



Research Article

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PHYSICOCHEMICAL AND PHYTOCHEMICAL ANALYSIS OF HERBO-MINERAL HAIR DYE FOR PREMATURE HAIR GRAYING

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ABSTRACT

Background: Premature Hair Graying (PHG/ Canities) is the burning problem of today's society. Despite extensive research, the treatment for PHG is still unsatisfactory. Ayurveda considers Palitya (premature hair graying) as an ailment of vitiated Pitta dosha (deranged functional factor of the body responsible for digestion and metabolism). Ayurveda therapeutics can provide the natural solution to this problem without adverse effects and also can prevent the recurrence. Objective: In present research work, attempts are made to formulate unique, safe and effective herbomineral hair dye (Keshranjak yog) an Ayurvedic formulation. The prepared formulation is also evaluated for its efficiency through various organoleptic, phytochemical and physicochemical parameters. Results: The pH of prepared herbomineral hair dye was found to be 6 which is close to neutral; to suit the requirements of different scalp and hair types. Total phenolic content (8.1 ± 0.7), total flavonoid content (6 ± 1.2) of prepared sample were found to be acceptable and within limit. Conclusion: The dyeing effect of the developed dye may be attributed to keshya (good for hair), Kesharanjak (hair coloring) properties according to Ayurveda and presence of tannins, phenols as per pharmaceuticals. Nowadays natural remedies are widely accepted as they are safer with minimal side effects as compared to the chemical hair dye. Kesharanjak yog may provide vital nourishment to the scalp skin and can color the hair in an utmost gentle manner. Due to nontoxic nature can act as public healthcare product.

Keywords: Herbo-mineral Hair dye, Kesharanjak yog, Premature hair graying, Palitya, *Eclipta alba*, Kasees bhasma, *Indigofera tinctoria*

INTRODUCTION

Hair is the most noticeable physical character of human appearance, which enhances the beauty of a person. Not just beauty but the colour, texture, thickness and growth of hair reflect overall good health status of the person. Charaka Samhita emphasized daily care of hair from both health and beauty perspective; by terms Ayushya (life promoting) and Rupavirajan¹ (enhancing the beauty). Graying of hair is a natural age associated, inevitable feature. There exist the "50" thumb rule which states that at least 50% of individuals have 50% gray hair by the age of 50 years². PHG is defined as greying of hair before the age of 20 years in Caucasians and before 30 years in Blacks³. Owing to its diverse causative factors, exact prevalence rate is unknown. Other than genetics, PHG is said to be associated with autoimmune diseases, high oxidative stress, smoking, environmental factors and some mineral-vitamin deficiencies^{4,5}.

Despite extensive research like formulations including p-aminobenzoic acid (PABA), calcium pantothenate, nutritional supplements etc.; the treatment for PHG is still unsatisfactory with inconsistent results. Hair dyes available in market includes dye modifiers, antioxidants, alkalizers, soaps, ammonia, wetting agents, fragrance, and a variety of other chemicals though impart special qualities to hair such as softening the texture, colour to the

hair but continuous usage of such compounds containing dye on natural hair causes many side effects such as skin irritation, erythema, loss or damage of hair and skin cancer⁶. This emphasizes the need of research for remedies over this annoying, ever increasing, anxiogenic condition.

Ayurveda considers Palitya (premature greying of hair) as an ailment of vitiated Pitta dosha⁷ (regulatory functional factors of the body for metabolic activities). According to Ayurveda, increasing ushma (heat) in a body finds its roots in shira (head region), that results in greying of hair. One must understand this difference between palitya as a disease caused by pitta dosha and akal palitya (PHG) as a result of fastened process of ageing that may be associated with hormonal imbalance. Though in nutshell, both these conditions arise from systemic dosha vitiation and is not just localized pathology occurring in scalp.

Many review and research articles are published in the field of Ayurveda⁸⁻¹⁰ Pharmacy¹¹ and trichology, dermatology¹² related to this problem. All the above studies are focused on to study etiopathology of Palitya / PHG, herbs for coloring hair, formulate herbal dyes and in vitro studies of prepared herbal dyes. In present research work, an attempt is made to formulate ayurvedic herbomineral hair dye Kesharanjak yog, and evaluate for its probable efficiency through various organoleptic, phytochemical

and physicochemical parameters. Ayurveda as a holistic science can provide the effective solution to PHG without adverse effects and also prevent the recurrence.

MATERIALS AND METHODS

All the herbal and mineral drugs were procured, authenticated and processed according to the proposed composition at GMP certified pharmacy Dhanwantari Ayurved, Pune, Maharashtra, India. The final preparation was evaluated at Pharmacy of the Institute, Pimpri, Pune, Maharashtra, India. This project was approved by IEC of DPU with reference no. DYPV/EC/90/18 dated 05/06/2018.

Formulation of Herbomineral Hair Dye

For the preparation of the formulation, various drugs from Ayurveda texts were referred which are said to be beneficial for hair graying. After a thorough review a powder formulation Keshranjak yog was prepared by mixing the following ingredients in appropriate proportion (Table 1). All the drugs were mixed and then the mixture was further processed in Bhrungaraj swaras (*Eclipta alba* plant juice) for 7 days and then dried and powdered. All powder material was passed through the sieve (No. 80). The sample of the final preparations was assessed for physicochemical and phytochemical evaluation.

Table 1: Ingredients of the Herbomineral Hair Dye

Ingredients	Parts
Yasthimadhu (<i>Glycyrrhiza glabra</i>)	2
Bhrungaraja (<i>Eclipta alba</i>)	2
Neeli (<i>Indigofera tinctoria</i>)	2
Krushna tila (<i>Sesamum indicum nigrum</i>)	2
Amrasthi (seeds of <i>Mangifera indica</i>)	1
Triphala	1
Kasees bhasma (FeSO ₄)	1/4 th

The different drugs in above formulation possess different chemical composition leading to goal of hair dyeing. The dyeing effect of the developed dye may be attributed to properties mentioned as follows,

*Eclipta alba*¹³ - The exact role of the drug and its mechanism is still unclear but it is considered keshya i.e. it improves quality of hair and provides nourishment.

*Sesamum indicum nigrum*¹⁴ - It is a rich source of antioxidants leading to free radical scavenging and promoting hair growth.

*Glycyrrhiza glabra*¹⁵ - The studies on animal and human subjects have proven that, it possesses hair growth stimulatory effects.

*Indigofera tinctoria*¹⁶ - It contains Indigotin which is a colouring agent helpful for hair dyeing.

*Mangifera indica*¹⁷ - Mango seed kernels are also equally rich in polyphenols with potent antioxidant activity. As reported by, Jahurul *et al.* mango seed kernels contain tannin, gallic acid, coumarin, caffeic acid, vanillin, mangiferin, ferulic acid, and cinnamic acid.

Triphala¹⁸ - It is rich in anti-oxidants. It exerts protective anti-ageing effect on body, leading to prevention of hair greying.

Kasees¹⁹ - (Iron sulphate) - The bhasma or micro fine powder of FeSO₄ can produce fused black particles or chelates with herbs which can help in hair colouring.

Analysis: Following analysis were conducted on the final preparation,

Organoleptic Evaluation: The formulation powder was studied for organoleptic and morphological characters like colour, odour, texture and appearance.

Physicochemical Analysis: The physical and chemical features of Kesharanjak yog were evaluated to determine the average particle size, solubility, pH, and moisture content and ash value. Determination of particle size: Particle size was analyzed with particle size analyzer.

Determination of ash value: One gram of the powdered material was taken in a silica crucible. The material was spread in an even layer in the crucible and ignited by gradually increasing the heat until complete incineration and powder turns white, indicating the absence of carbon. The material was cooled and weighed. The Ash value was calculated in percentage.

Qualitative Phytochemical Studies

The phytochemical screening was done of each individual ingredient as well as final formulation; to test the presence or absence of various phytoconstituents as Glycosides, Alkaloids, Flavonoids, Tannins and Phenols.

Test for Glycosides: Appropriate amount of glacial acetic acid and a few drops of 5% ferric chloride solution to a individual drug extract. Further addition of 0.5 ml Conc. sulfuric acid results in the formation of blue color in acetic acid layer that confirmed the presence of glycoside.

Test for Tannins and Phenols: To the drug extract, small amount of 5% ferric chloride was added. The presence of tannins was confirmed by the formation of dark blue coloration.

Test for Alkaloids: In 2-3 ml filtrate, add Hager's reagent that results in formation of yellow precipitate.

Test for Flavonoids: In the filtrate, add 95% ethanol, few drops of Conc. HCl and Mg metal, an orange, violet/red coloration appears as a confirmation for presence of flavonoids.

Quantitative Phytochemical Studies

Reagents and Chemicals: Gallic acid and Quercetin standards were procured. Water was purified using a Milli-Q system (Millipore).

Preparation of Sample for Phytochemical Testing: The formulation is dissolved in 5 ml of water, sonicated and then filtered. The filtrate was used for UV-spectrophotometric assay for quantification of phenolic and flavonoids content.

Total Phenolic Content: The total phenolic content was calculated considering gallic acid as standard. The standard calibration curve of gallic acid was plotted in the range of 5-50 µg/mL with R² = 0.998 and Y=0.0104 x +0.194 as equation. The phenolic content in the formulation is calculated on the basis of a standard curve of gallic acid.

Total Flavonoids determination: For total flavonoid determination, quercetin was used to make the standard calibration curve. Stock quercetin solution was prepared by dissolving 10 mg quercetin in 10ml of methanol, then the standard solutions of quercetin were prepared by serial dilutions using methanol (10-50 µg/mL) to plot standard curve. The concentration of total flavonoid content in the test samples was calculated from the calibration plot (y= 0.2615x + 0.076, R² = 0.997)

Patch Test

Patch test was performed to determine which substances in the formulation can cause allergic inflammation on skin where it is applied. This usually entails applying a tiny bit of the hair dye's aqueous solution behind the ear or on the inner elbow in an area of measured 1sq.cm. If there are any indications of discomfort or

feeling unwell, they are documented. We monitored and recorded swelling, redness, and irritation at regular intervals for up to 24 hours¹².

RESULTS

Qualitative Analysis: Organoleptic and physicochemical evaluation of prepared formulation is illustrated in Table 2.

The phytochemical analysis of individual extracts is shown in the Table 3. From the qualitative findings presented in Table 3, it is observed that the different extracts confirmed the presence or absence of glycosides, tannins, alkaloids and flavonoids.

Quantitative Analysis: The UV spectrophotometric assay indicates that the prepared formulation contained 8.1% of phenolic content. It also indicated the presence of 6% of flavonoid content as presented in Table 4.

Table 2: Organoleptic and Physicochemical Evaluation of Hair Dye

Parameter	Results
Color	Black
Odor	Characteristic ferrous
Texture	Fine
Appearance	Powder
pH	6.0
Ash value	12 %
Solubility Test	Acid Soluble
Average Particle Size	6.66 μ m

Table 3: Phytochemical Evaluation of individual Ingredients of the Hair Dye

Name of Drug	Glycosides	Tannin and phenols	Alkaloids	Flavonoids
<i>Eclipta alba</i>	-	+	+	-
Triphala	-	+	+	-
<i>Glycyrrhiza glabra</i>	+	-	+	+
<i>Mangifera indica seeds</i>	-	+	+	+
Iron sulphate	-	-	-	-
<i>Indigofera tinctoria</i>	-	-	-	-
<i>Sesamum indicum nigrum</i>	-	-	+	-
Final Formulation	+	+	+	+

- indicates the content to be absent, + indicates the content to be present

Table 4: Total Phenolic and Flavonoid Content in Aqueous Extract of Formulation

Total Phenolic content	8.1%±0.7
Total flavonoid content	6±1.2

Patch Test: The results of patch tests for the signs of swelling, redness and irritation were found to be negative. No swelling, redness or irritation was observed at the sight of application.

DISCUSSION

Kesharanjan (dying) action of drugs is very important to maintain aesthetic value of hair in very common keshavikar Palitya (PHG). Hence the prepared formulation Kesharanjak yog was tested analytically for primary outcome measure i.e. its safety and efficacy. Organoleptic evaluation findings revealed that the formulation is smooth and smells like ferrous content; due to presence of Kasees in it. The physicochemical parameters like ash value, solubility, pH and particle size were also performed and are found to be within the specified limits, with the minimal moisture content. Ash value was found to be 12% signifying the presence of inorganic radicals in appropriate amounts. The pH of the preparation was found to be 6 (i.e. close to neutral) to suit the requirements of different scalp and hair types. The pH is found similar to other previous works on dyes^{5,9}. The current studies show that, hair products with pH less than 5.5 are considered healthy for hair¹⁹. A pH of 6.69 (H.H.D-IV) was found to be best for penetration of hair colorant⁵. The qualitative analysis shows the natural presence of major phytoconstituents, which acts as true coloring agents and nourish the scalp as well as hair. The formulation was found to be water soluble as well as acid soluble.

All the components of the Kesharanjak yog preparation were subjected to Phytochemical screening to identify the phytoconstituents present in it. Only *Glycyrrhiza glabra* /Liquorice

showed the presence of glycosides. *Mangifera indica* and *Glycyrrhiza glabra* tested positive for flavonoids. It was observed that all the components showed presence of alkaloids except *Indigofera tinctoria*. Also, all components showed presence of tannins and phenols except *Glycyrrhiza glabra*, *Sesamum indicum nigrum*. The quantitative analysis indicates that the proposed formulation showed presence of 8.1% phenols and 6% flavonoids that may support its activity as an herbal hair dye.

A Patch test conducted for any local side effect indicated unfavorable outcomes for irritation, redness, etc. because the mixture contains herbs in their natural state without the use of synthetic compounds. It is convenient to use and store at room temperature. It is free of any harmful effects like those of artificial dyes based on ammonia, because it is a naturally derived herbal composition. Given that natural ingredients are recognized for their non-toxic and non-habit-forming qualities and that the dye contains no artificial colors, fragrances, preservatives, or chemicals, the likelihood of negative consequences is practically negligible. This ensures that the dye can be easily acceptable as substitute over synthetic hair dyes. A study published, pertaining to use of hair dye and adverse reactions; mentions that 90.82% of the participants perceived that coloring of hair was unsafe yet still 63.27% used chemical dye and only 36.74 % used herbal dye²⁰. Thus, it is the need of the hour to provide herbal safe and effective solution to the burning issue of PHG.

Preparation of hair dye that is devoid of any synthetic agent and may give natural hair color with better dyeing effect, was the

secondary outcome of this work. The dyeing effect of the developed dye may be attributed to the properties of each ingredient present in the formulation. The different drugs in above formulation possess different chemical composition leading to same goal of hair colouring. Hence the combined effects of all these separate herbs and minerals can increase the efficiency of hair coloring along with improving the hair growth.

The present study limits to analytical study of formulation. Further there is scope for clinical study on patients of PHG to evaluate exact dyeing efficiency of the formulation, with varying proportions of herbs and methods of application.

CONCLUSION

The developed hair dye formulation, Kesharanjak yog is prepared according to principles of Ayurveda, purely from herbs and minerals. As the preparation is free from chemicals, can be considered as nonirritating, nontoxic and devoid of any side effects on scalp skin and hair. When different herbomineral drugs are combined and are further processed with aqueous extracts, it decreases any side effects of individual drugs. Many phytochemical constituents suitable for coloring hair that were absent in individual drugs were found to be present in final preparation, which suggests probable efficiency of the product. The obtained pH value of formulation is good to preserve the health of hair as compared to market dyes which cause harmful effect on cortex of hair.

This study will prove advantageous to pharmaceutical industry as well as public health.

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