning modes, suspected for the metal chelates. 1300-1365 Cm^{-1} for C=C stretching and 1150 - 1230 Cm^{-1} medium weak C-O stretching. The 1365 Cm^{-1} from the raw dye sample can be assigned to C=C chelate ring of some metal chelate complexes within the sample.

Thin layer chromatography results

The Presence of carbohydrates, tannins, alkaloids, amino acids and flavonoides has been proved and confirms the dyeing property on hair. The presence of these compounds have been confirmed by thin layer chromatography[18] showing spots with R_f values of 0.8 and 0.56 inferring the presence of flavanoids.[19]

Colorant performance can be modified by the presence of metals, by pH and by interaction with colorless flavonoids.[20] The dyeing effect of aqueous mixture of dried powder sample with and without Iron filings has been prepared by varying the pH and applied on hair strand to check the color intensity as given in table 4.

Table 4: Water extract, dried extract with iron filings

Water Extract at different pH	Dried Extract	Dried Extract along with Iron Filings
Acidic pH 4.0	Light color	No color
Alkaline pH 7.2	Brownish tint	Blackish brown

Alkaloids, phenolic compounds and tannins are generally present in the water extract of the dyeing composition. Further it was confirmed that dyeing activity was pH dependant. The color developed at lower pH was unable to sustain on washing. The brown color developed at higher pH was of permanent in nature. This further improved to blackish brown with the addition of Iron filings.

Effect of application of plant material and also the formulated organic dye

Each powdered plant material has been applied to the hair by mixing in water and the contact time has been limited to 30 minutes. Among the twelve plant sources *lawsonia Inermis, Murraya Koenigii, Eclipta alba, Punica granatum, Embilica officianalis* and Juglone have shown the dying effect on the hair without damaging the

property of hair as traditional cosmetics as given in Figures 1,2,3,4,5 & 6. The fastness has been observed to be good after washing with *Sapindus Laurifolia, Sapindus Mukorossi* or Soap nut (rita) powder.

The dying effect and fastness of the alkaline formulation has been observed to be excellent after washing with soap nut (rita) powder without disturbing the hair structure as given in figures 7 & 8.

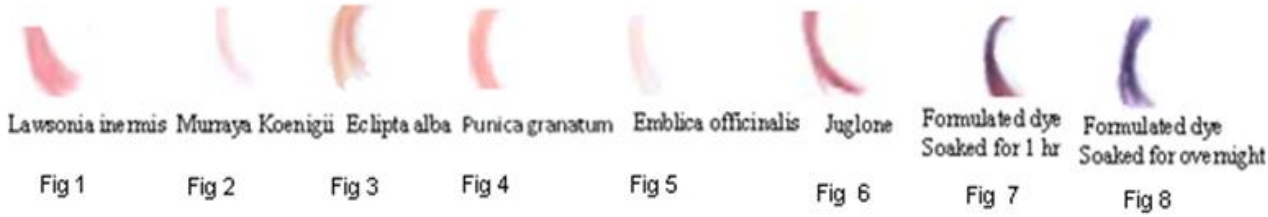

Fig 1 Fig 2 Fig 3 Fig 4 Fig 5 Fig 6 Fig 7 Fig 8

Anti bacterial activity

The antibacterial activity of these plant material selicited interests of chemists and biologists to investigate components responsible for this activity. The chemical components like flavonoids or tannins are known to inactivate the susceptible biological processes.

The water extract of each individual herb was studied for anti- bacterial activity using E. Coli bacteria following the procedure of Manoj Kumar Pandey et al. [21]

In agar well diffusion method, the media and the test bacterial culture was inoculated on a petridish. The pH of the medium and test sample has been maintained at 7. The test stain of 0.25 ml was inoculated into the media. After the medium solidified, a well was made in the plates. The extract compound of 50 µl was introduced into the well and the plates were incubated at 37^0C for 24 hrs. It was observed that out of 12 plant extracts *Murraya Koenigii, Emblica officinalis, Eclipta alba* and *Azadirachta indica* have shown the maximum inhibitory effect. Hibiscus flower, leaf and *Lawsonia inermis* have shown only mild inhibiting effect indicating low anti bacterial activity. The anti bacterial activity has been attributed to its different components like polyphenolic compounds, alkaloids, flavonoids and terpenoids. These extracts are active in inhibiting the growth of bacteria.

CONCLUSION

The present study evaluates the formulation of organic hair dye comprising a mixture of powdered plant materials having natural products useful for dying the hair. It is evident from the results that this formulation is highly effective at slightly alkaline pH without causing hair damage and skin irritation.[22,23]

A fixative Iron filing with these powders gives darker and stable shade preferred in hair dying.[24]

Efficacy data shows that all these active constituents have prolonged dying effect on hair.

The surfactant used cause dry scalp and loss of fat under the skin, which enhances the drying and damage of the hair follicle, thus hair fall starts. Advantage of this formulation is that the plant pigments penetrate into the cortex region without rupturing the hair follicles. The formulation contains 100% water soluble herbal extracts which are highly environment friendly.

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