"FORMULATION, EVALUATION AND STABILITY IMPROVEMENT OF HEENA BASED HAIR DYE"

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ABSTRACT:

Background: Herbal hair dyes have become much more popular as a result of their many benefits in reducing the negative effects of chemical-based alternatives. In order to ensure the quality and stability of a herbal hair dye, We tried to develop a premium herbal hair dye that addresses consumer concerns about long-term stability through meticulous preparation and standardization processes.

Objective: This study set out to develop a natural hair dye and test its efficacy and shelf life using a variety of criteria. The study aimed to create a hair dye product and compared with the marketed product while ensuring its efficacy, dying ability and long-term stability.

Materials and Methods: The herbal dye was made in accordance with a standard proposed composition using only natural ingredients. The dye was evaluated and compared with marketed product for its organoleptic, Physico-chemical and stability parameters.

Results: The parameters examined in this study were discovered to be comparable and appropriate for assessing the herbal dye. The outcomes of the various analyses strongly supported the optimized formulation formulation's efficacy and dependability and showed that it could be used as a hair dye for an extended period of time. The findings suggest that the prepared formulations possess favorable properties and offer improved performance compared to existing market option. These results provide strong support for the stability and quality of the herbal dye, demonstrating its suitability for long-term use.

Conclusion: The various parameters have been used to prepare and evaluate herbal-based hair dye. It presents a risk-free natural alternative that can be used for long-term stability.

Keywords: Herbal hair dye, Organoleptic, Physico- chemical evaluation, Herbal drugs, Chemical hair dye.

1. INTRODUCTION:

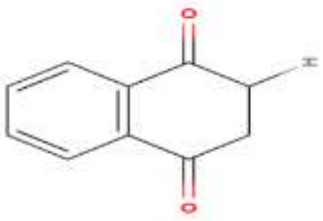
Compared to chemical-based hair dyes, which can cause skin diseases and other skin-related ailments, natural herbal dyes are currently preferred[1]. In today's society, individuals are highly concerned about their beauty, and

hair plays a significant role in this regard. To maintain healthy hair, people are turning to herbal remedies that have no adverse effects. Approximately 70% of individuals over the age of 50 struggle with the issues of balding and graying hair, with some experiencing these symptoms even earlier. Gray hair typically appears on the scalp around the age of 40, starting from the spreading to the beard, mustache, and eventually the chest. The onset of graying is heavily influenced by heredity, but premature depigmentation in adults can be attributed to various factors, such as illness, specific medications, or shock[2, 3].

Natural dyes have been utilized since ancient times for dyeing carpets and clothing, using the roots, stems, barks, leaves, berries, and flowers of dye-producing plants[4]. The demand for herbal-based natural remedies is rapidly increasing due to their inherent goodness and lack of side effects. Ayurvedic herbal plant such as Amla, Bhringraj, Henna, Mandara, Jatamansi, Reetha, Sariva, Curry leaves, and Methi are well-known for their use as hair colorants and for promoting hair growth[5]. Before the invention of modern dyes, various plant extracts were used in Europe and Asia for hair dyeing. Indigo, an initial fabric dye, could be combined with henna to create shades ranging from light brown to black[6]. However, the use of chemical dyes can lead to unpleasant side effects, including skin irritation, allergies, hair breakage, skin discoloration, and unexpected hair colors[7,8]. Repeated application of such compounds to natural hair can result in multiple side effects, such as skin irritation, allergies, hair loss, dry scalp, erythema, and even skin cancer[9].

In India, henna has traditionally been used to color palms and hair. There are numerous herbs, such as henna, Bhringraj, reetha, shikakai, hibiscus, Amla, coffee, jatamansi, and methi, which are widely used in hair care preparations, primarily for hair dyeing purposes[10]. Henna has been a part of Islamic and Hindu cultures since the Bronze Age, used for coloring hair, decorating nails, and creating temporary skin tattoos during marriages and other social celebrations[11]. Medicinal products derived from plants are readily available, cost-effective, safe, efficient, and rarely have side effects. In the current era of eco-conservation, there has been a resurgence of interest in and exploration of natural dyes for coloring textiles and food products[12].

Figure.1 Molecular structure of Lawsone



2. ROLE OF INGREDIENTS USED IN THE FORMULATION

Henna: Henna, derived from the Lawsonia inermis plant, has long been utilized as a natural hair dye with remarkable benefits. With its inherent coloring properties and numerous advantages for hair health, henna has carved a prominent place in the realm of herbal hair dyes.[13]

As a natural coloring agent, henna owes its efficacy to lawsone, a pigment found in the leaves of the Lawsonia inermis plant. This compound binds to the proteins in hair, resulting in rich, reddish-brown, or coppery hues when applied. The availability of henna as a natural alternative to synthetic dyes makes it particularly sought after for achieving vibrant auburn, red, or burgundy shades.[14,15]

Amla: Amla, derived from the berries of the amla plant, offers numerous benefits for hair health. It is rich in calcium, aiding in the development of stronger hair. Amla also helps maintain hair color and prevents premature graying while strengthening the hair follicles.[16] The fruit is known for its high concentration of Vitamin C and tannins, making it a valuable ingredient in hair care products. The Vitamin C in amla binds with tannins, preserving its potency even when exposed to heat or light. Additionally, amla is rich in minerals like calcium, phosphorus, iron, and amino acids.[17,18]

Tea Decoction: Incorporating tea decoction into herbal hair dye offers several advantages, including natural color enhancement, improved hair health, scalp nourishment, a gentle and chemical-free approach, and sustainability.[19] Tea decoction not only provides vibrant and customizable hair color but also nourishes the hair and scalp, resulting in healthier and more vibrant locks. By embracing tea's natural properties, individuals can enjoy the benefits of herbal hair dyeing while maintaining a conscious and sustainable lifestyle.[20]

Hibiscus: The use of hibiscus in herbal hair dye holds significant importance due to its remarkable benefits. From stimulating hair growth and preventing premature graying to controlling dandruff and rejuvenating and conditioning the hair, hibiscus offers a holistic hair care solution.[21] With its rich nutrient composition and natural properties, hibiscus contributes to healthier, thicker, and more vibrant hair. Incorporating hibiscus into herbal hair dye formulations allows individuals to enjoy the beauty and benefits of this remarkable flower while enhancing their hair's natural vitality and radiance.[22]

Bhringraj: Significance of incorporating bhringraj in herbal hair dye. It emphasizes the exceptional benefits that bhringraj offers in terms of stimulating hair follicles, promoting hair growth, and imparting natural color to grey hair. These properties make bhringraj a valuable ingredient in hair care formulations.[23,24]

Manjistha: the significance of incorporating Manjistha in herbal hair dye due to its exceptional benefits. It highlights the Manjistha offers a holistic solution for various hair care needs, including enhancing hair color, promoting scalp health, and providing natural conditioning.[25,26]

Triphala: The significance of incorporating Triphala in herbal hair dye due to its remarkable benefits. It highlights how Triphala provides a holistic solution for various hair care needs, including nourishing the hair, promoting hair growth, preventing hair loss and premature greying hairs.[27,28]

Methi: The incorporation of Methi in herbal hair dye holds significant importance due to its remarkable benefits. From promoting hair growth and preventing hair loss to improving the texture of your hairs and offering a natural alternative to chemical dyes.[29,30]

Eucalyptus oil: Eucalyptus oil offers a multitude of advantages when used in hair dye formulations. Its stability-enhancing properties ensure the longevity of the product, while its antibacterial activity maintains a healthy scalp environment. Additionally, eucalyptus oil contributes to a pleasant sensory experience through its cooling sensation and aromatic fragrance. By incorporating eucalyptus oil in hair dye, individuals can enjoy the combined benefits of stability improvement, scalp health, and sensory satisfaction, embracing a holistic approach to hair care that promotes overall well-being.[31,32]

Tea tree oil: The incorporation of tea tree oil in hair dye formulations provides a dual benefit of stability improvement of dye and scalp health promotion. This versatile ingredient offers a unique combination of stability improvement, scalp health promotion, and sensory satisfaction. With its antibacterial properties, tea tree oil helps preserve the freshness of your hair dye formulation, ensuring a longer shelf life.[33,34]

Table 1. Description of the ingredients of the herbal hair dye

S.No.	Common and botanical name	Picture	Chemical constituents	Uses
1.	Henna: Lawsonia inermis		Lawsone, Flavonoids, gallic acid	Coloring agent, antifungal
2.	Indigo: Indigofera tinctoria		Glycosideindan, indigotin, indirubin flavonoid, tannins	Coloring agent, promote hair growth
3.	Hibiscus: Hibiscus rosa-sinensis		Alkaloids, tannins, anthocyanin, bata carotine	stimulating hair growth, preventing premature graying, controlling dandruff
4.	Amla: Phyllanthus embilica		Rich in Vitain C, Tannins	Antioxidant, maintains hair colour
5.	Bhringaraj: Eclipta alba		Flavonoids, triterpines, steroids	Imparting natural color to grey hair

6.	Tea: Camellia sinensis		Tannins, Isoflavone, aminoacids, carotenoids	Conditioner, mordant, darken the hair, and antioxidant
7.	Methi: Trigonella foenum-graecum		Alkaloids,saponins, flavonoids, amino acids, hydrocarbons	Promoting hair growth and preventing hair loss
8.	Manjistha: Rubia cordifolia		Quinones, oleananes triterpenoid	Enhancing hair color, providing natural conditioning.

3. MATERIALS AND METHODS:

Pure Lawsone was purchased from Carbanio, India. The important ingredients, namely Henna, Manjistha, Coffee, Tea, Amla, Hibiscus, Bhringraj, Triphala, Methi, Eucalyptus oil, and Tea Tree oil were procured from authorized local market stores, Hubli.

3.1 Procedure for extraction of Henna: 100 grams of dried henna powder is mixed with 1000 ml of methanol. This mixture is then subjected to agitation in a rotary shaker for 10 minutes to facilitate the extraction process. Next, the mixture is transferred to an ultrasonic bath set at a frequency of 40 kHz and allowed to undergo ultrasonic treatment for another 10 minutes. This step further enhances the extraction by promoting solvent penetration into the henna powder. Once the ultrasonic treatment is complete, the extract is filtered through Whatman No.41 filter paper to eliminate any solid particles or impurities. To concentrate the extract, the filtered solution is subjected to controlled evaporation, wherein the solvent is gradually removed. This process continues until the desired concentration of the henna extract is achieved.[35]

3.2 Phytochemical screening of Henna Extract:

The prepared herbal extract was subjected to phytochemical screening tp reveal the various phytoconstituents as carbohydrates, glycosides, flavonoids, tannins, alkaloids and saponins.[36]

3.2 Lawsone quantification: Accurately weighed quantity of henna extract (100 mg) was dissolved 10 ml methanol, filtered and lawsone content was determined by standard calibration curve of pure lawsone using spectrophotometer at 269nm.[37]

3.3 Method of Preparation of dye:[38,39]

Step1 Preparation of tea decoction:

Accurately weigh the 20 grams of tea powder and combining it with 50ml of distilled water in 100ml beaker. The mixture was then heated to a temperature of 100^{o}C and maintained at this temperature for a duration of 10 minutes. After boiling, the solution was filtered using a muslin cloth.

Step 2 Preparation of sodium alginate solution:

Accurately weigh the 2gm of sodium alginate and combining with 100ml of distilled water in 250ml beaker. The mixture was then heated to a temperature of 60-80^{o}C with continued stirring until it completely dissolved.

Step 3 Preparation of sugar solution:

Accurately weight the 5gm of sugar and dissolve in 20ml of distilled water in 50ml beaker.

Step 4 Preparation of dye:

Take all the required ingredients according to formulation table 2. in powder form and pass all the ingredients separately through sieve no. 120. Then accurately weigh the all ingredients and mix it by geometric ratio in 50 ml of tea decoction by using remi mixture. Then add slowly 20ml of sugar solution which is already prepared with continuing mixing. Then add slowly sodium alginate solution until it became sticky paste. If necessary, then add distilled water to become smooth paste.

Table 2. Optimized formulation of herbal hair dye. (200gm)

INGREDIENTS	Weight (gm)
Henna	23.98
Henna extract	47.02
Amla	10
Manjistha	50
Hibiscus	6
Bhringaraj	7
Methi powder	5
Tea decoction	42
Sodium alginate (%)	2%
Sugar solution (%)	5%
Tea tree oil	2%

3.4 Application of Hair Dye:[40]

The hair dye should be applied following these steps:

- Ensure that the hair is dry before applying the hair dye. The dye comes in the form of a paste in the pack.

- The ready-made paste has an ideal consistency for application. Take the required amount of paste and make sure it is evenly mixed.
- Use a brush to apply the hair dye evenly from the roots to the tips of the hair. Make sure to cover the entire scalp.
- Once the hair dye is applied, it should be left on the scalp for a period of 2-3 hours to allow it to completely dry.
- After the drying time, the hair dye can be removed by washing the hair with plain water. Rinse the hair thoroughly until the water runs clear.
- Following these steps will help in the proper application of the hair dye, ensuring an even coverage and desired results.

4. EVALUATION OF THE HERBAL HAIR DYE:

The prepared herbal hair dye was evaluated for its various parameters, such as organoleptic, physico-chemical, phytoconstituents and the rheological aspects.

4.1 Physical Evaluation[3,8]

The evaluation of henna dye's organoleptic properties, which include sensory qualities like color, pH, odor, and more, is shown in Table 3. These sensory characteristics were carefully observed and noted during the assessment, as shown in the table.3

4.2 Physico-Chemical Evaluation[1,12]

The physical and chemical features of the herbal hair dye were evaluated to determine the pH, its viscosity and lawsone concentration for the purpose of stability, compatibility results present in it. Table 4 reflects the above findings

4.3 Stability studies[3,27]

The stability of the prepared formulation was assessed through a comprehensive stability test. The formulation was stored under various temperature conditions for a duration of 3 months. Self-sealing aluminum pouches were used to store the formulation at different temperatures, including room temperature (25°C±2°C) and cool temperature (5°C ± 3°C). The evaluation focused on key physical parameters such as color, odor, pH, viscosity, smoothness and lawsone concentration. The results of this evaluation can be found in Table 5.

4.4 Dye test on artificial hair:[18,22]

A dye test was performed on Remmy hair to evaluate the effectiveness of hair dyes and their ability to achieve desired color outcomes. The process involved applying the dye to a small section of clean Remmy hair, following the manufacturer's instructions. After rinsing and drying, the different color was assessed for accuracy, vibrancy, and overall appearance. The test helped in selecting suitable dyes and ensuring satisfactory results. Conducting a dye test on Remmy hair is essential for making informed choices and achieving desired hair color outcomes.

4.5 Fastness property:[2,8]

The fastness properties of henna hair dye on Remmy hair were examined with regards to light exposure and water wash. The study aimed to determine the dye's resistance to fading and color transfer. Dyed Remmy hair samples were subjected to controlled light exposure and water washing in to assess their durability and color retention. This investigation contributes to understanding the longevity and performance of henna hair dye on Remmy hair under different conditions.

4.6 Comparison with marketed brand:

The dyeing effect and fastness study of formulated dye was compared with marketed 'nat habits' hair dye. The color comparison was noted down and color fading of both dyed hair compared with wash fading and sun light exposure fading. The result was noted down in table .

4.6 Antidandruff activity of the formulation:[41]

in vitro anti-dandruff activity of "formulation 4" was tested using the Agar disc diffusion method. In this study, a suspension containing 1.2×10^6 colony-forming units per milliliter (CFU/ml) of the dandruff-causing agent *Malassezia furfur* was used. The APX laboratories in Mumbai conducted the investigation to evaluate the antidandruff activity of the hair dye.

4.7 Antibacterial activity of the formulation:[42]

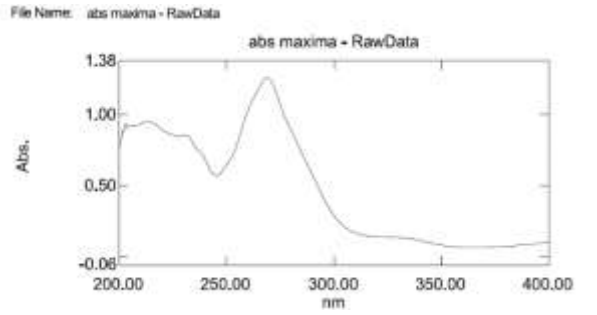
in vitro anti-bacterial activity of "formulation 4" was evaluated using the Agar disc diffusion method. The study involved testing the formulation against various gram-positive and gram-negative bacterial strains, including *Staphylococcus epidermidis* and *E. coli*. The results of the experiment are presented in Table 7.

5. RESULTS AND DISCUSSIONS:

Development and optimization of the spectrophotometric method:

Proper wavelength selection of the methods depends upon the nature of the sample and its solubility. To develop a rugged and suitable spectrophotometric method for the quantitative determination of lawsone, the analytical condition was selected after testing the different parameters and other chromatographic conditions.

Selection of wavelength:

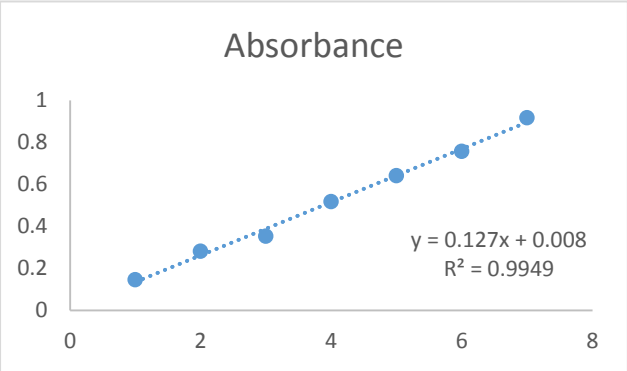


Wavelength nm	Absorbance
269.0	1.258
230.5	0.853
214.0	0.948

Table 3. λ max

Fig .2 800nm-200nm absorbance maxima of pure Lawsone in methanol

Linearity: Seven points calibration curve were obtained in a concentration range from 0-70 ppm for Lawsone. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.127x+0.008 with correlation coefficient 0.994 **(**Table 4**,** Figure 3).



Conc. µg/ml	Absorbance
1	0.145 ± 0.00115
2	0.281 ± 0.00051
3	0.353 ± 0.00057
4	0.518 ± 0.0001
5	0.641 ± 0.00152
6	0.757 ± 0.001
7	0.917 ± 0.00173

Table 4. UV estimation of Pure Lawsone in methanol at 269nm

Fig 3. Standard calibration curve of pure Lawsone in methanol at λ_{max} 269nm

Table 5: UV estimation of Henna Extract in Methanol at 269nm

Concentration (µg/ml)	Absorbance at 269nm			Mean ± SD N = 3
	I	II	III	
10	**0.272**	**0.273**	**0.275**	**0.273 ± 0.00152**

The technique for estimation of herbal extract of Henna Extract was done by UV-Visible spectrophotometry and the technique was confirmed for its reproducibility and accuracy.

The estimation of Pure Lawsone was standardized by using UV-Visible spectroscopy.

Linearity range from 1-7 µg/ml at 269nm in Methanol. Standard calibration curve was plotted Absorbance v/s Concentration in µg/ml.

Equation was developed for the calculation of concentration

Absorbance (y) = 0.127x - 0.008

Regression coefficient (R^2) = 0.9949

Percentage of Lawsone present in extract = 20.86%

Table 6: Phytochemical screening Henna extract

Sl.no.	Phytoconstituents	Henna extract
1	Carbohydrates	Present
2	Glycosides	Present
3	Flavonoids	Present
4	Tannins	Present

5	Alkaloids	Absent
6	Saponins	Absent

Table 7. Physico-chemical Parameters of optimized formulation and Marketed product

Sl No.	Parameters	Results (optimized formulation)	Results (Marketed Product)
1	Colour	Brownish red	Brownish
2	Odour	Pleasant mint	Pleasant mint
3	Consistency	Excellent	Good
4	Viscosity	3576 ± 0.019cps	3462 ± 0.019
5	Appearance	Smooth Paste	Smooth Paste
6	pH	5.8 ± 0.0013	5.8 ± 0.0028
7	Lawsone Concentration	9.44 %	7.89%

Table 8. Stability studies of optimized formulation

Sl No.	Parameters	DAY 0 25°C±2°C 60% ± 5% RH	DAY 0 5°C ± 3°C	DAY 90 25°C±2°C 60% ± 5% RH	DAY 90 5°C ± 3°C
1	Colour	Brownish red	Brownish red	Brownish red	Brownish red
2	Odour	Pleasant mint	Pleasant mint	Pleasant mint	Pleasant mint
3	pH	5.8 ± 0.0013	5.8 ± 0.0013	5.8 ± 0.0021	5.8 ± 0.0021
4	Viscosity	3576 ± 0.019cps	3576 ± 0.019cps	3576 ± 0.019cps	3576 ± 0.019cps
5	Appearance	Smooth Paste	Smooth Paste	Smooth Paste	Smooth Paste
6	Consistency	Excellent	Excellent	Excellent	Excellent
7	Lawsone Concetration	09.44 %	09.44 %	09.44 %	0. 9.44 %

In the present study, stability assessment of a ready-made henna hair dye was conducted over a period of 3 months. The formulation was subjected to various temperature conditions, and the results were compared at 10-day intervals. It was observed that the **optimized formulation** variant consistently demonstrated the best results across all tested parameters. Based on these findings, it can be concluded that our henna dye formulation can be stored under the mentioned conditions for a duration of 3 months while maintaining its stability.

Dye tests on artificial hairs:

The dye test was performed on both optimized henna dye and marketed product 'Nat habit' by using remi hairs.

Optimized formulation

Figure 4: Dyed remi hair of optimized formulation

Marketed product

Figure 5: Dyed remi hair of 'marketed product'

By comparing the both optimized formulation and marketed product's dyed remi hair it concluded that the formulated henna dye can give the equal results of the marketed product.

Figure 6: Sun Light exposure fading study on dyed remi hair

Dyed remi hair at day-0

Dyed remi hair of at day 0

Dyed remi hair of optimized formulation after 60 days

Dyed remi hair of marketed product after 50 days

In the present study, the sun light fading tests were conducted by exposing colored hair samples of both optimized formulation and marketed product to sunlight for a duration of 3 hours daily over a period of 70 days. Based on the observations, it was found that the optimized formulation shade of color began fading after the 60^{th} day of sun exposure while the marketed product shade of color began fading after 50^{th} day. Therefore, it can be

concluded that optimized dye formulation is capable of retaining its color on the hair for approximately 60 days under sunlight exposure.

Figure 7: Water washing fading study of optimized formulation and nat habit

Optimized formulation after 10th water wash

Optimized formulation after 40th water wash

Marketed product after 10th water wash

Marketed product after 30th water wash

In the present study, water wash fading tests were performed on optimized formulation and marketed product by washing the colored hair samples with normal tap water. It was observed that the shade of color started fading after the 30th wash for marketed product whereas the optimized formulation dyed hair starting fading the color after 40th wash. Based on these findings, it can conclude that optimized dye formulation is capable of retaining its color on the hair for approximately 40 normal water washes.

Figure 8: Shampoo washing fading study of optimized formulation and nat habit

Optimized formulation after 10th shampoo wash

Optimized formulation after 25th shampoo wash

Marketed product after 10th shampoo wash Marketed product after 20th shampoo wash

In the present study, shampoo wash fading tests were performed on both Optimized formulation and Marketed product by washing the colored hair samples with shampoo. It was observed that the shade of color started fading after the 20th wash for Marketed product dyed remi hair whereas optimized dyed remi hair started fading after 25th shampoo wash. Based on these findings, we can conclude that optimized dye formulation is capable of retaining its color on the hair for approximately 25 shampoo washes.

Fig 9. Dye test on artificial hair

a) Before applying dye b) Black Tone c) Brown Tone d) Copper red Tone

The dye test performed on Remmy hair, three different tones were achieved by incorporating varying proportions of ingredients in optimized formulation. These tones included a black color tone, a brown color tone, and a copper red tone. the following images (Figure 9) shows the 3 tons of dye.

Bacterial strain	Sample	Concentration	Zone of inhibition
Malassezia furfur	Optimized formulation	4000µg/ml	22mm

Table 10. Anti-dandruff activity of optimized formulation

The APX laboratories in Mumbai conducted this investigation to evaluate the antidandruff activity of the hair dye formulation.

The diameter of the zone of inhibition was measured after 24 hours of incubation and compared to the reference drug. The sample (optimized formulation) demonstrated stronger anti-dandruff activity against the microbial agent (*Malassezia furfur)*. The agar disc diffusion method was used to assess the zone of inhibition of the test sample at a concentration of 4000µg/ml. the results are shown in table 11 and figure 6.

Fig 6. Antidandruff activity of on *Malassezia furfur*

Table 11: Anti-bacterial activity of optimized formulation against standard amoxicillin

Sl no	Pathogen tested	Inhibitory diameter (mm)	
		Optimized formulation optimized formulation	Standard ciprofloxacin
1	Staphylococcus epidermidis	13	17
2	Escherichia coli	10	16

Staphylococcus epidermidis

Escherichia coli

Figure 25: Antibacterial activity of optimized formulation on two different bacterial strain against standard amoxicillin

The diameter of the zone of inhibition was measured after 24 hours of incubation and compared to the reference drug. The test sample demonstrated stronger antibacterial activity against the microbial agent (*Staphylococcus epidermidis, E-coli).* The agar disc diffusion method was used to assess the zone of inhibition of the test sample at a concentration of 2000µg/ml.

Antibacterial activity is evaluated by zone of inhibition diameter measured in mm where the bacterial growth doesn't occur.

- Optimised formulation showed sensitivity to
  Staphylococcus epidermidis → 13mm, *Esherichia coli* → 10mm
- Standard ciprofloxacin disc showed sensitivity

Staphylococcus epidermidis → 1mm, *Esherichia coli* → 16mm

6. CONCLUSION:

In conclusion, this study successfully developed a method for evaluating and characterizing a ready-to-apply natural hair dye formulation. The formulated henna dye demonstrated favorable physical and chemical properties, such as a desirable color, consistency, and appearance. The concentration of lawsone in the prepared formulation (optimized formulation) exceeded that of the marketed product, indicating its potency. Stability studies revealed that optimized formulation maintained its properties for a duration of 3 months under different storage conditions. Dyeing tests demonstrated excellent color retention even when exposed to sunlight and subjected to water washes. Moreover, optimized formulation exhibited additional benefits, including antidandruff and antibacterial activity. These findings provide valuable insights for the development of natural hair dye products, as they offer improved performance while addressing consumer concerns regarding safety and stability. The herbal hair dye represents a safer and more sustainable alternative to chemical-based dyes, contributing to the growing popularity of herbal hair care products. Additionally, the study highlights the effective properties of herbal hair packs and their potential to meet the evolving demands of the global hair care market.

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8. DECLARATION -

8.1 CONFLICT OF INTEREST:

The authors declare no conflict of interest in relation to the present research paper.

7.2 ETHICS APPROVAL AND CONSENT TO PARTICIPATE:

Not applicable.

7.3 HUMAN AND ANIMAL RIGHTS:

No Animals/Humans were used for studies.

7.4 AUTHORS CONTRIBUTIONS:

Author 1 and Author 2 made significant contributions throughout the entire project. Their joint efforts encompassed all aspects of the research article, including conception, design, experimentation, data analysis, and interpretation.

Author 1 and 2 played a crucial role in the initial stages of the project, contributing to the formulation and development of the herbal hair dye. They conducted extensive literature reviews to gather relevant information on natural ingredients and their potential benefits for hair care. Author 1 was also involved in the experimental design, overseeing the preparation of the herbal dye and conducting various tests to assess its efficacy and stability. Additionally, they contributed to data analysis and interpretation, ensuring the accuracy and reliability of the study's findings.

Author 3, and 4 collaborated closely with Author 1 and 2, providing valuable insights and expertise throughout the project. They actively participated in the experimental work, assisting in the formulation and standardization of the herbal hair dye. Author 1 and 1* played a key role in conducting organoleptic, physico-chemical, and stability tests, carefully analyzing the results and drawing meaningful conclusions. Their contribution extended to data interpretation, contributing to the overall validity of the study.

All authors collaborated in writing the research article, sharing responsibilities in drafting and revising the manuscript. They critically reviewed each section, ensuring the accuracy of the content and the coherence of the research narrative. Author 1 and Author 2 worked together to address reviewer comments and incorporate revisions, ultimately producing a comprehensive and well-structured research article.

7.5 FUNDING:

Not applicable

8 REFERENCES:

1. Packianathan N, Karumbayaram S. Formulation and evaluation of herbal hair dye: an ecofriendly process. Journal of Pharmaceutical Sciences and Research. 2010 Oct 1;2(10):648.
2. Singh V, Ali M, Upadhyay S. Study of colouring effect of herbal hair formulations on graying hair. Pharmacognosy Research. 2015 Jul;7(3):259.
3. Pal RS, Pal Y, Rai AK, Wal P, Wal A. Synthesis and evaluation of herbal based hair dye. The Open Dermatology Journal. 2018 Oct 18;12(1).
4. Arora J, Agarwal P, Gupta G. Rainbow of natural dyes on wool using plants extracts: Sustainable and eco-friendly processes. Green and Sustainable Chemistry. 2017 Jan 26;7(1):35-47.
5. Vaidya AD, Devasagayam TP. Current status of herbal drugs in India: an overview. Journal of clinical biochemistry and nutrition. 2007;41(1):1-1.
6. PK J, Dass DJ. Evaluating hair growth potential of some traditional herbs. Asian Journal of Pharmaceutical and Clinical Research. 2015;8(6):150-2.
7. Shukla MK, Srivastava S, Srivastava H, Gupta N, Yadav R. Development and Assessment of An Anti-Dandruff Herbal Hair Mask. International Journal of Advanced Research In Medical & Pharmaceutical Sciences. 2021 Jan 1;49(1):48-60.
8. Rao, Y. Madhusudan, and P. Sujatha. "Formulation and evaluation of commonly used natural hair colorants." Indian journal of natural product and resources. 2008; NPR Vol. 7(1). 45-48
9. Mielke HW, Taylor MD, Gonzales CR, Smith MK, Daniels PV, Buckner AV. Lead-Based Hair Coloring Products: Too Hazardous for Household Use. Journal of the American Pharmaceutical Association (1996). 1997 Jan 1;37(1):85-9.

10. Wilkinson, John Bernard, and Raymond Jack Moore, eds. *Harry's cosmeticology*. Chemical Publishing, 1982. Vol. 749.
11. Yazganoglu K, Arda A, Topkarci Z. The Henna . Indian Journal of Dermatology, Venereology, and Leprology. 2013 Mar 1;79(2):254.
12. Kumar KS, Begum A, Shashidhar B, Meenu M, Mahender C, Vamsi KS. Formulation and evaluation of 100% herbal hair dye. International Journal of Advanced Research In Medical & Pharmaceutical Sciences. 2016 March; Vol 2 (1):2455-6998.
13. Patel MM, Solanki BR, Gurav NC, Patel PH, Verma SS. Method development for Lawsone estimation in Trichup herbal hair powder by high-performance thin layer chromatography. J Adv Pharm Technol Res 2013; 4(3): 160-5.
14. S.G. Design and evalution of herbal hair oil formulations by using ethanolic extract OF Ziziphus jujuba Mill. LEAVES Int J Pharma Bio Sci 2017; 8(3): 322-7.
15. Grabley S, Thiericke R. Bioactive agents from natural sources: Trends in discovery and application. Adv Biochem Eng Biotechnol 1999; 64: 101-54.
16. Nisha P, Singhal RS, Pandit AB. A study on degradation kinetics of ascorbic acid in amla (Phyllanthus emblica L.) during cooking. Int J Food Sci Nutr 2004; 55(5): 415-22.
17. Singh E, Sharma S, Pareek A, Dwivedi J, Yadav S, Sharma S. Phytochemistry, traditional uses and cancer chemopreventive activity of amla (Phyllanthus emblica): The sustainer. J Appl Pharm Sci 2011; 2: 176-83.
18. Turner DM. Natural product source material use in the pharmaceutical industry: The Glaxo experience. J Ethnopharmacol 1996; 51(1-3): 39-43.

19. Upadhyay VP. International Seminar on Medicinal Plants. Plants as cosmetics 1985. In: Mungpoo, Govt. of West Bengal: Publication and Information Directorate, CSIR,; New Delhi. 1985.
20. Kitrikar K, Basu BD. Indian Medicinal Plants 2nd ed. 1993; Vol. I: 335-6.
21. Trüeb RM. Pharmacologic interventions in aging hair. Clin Interv Aging. 2006;1(2):121-9. Doi: 10.2147/ciia.2006.1.2.121. PMID: 18044109.
22. Lurie R, Ben-Amitai D, Laron Z. Laron syndrome (primary growth hormone insensitivity): a unique model to explore the effect of insulin-like growth factor 1 deficiency on human hair. Dermatology (Basel) 2004; 208(4): 314-8.
23. Banerjee PS. Spectrophotometric methods for the determination of selected drugs in pharmaceutical formulations. J Chem Pharm Res 2009; 1(1): 261-7.
24. Dweck AC. On the Centella asicatica trail. Soap. Perfumery and Cosmetics Asia 1996; 1: 41-2.
25. Porwal P, Sharma A, Gupta SP. Henna based cream preparation, characterization and its comparison with marketed hair dyes. J Herbal Med Tech 2011; 5(1): 55-61.
26. Banerjee P, Sharma M. Preparation, evaluation and hair growth stimulating activity of herbal oil. J Chem Pharm Res 2009; 1(1): 261-7.
27. Baziga KA, Heyan SA. Formulation and evaluation of herbal shampoo from zizyphus spine leaves extract. Int J Res Ayurveda Pharm 2011; 2(6): 1802-6.
28. Anjali J¸. Hair care formulations. World J Pharm Pharm Sci 2016; 5(6): 630-48.
29. Turner DM. Natural product source material use in the pharmaceutical industry: The Glaxo experience. J Ethnopharmacol 1996; 51(1-3): 39-43.
30. Fatima A, Alok S, Agarwal P, Singh P, Verma A. Benefits of herbal extracts in cosmetics: A review. Int J Pharm Sci Res 2013; 4(10): 3746-60.
31. Sharma AK. Medicinal properties of apamarg (achyranthes aspera linn.). Int. J. Ayur. Pharma Research. 2013;1(3):4-12.
32. Maurya SK, Seth AN. Potential medicinal plants and traditional ayurvedic approach towards hair care. Int J Pharm Pharm Sci. 2014;6(5):172-7.
33. Shivaprasad BM, Patil P, Nair SK, Singh N, Shivanand S, Sameera U. *Triphala*: A phytomedicine for local drug delivery - A strategic intervention. Ayu. 2019 Jan-Mar;40(1):53-57. doi: 10.4103/ayu.AYU_40_17. PMID: 31831970; PMCID: PMC6891999.

34. Idris S, Mishra A, Khushtar M. Recent Therapeutic Interventions of Fenugreek Seed: A Mechanistic Approach. Drug Res (Stuttg). 2021 Apr;71(4):180-192. doi: 10.1055/a-1320-0479. Epub 2020 Dec 30. PMID: 33378775.

35. Trividic M, Gauthier ML, Sparsa A, Ploy MC, Mounier M, Boulinguez S, Bedane C, Bonnetblanc JM. Staphylococcus aureus métirésistant en dermatologie (Methi-resistant Staphylococcus aureus in dermatological practice: origin, risk factors and outcome). Ann Dermatol Venereol. 2002 Jan;129(1 Pt 1):27-9. French. PMID: 11937926.

36. Dhakad AK, Pandey VV, Beg S, Rawat JM, Singh A. Biological, medicinal and toxicological significance of Eucalyptus leaf essential oil: a review. J Sci Food Agric. 2018 Feb;98(3):833-848. doi: 10.1002/jsfa.8600. Epub 2017 Sep 11. PMID: 28758221.

37. Wińska K, Mączka W, Łyczko J, Grabarczyk M, Czubaszek A, Szumny A. Essential Oils as Antimicrobial Agents-Myth or Real Alternative? Molecules. 2019 Jun 5;24(11):2130. doi: 10.3390/molecules24112130. PMID: 31195752; PMCID: PMC6612361.

38. Carson CF, Hammer KA, Riley TV. Melaleuca alternifolia (Tea Tree) oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev. 2006 Jan;19(1):50-62. doi: 10.1128/CMR.19.1.50-62.2006. PMID: 16418522; PMCID: PMC1360273.

39. Pazyar N, Yaghoobi R, Bagherani N, Kazerouni A. A review of applications of tea tree oil in dermatology. Int J Dermatol. 2013 Jul;52(7):784-90. doi: 10.1111/j.1365-4632.2012.05654.x. Epub 2012 Sep 24. PMID: 22998411.

40. L Qadariyah, N Azizah, A Q Syafa atullah, D S Bhuana and M Mahfud**;** The Extraction of Natural Dyes from Henna Leaves (Lawsonia Inermis L.) by Ultrasound-assisted Method; IOP Conference Series: Materials Science and Engineering, Volume 543. Oct 2018;543

41. Mansoor S, Mehfooz S, Mustafa H. Anti-bacterial, anti-oxidant and cytotoxic potential of aqueous and organic extracts of lawsonia inermis. World J. of Pharmaceutical Research. 2016 Mar 2;5(5):686-703.

42. Amit, & Singh, N. (2022). Studies on antimicrobial activity of Lawsonia inermis L. against different strains of bacteria and fungi. Journal of Applied and Natural Science, 14(1), 247–253. https://doi.org/10.31018/jans.v14i1.3337