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MURRAYA KOENIGII LINN SPRENG: A NATURAL SOURCE FOR HERBAL HAIR DYE GEL INNOVATIONS

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ABSTRACT

Pharmacognosy, the study of bioactive substances from natural sources, is vital for identifying therapeutic agents and sustainable products. Advances in herbal pharmacognosy, incorporating molecular and genomic techniques, have enhanced the understanding of traditional remedies. Natural colorants from plants, minerals, and animals offer safer, eco-friendly alternatives to synthetic dyes, with growing demand in the food, cosmetic, and textile industries due to their safety and reduced environmental impact. *Murraya koenigii L* (curry leaves), a key ingredient in South Asian cuisine and traditional medicine, is rich in phytoconstituents, including carbazole alkaloids with antioxidative, anti-inflammatory, and antidiabetic properties. This study investigates its pharmacognostic properties, including morphological, proximate, and rheological analyses. Phytochemical screening revealed polyphenols, flavonoids, and anthocyanins in aqueous, ethanol, and ethyl acetate extracts. The dyeing potential of *M. koenigii* extracts was evaluated on cotton, sheep wool, and human hair using mordanting techniques. Tests for colorfastness, intensity, and light fading demonstrated the dyes' effectiveness and stability. An herbal hair dye gel incorporating *M. koenigii* extracts, *R. indica* extracts, aloe vera, and rose oil was developed, exhibiting desirable physicochemical properties such as pH, spreadability, and stability. Toxicity evaluations, including brine shrimp lethality and animal skin irritation tests, confirmed the gel's safety. This study highlights *M. koenigii's* role as a natural dye and a health-promoting agent and emphasizes its potential to create sustainable, safe alternatives for the cosmetic industry.

KEYWORDS: *Murraya koenigii*, Natural colorants, Carbazole alkaloids, Herbal hair dye gel, and Sustainability.

1. INTRODUCTION

In contemporary medicine, pharmacognosy is the study of natural remedies that originate from microbes, plants, and animals which is essential because it can assess the therapeutic qualities of phytochemical molecules. It has grown to encompass herbal pharmacognosy, which uses genomic and molecular techniques to investigate traditional therapies and their modes of action.[1] In Ayurveda, Traditional Chinese Medicine, and other conventional therapies, herbal medications are very significant. Pharmacognosy involves addressing difficult health challenges by bridging the gap between traditional knowledge and cutting-edge research. Around the world, 70–80% of people still use herbal treatments, particularly in low-income regions. Because of interests in integrative health and environmental concerns, these treatments are growing in popularity.[2]

Natural colorants found in herbal resources become increasingly popular because of their positive health effects and less negative environmental effects. Plants, minerals, and animals provide safer sources of carotenoids, curcumin, and anthocyanins. India is home to around 450 plants that produce dyes, some of which are used medicinally. Natural dyes provide anti-aging and UV protection in food, medicine, and cosmetics.[3] In 2022, the global market for natural colors and dyes was projected to reach USD 38.2 billion. Natural colors are becoming more common as a sustainable substitute for synthetic ones, even if synthetic dyes still predominate. This is because of concerns about safety, health, and the environment.[4]

Hair is a protein filament that develops from dermal follicles and is comprised primarily of keratin. Its three structural layers are the cuticle, cortex, and medulla, and perform sensory, regulatory, and protective roles. Each strand of hair regenerates during the three stages of the hair development cycle: anagen (growth), catagen (transition), and telogen (rest).[5,6]

Hair dyes, ranging from temporary to permanent, allow individuals to express themselves, cover gray, or enhance appearance. Permanent dyes penetrate the hair shaft using ammonia and peroxide, but semi-permanent and demi-permanent dyes have less pronounced effects. Gradual and temporary dyes either stay on the surface or undergo color changes after several applications. Natural colors derived from plants, such as blackcurrant and henna, are gentle on tender hair.[7]

The dyeing mechanism involves direct and mordant dyeing:
Direct dyeing enables dye molecules to form a link with hair keratin, whereas to enhance color persistence, mordant dyeing employs metallic ions like iron or copper. The revival of natural colorants has been aided by environmental worries over synthetic dyes. In textiles, cosmetics, and dietary supplements, natural pigments such as plant anthocyanins act as environmentally benign colors, minimizing hazards to human health and the environment.[8]

CURRY LEAVES (*Murraya koenigii Linn Spreng*) is a vital ingredient in South Asian cuisine and have a longstanding role in traditional medicine. Indigenous to India, Sri Lanka, and Southeast Asia, they flourish in tropical regions. Known in Ayurveda as "Girinimba," these leaves are packed with essential oils, antioxidants, and carbazole alkaloids, which support digestion, fight oxidative stress, and reduce inflammation. Traditionally, curry leaves are used to address and improve hair and skin health, and aid in managing diabetes. They have a nutrient-dense composition that benefits immunity, liver health, and oxidative damage reduction due to their high iron, calcium, and vitamin A, B, C, and E

content. Curry leaves are valued for combining culinary and therapeutic benefits since they maintain taste even after drying.[9,10]

SYNONYMS

English- Curry leaves, **Kannada**- Karibevu, **Hindi**- Karipatta, Mitha neem

TAXONOMICAL CLASSIFICATION	
Kingdom	Plantae
Sub kingdom	Tracheobionta
Infra kingdom	Angiosperms
Phylum	Magnoliophyta
Subdivision	Spermatophytina
Infra division	Angiospermae
Class	Magnoliopsida
Subclass	Rosidae
Order	Sapindales
Superorder	Rutinae
Family	Rutaceae
Subfamily	Aurantioideae
Genus	*Murraya*
Species	*M. koenigii L. Spreng*
Binomial name	*Murraya koenigii Linn*
Common name	Curry leaves

A minuscule tree or semi-deciduous aromatic shrub belonging to the Rutaceae family, *Murraya koenigii L.* is renowned for its savory and therapeutic virtues. Its dark, rough bark peels off to expose smooth, white timber, and it may reach a height of 7 meters. The complex leaves, which have nine to twenty-five elliptic leaflets, are strongly fragrant. It has tiny, black fruits with smooth, green seeds that are essential for proliferation, as well as attractive white blooms with ten stamens. The seeds sprout on damp, shaded soil and are best cultivated in tropical regions. It tends to thrive in gardens because of its unique odor and aesthetic attractiveness.[11,12]

Murraya koenigii leaves are abundant in nutrients, including proteins, carbs, minerals, vitamins A and C, and a variety of phytoconstituents, including carbazole alkaloids (e.g., girinimbine and koenigine). A multitude of alkaloids, including mahanimbine, and coumarins are found in the stem bark. Pharmacologically active alkaloids are also produced by root extracts. While the seeds contain lipids and carbazole alkaloids, the fruit pulp is rich in moisture (64.9%) and contains a significant quantity of vitamin C (13.35%). The monoterpenoids and sesquiterpenoids that make up the majority of the essential oils that are extracted from the leaves include β-caryophyllene. Every component of the plant has therapeutic significance, highlighting its use in traditional medicine.[13,14]

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Samples

Murraya koenigii Linn samples were collected from a home garden in Sullia, Karnataka, India, and authenticated by DR. V. Rama Rao from the Central Ayurveda Research Institute in Bengaluru, Karnataka.

2.2 Pharmacognostical Evaluation[15]

Murraya koenigii Linn leaves underwent morphological and powder microscopy evaluation, revealing moisture content, total ash value, water soluble ash value, acid insoluble ash value, water soluble extractive value, and alcohol soluble extractive values.

i. Morphological investigation of plant material

Murraya koenigii (Linn.) Spreng leaves were examined for a macroscopic evaluation as per WHO Quality Control procedures for herbal medicines. The color, measurements, structure, taste, aroma, and surface features were all noted in Table No. 3.

ii. Powder microscopy

Powdered *Murraya koenigii* leaves were prepared on a microscopic slide with 0.1% w/v phloroglucinol and concentrated hydrochloric acid, mounted in glycerol, and examined microscopically. The powder exhibited starch grains and calcium oxalate crystals, confirmed by the blue coloration with iodine solution. Detailed cell structures and components were observed and documented in photographs, with the microscopic features summarized in Figure No. 1.

2.3 RHEOLOGICAL PARAMETERS[15]

Bulk and tapped densities were measured to assess powder flow. Carr's Index and Hausner Ratio were calculated, with values indicating good flow (<15% Carr's, <1.25 Hausner) or poor flow (>25% Carr's, >1.5 Hausner). The angle of Repose, measured by the height and radius of a powder heap, further assessed flowability: <30° for excellent flow, 30°–40° for good/fair, and >40° for poor. Results appear in Table 04.

2.4 PROXIMATE ANALYSIS[15]

Proximate values such as moisture content, total ash value, water soluble ash value, acid insoluble ash value, alcohol soluble extractive value, and water-soluble extractive value, were determined for the powdered drug. The pH of 1% and 10% suspension was recorded. Results are represented in Table No. 05.

i. Moisture content

The Loss on drying method was used to determine the moisture content of drug samples. 2g of drug powders were placed in a weighted Petri dish and dried at 105°C for 2 hours. The difference in weight was considered a loss of moisture. The drying and weighing were repeated for 30 minutes until a constant weight was obtained. Results were expressed as % w/w in Table No. 05. The calculation involved weighing the empty Petri dish, sample, and Petri dish after drying at 105°C.

ii. Determination of Ash values

The total ash, acid-insoluble ash value, and water-soluble ash values were determined for air-dried samples using the procedure described in quality control methods for medicinal plant materials. Results are shown in Table No. 05.

Total Ash Value

The total ash value indicates the presence of inorganic compounds and contaminants in plant materials. To determine this, 2g of the powdered sample was incinerated in a pre-weighed silica crucible at 450°C and gradually increased to 675°C until carbon was fully removed. The cooled crucible was weighed to calculate the ash percentage.

Acid-Insoluble Ash

The total ash was boiled with 25ml of 2M HCl, filtered, and the insoluble residue was ignited at 450°C. The ash percentage was calculated to reflect acid-insoluble components.

Water-Soluble Ash

The ash was boiled in 25ml water, filtered, and the insoluble residue ignited. The weight difference indicated the water-soluble ash, calculated as % ash.

iii. Extractive value

The extractive values of powdered *Murraya koenigii* were assessed for alcohol-soluble extractive and water-soluble extractive values according to the Indian Pharmacopoeia, serving as indicators of the drug's quality and purity.

For alcohol and water-soluble extractives, 5g of powdered sample was macerated with 100 ml of solvent (alcohol or water) for 24 hours, shaken periodically for the first 6 hours, and then filtered. 20 ml of filtrate was evaporated in a water bath, dried at 105°C, and weighed to obtain a constant weight. The extractive percentage was calculated for each solvent, with results shown in Table No. 05.

iv. Determination of pH

Using a digital meter, the pH of the drugs was determined *M. koenigii* powder and distilled water were mixed to create suspensions at 1% and 10% concentrations for two hours. The mixture was then tested three times, and an average was determined, the same is represented in Table No.05.

2.5 PREPARATION OF EXTRACTS

Curry leaf powders were extracted by maceration extraction with water and Soxhlet extraction with ethanol and ethyl acetate solvents. After 15 days of sun drying, *Murraya koenigii L* leaves were ground into a powder and sieved. To stop degradation, powders were kept in a refrigerator.

i. Soxhelation

Using a Soxhlet apparatus, *Murraya koenigii L* powders were weighed and packed before being extracted for eight hours using ethanol and ethyl acetate (1:4). After that, the extract was concentrated below 60^0C using a rotating vacuum evaporator. The extracts' color, consistency, and yield % were displayed in Table No. 06.

ii. Maceration

Murraya koenigii L powder was macerated by weighing and packing 100 gm in a conical flask, extracting with distilled water (1:15), and then adding chloroform to cease the development of microorganisms. The mixture was macerated for seven days while being mixed occasionally. A rotary vacuum evaporator was used to filter and concentrate the extract, and the extracts' color, consistency, and yield percentage were noted in Table No. 06.

2.6 QUALITATIVE PHYTOCHEMICAL EVALUATION OF EXTRACTS[16]

Individual aqueous, ethanolic, and ethyl acetate extracts were subjected to qualitative chemical tests to detect phytoconstituents such as proteins, carbohydrates, alkaloids, glycosides, tannins, sterols, flavonoids, and triterpenes. Using standard procedures, preliminary phytochemical analyses were conducted for every extract. Results are shown in Table No. 07.

2.7 QUANTITATIVE EVALUATION OF EXTRACTS

Curry leaf extracts, obtained through soxhelation and maceration, showed good yield in all solvents. Polyphenols and anthocyanins were detected in ethanolic, ethyl acetate, and aqueous extracts, indicating their phytochemical content.

i. Estimation of total polyphenols content by Folin Ciocalteu colorimetric method[17]

The TPC in ethanolic, ethyl acetate, and aqueous extracts of curry leaves was determined, with gallic acid as the standard. A stock solution (1 mg/ml) of gallic acid was diluted to prepare a 200 µg/ml. Extract samples (2 mg/ml) were prepared by dissolving 50 mg of each extract in distilled water using ultrasonication to obtain 40 µg/ml. For analysis, both standard and sample solutions were mixed with 0.25ml of Folin-Ciocalteu reagent and 1.25ml of sodium carbonate and then left for 30 minutes at room temperature, and a blank was prepared without any standard or sample. Absorbance was measured at 670 nm using a spectrophotometer. Polyphenol content was expressed in gallic acid equivalents (GAE), and a calibration curve was used to quantify it. Results are shown in Table No. 08.

ii. Estimation of total anthocyanin content by the UV-Spectrometric method[18]

Curry leaf extracts in ethanol, ethyl acetate, and water were tested for their total anthocyanin concentration. pH 1.0 buffer (0.025 M potassium chloride) and pH 4.5 buffer (0.4 M sodium acetate) were used to prepare the buffers. The extract was diluted with pH buffer to prepare test solutions until the absorbance fell within the linear range (0.2-1.4 AU). Using a spectrophotometer set at 520 nm and 700 nm, absorbance was measured for test solutions with pH values of 1.0 and 4.5 within 20 to 50 minutes after preparation. The formula A x MW x DF x103/ ε x l was used to determine the concentration of anthocyanin pigment. The outcome provides the sample's total anthocyanin content, which is given as mg/L. Results are shown in Table No. 0.

2.8 TLC ANALYSIS

The TLC fingerprint method is widely used to quickly assess the quality and authenticity of crude drugs by identifying phytoconstituents in herbal extracts. In this study, TLC patterns were developed for aqueous, ethanolic, and ethyl acetate extracts of *Murraya koenigii* leaves to identify coloring compounds. Sample extracts were prepared at 10 mg/mL concentration by dissolving 100 mg in 10 mL of the respective solvent via 15 minutes of ultrasonication. TLC solvent system used toluene: ethyl acetate: methanol: formic acid (9:8:2:1), a silica gel GF 254 plate, and UV detection at 254 nm and 366 nm, as well as visible light observation. Results are shown in Table No.10. and Figure No. 02.

2.9 UV SPECTRAL DATA ANALYSIS[19]

0.1mL of each sample (Curry Water, Curry Ethanol, Curry Ethyl acetate) was transferred into separate 10 mL volumetric flasks and diluted to volume with the appropriate solvent. For a 1:100 dilution, 0.1 mL of stock solution was diluted to 10 mL. Absorbance was recorded from 190 to 800 nm after baseline calibration, and λmax was determined. Results are shown in Table 11.

2.10 DYEING OF COTTON AND HAIR

i. Extracts for dyeing properties on the cotton fabric[20]

Cotton fabrics were sourced locally in Bangalore, cut into 5 cm × 5 cm pieces, and pre-treated by soaking in hot water to remove impurities and improve dye absorption. Dyeing was conducted using extracts at 60°C for 40 minutes, with a mass-to-liquor ratio of 1:30 and pH 6.5, followed by a cold-water rinse to remove the unfixed dye. Mordanting was then performed at room temperature with ferrous sulfate and potassium aluminum sulfate for 10 minutes. Finally, the dyed fabric was washed with a soap and sodium carbonate solution, rinsed, and dried at 100°C for 5 minutes. Results are shown in Table No. 12.

ii. Extracts for dyeing properties on the sheep hair and human hair[21]

Sheep and human hair samples were collected from butcher and barber shops in Bangalore, Karnataka, and then thoroughly cleaned by washing daily with water and shampoo for a week to remove impurities and oils. After washing, the hair was left to air-dry for an additional week. For dyeing, 0.2-gram hair swatches were immersed in dye solutions within screw-capped bottles for six hours, followed by a water rinse and natural drying. Results are shown in Table No. 12.

2.11 EVALUATION OF DYES [21]

i. Color fastness by grey scale evaluation

Colorfastness measures how well a dye retains its color when exposed to conditions like washing, light, and perspiration. To test this, dyed hair samples underwent a daily shampooing, rinsing, and air-drying cycle for seven days. Color retention was then assessed by comparing the washed samples to their original state using a standardized grey scale (ranging from 0 to 5), which rates the degree of color change.

0-White (no change), **1**-Very faint color change, **2**-Slight light color change,

3-Light color change, **4**-Slight dark color change, and **5**-Dark color change.

Results are shown in Table No. 13.

ii. Measurement of color intensity

Hair swatches from humans and sheep were labeled and dyed, soaking for 12 hours to ensure full-color penetration. Post-dyeing, they were rinsed until clear and then shampooed daily for a week. Afterward, color intensity (chroma) was measured using the CIE L*a*b* system at the Central Silk Board, Bangalore. This method quantified lightness (L*), green-red (a*), and blue-yellow (b*) spectra, chroma (C*), and hue (h°), with ΔE calculations assessing dye permanence and color change. Results are shown in Table No. 13.

iii. Light fading test Using Xenon Arc Lamp 2454

Dyed human and sheep hair samples, were subjected to continuous light exposure for 24 hours using a Xenon Arc Lamp 2454 at the Central Silk Board, Bangalore. The test aimed to simulate extended sunlight exposure, evaluating dye durability by comparing color fading against British Blue Wool standards. Results were scored on a 6-point scale, where higher scores (5-6) signified strong color retention, indicating the dye's resilience and effectiveness under prolonged light conditions. Results are shown in Table No. 13.

2.12 FORMULATION OF HERBAL HAIR DYE GEL[22]

To prepare the gel, dissolve 2 g guar gum in aloe vera gel with vigorous stirring. Weigh 1% ethanolic and ethyl acetate extracts of rose and curry leaves, dissolving each in a 1% $FeSO_4$ and PAS solution. Gradually mix this with the guar gum solution, maintaining constant stirring. Adjust pH to 7 using sodium hydroxide, then blend in rose oil. Add more aloe vera gel to reach 100 g total, stirring until smooth, then store in a sterile container.

Table No. 1: Formulation using various ingredients.

INGREDIENTS	QUANTITY	ROLE
GUAR GUM	2 gms	Thickening agent
ALOE VERA GEL	50 ml	Base
CURRY ETHANOL EXTRACT	1 g	Dye
CURRY ETHYL ACETATE EXTRACT	1 g	Dye

ROSE ETHANOL	1 g	Dye
ROSE ETHYL ACETATE EXTRACT	1 g	Dye
1% $FeSO_4$	10 ml	Mordant
1% PAS	10 ml	Mordant
SODIUM HYDROXIDE	Q. S	pH neutralizer
ROSE OIL	Q. S	Perfuming agent

2.13 EVALUATION OF HERBAL HAIR DYE GEL FORMULATION[23]

The herbal hair gel was assessed through several tests:

1. Physical Appearance: Evaluated for color, transparency, odor, appearance, and any foreign particles.
2. pH Determination: One gram of gel in 100 ml distilled water was measured for pH after standing for two hours.
3. Homogeneity: Visually inspected for uniformity and absence of lumps or aggregates.
4. Extrudability: Checked by squeezing gel from a collapsible tube.
5. Spreadability: 2 g of gel sandwiched between slides with weight applied; spread diameter was measured.
6. Open-Patch Test: Applied to skin, observed for irritation or rashes.
7. Viscosity: Measured using a Brookfield viscometer at 100 rpm with spindle no. 6, repeated thrice.
8. Washability: Assessed by applying and rinsing gel with water.
9. Stability Studies: Monitored for color, odor, appearance, and consistency changes over 30 days at room temperature.

   Results are shown in Table No. 14.

2.14 BRINE SHRIMP LETHALITY ASSAY[24]

To prepare seawater, dissolve 38 g of non-iodized sea salt in one liter of distilled water and filter. Hatch Artemia salina eggs in a seawater tank with constant aeration; after two days, collect light-attracted, egg shell-free nauplii from the illuminated side. Prepare test samples (dye extracts, mordants, and formulations) in various concentrations (50–300 µg/mL) and add 0.5 mL of each to the brine solution containing 10 nauplii. Incubate for 24 hours under light, then count surviving larvae. Conduct trials in triplicate, using LC50 values to determine toxicity: highly toxic (<10 µg/mL) to non-toxic (>500 µg/mL). Results are shown in Table No. 15.

2.15 ANIMAL ACTIVITY[25]

Albino rats (n = 6, 200-250 g) were kept in controlled conditions (12 h light/dark, 37 ºC, 40-60% RH). After IAEC approval (AACP/IAEC/42/FEB2024/03), the rats were acclimated for 7 days. Their dorsal abdominal skin was shaved 24 hours prior, and 200 mg of the formulation was applied with a cotton bandage, and removed after 24 hours. Erythema was scored: 1 (no reaction) to 5 (severe erythema with/without edema). Results are shown in Table No. 16.

Table No.2: Grouping of animals for Skin irritation test.

Group	No. of Animals	Treatment	Dose
Group – 1	N = 6 animals	Control group	
Group – 2	N = 6 animals	Standard Group – Boutique herb color	200mg/ rat
Group – 3	N = 6 animals	Sample group – Formulated herbal hair dye gel	200mg/rat

3. RESULTS AND DISCUSSION

3.1 COLLECTION AND AUTHENTICATION OF SAMPLES

The leaves of *Murraya koenigii Linn Spreng* (Rutaceae) were collected from a house garden in Sullia, Karnataka. The plant used for the current study was authenticated by Dr. V. Rama Rao at Central Ayurveda Research Institute Bengaluru, Karnataka with authentication certificate no (RRCBI-mus290).

3.2 PHARMACOGNOSTIC*AL* EVALUATION

i. Morphological evaluation

The Murraya koenigii Linn Spreng (Rutaceae) leaves were subjected to morphological evaluation such as color, odor, taste, size, and shape. The same description is recorded in **Table No.3.**

Table No. 3 Morphological investigation of *R. indica, C. ternatea,* and *M. koenigii.*

Evaluation Parameters for *Murraya Koenigii L*	Color	Odor	Taste	Size	Shape
	Light green – dark green	Sweetly scented	Slightly acidic, pungent, bitter	2-4 cm long and 1-2 cm broad	Slim, elongated, and has a pointed tip

ii. Powder Microscopy

The leaves of *Murraya koenigii L* were examined for microscopical characteristics. The photographic illustrations are seen in **Figure no: 1** respectively.

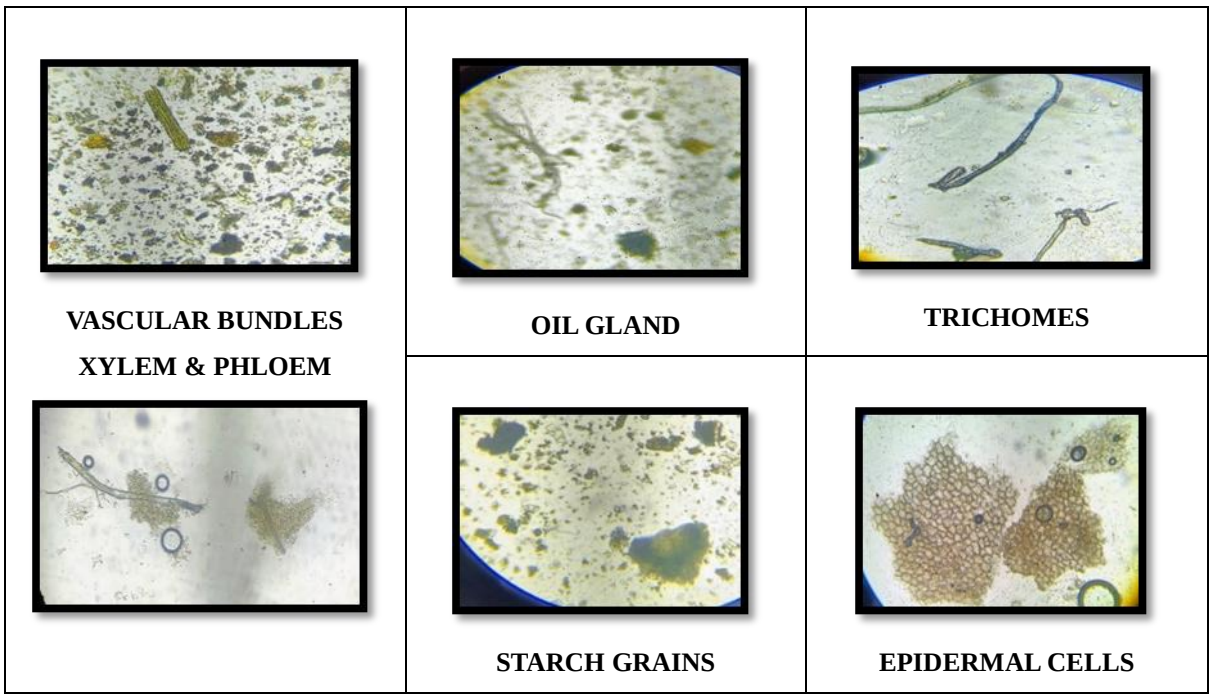

Figure no. 1: Powder microscopy of *Murraya koenigii Linn.*

3.3 RHEOLOGICAL PARAMETERS

The bulk density, tapped density, Carr's index, Hausner's ratio, and Angle of repose were carried out for *Murraya koenigii L* powder. The readings have been recorded in **table no. 4.**

Table No. 4: Rheological parameters of *M. koenigii* powders.

RHEOLOGICAL PARAMETERS FOR *MURRAYA KOENIGII L.*	Bulk density	Tapped density	Carr's index %	Hausner's ratio	Angle of repose
	0.28	0.3	7.1%	1.07	39

3.4 PROXIMATE ANALYSIS

The moisture content, total ash values, water-soluble ash values, Acid-insoluble ash value, Alcohol soluble extractive value, Water-soluble extractive value, and pH of the leaves of *Murraya koenigii L* powder were determined as per Indian Pharmacopoeia. The values obtained are shown in **Table no: 05.**

Table No. 5: Proximate analysis *of M. koenigii* powders.

PROXIMATE ANALYSIS OF *MURRAYA KOENIGII L*	Moisture content % w/w	Total ash value % w/w	Acid-insoluble ash value % w/w	Water soluble ash value %w/w	Water soluble extractive value % w/w	Alcohol soluble extractive value %w/w	ph-1%	ph-10%
	6%	7.9%	1.81%	5.34%	4.01%	5%	6.15	4.38

3.5 PREPARATION OF EXTRACTS

The *Murraya koenigii L*. were extracted using both Soxhlet and maceration methods, and evaluated for its nature, color and percentage yield were recorded. The results are presented in **Table No. 6.**

Table No. 6: Extraction Yields and Characteristics of *M. koenigii* using Soxhlation and maceration.

Drug	Extracting solvent	% yield of extract	Colour of extract	Nature of extract
Murraya koenigii Linn	Water extract	7.8%	Green	Sticky nature
	Ethanol extract	5%	Dark green	Resinous substance
	Ethyl acetate extract	11.6%	Dark green	Resinous substance

PHYTOCHEMICAL EVALUATION OF EXTRACTS

3.6 Qualitative chemical test

Extracts prepared were subjected to qualitative chemical tests to find out the chemical constituents present. Results are tabulated in **Table No. 7.**

Table No. 7: Phytochemical screening of *M. koenigii* extracts.

+ - Present and - - Absent

Phytochemical screening	Curry water extract	Curry ethanol extract	Curry ethyl acetate extract
Alkaloids	+	+	-
Carbohydrates	+	+	+
Glycosides	-	-	-
Saponins	+	-	-
Triterpenes and phytosterols	-	+	+
Fixed oils and fats	+	+	+
Resins	-	+	+
Phenols	+	+	+
Flavonoids	+	+	+
Proteins and amino acids	+	+	+
Mucilage	+	+	+
Anthocyanins	+	-	-

3.7 Quantitative evaluation of phytochemical constituents

- **Total phenolic content**

The data for the linearity curve of gallic acid for the determination of polyphenol content is represented in **Table No. 8.**

Table No. 8: Spectrophotometric determination of polyphenol content of *M. koenigii* extracts.

Volume of gallic acid	CONC (mg/ml)	FCR	Na_2CO_3	Volume up to	Absorbance nm	mg GAE/g
Blank	0	0.25 ml	1.25 ml	25 ml	0	
0.2	1.6				0.115	
0.4	3.2				0.279	
0.6	4.8				0.401	
0.8	6.4				0.526	
1	8				0.633	
1.2	9.6				0.801	
1.4	11.2				0.961	
1.6	12.8				0.999	
Curry water extract	**4.97**				**0.408**	**2.49**
Curry ethanol extract	**1.67**				**0.139**	**0.83**
Curry ethyl acetate extract	**1.02**				**0.086**	**0.51**

- **Total anthocyanin content**

The data for the bar graph of extracts for the determination of anthocyanin content is represented in **Table No. 9.**

Table No. 9: Spectrophotometric determination of anthocyanin content of *M. koenigii* extracts.

Extracts	1%		4.5%		TAC (mg/L)
	520 nm	700 nm	520 nm	700 nm	
Curry water extract	0.145	0.068	0.079	0.026	**30.03**
Curry ethanol extract	1.734	1.615	0.785	0.764	**81.79**
Curry ethyl acetate extract	0.038	0.031	0.054	0.028	**79.8**

3.8 THIN LAYER CHROMATOGRAPHY ANALYSIS

TLC was performed to identify coloring entities in extracts from *Murraya koenigii L*. leaves. The developed TLC plate was analyzed under UV light at 254 nm and 366 nm, and visible light, as shown in **Table No. 10** and **Figure No. 2.** Results indicated the presence of distinct spots in each sample, with varying R_f values by identifying significant coloring entities.

Table No. 10: TLC Analysis of Extracts for *Murraya koenigii.*

Content	UV visualisation				Visible light	
	254nm		366nm			
	No. of spots	R_f Value	No. of spots	R_f Value	No. of spots	R_f Value
Track 7 (curry water extract)	1	0.43	1	0.46	1	0.33
Track 8 (Curry Ethanol extract)	7	0.5,0.52, 0.56, 0.57,0.6,0.7,0.8	8	0.34, 0.56, 0.6, 0.65, 0.71, 0.76, 0.81, 0.86	5	0.51, 0.58, 0.61, 0.67, 0.7
Track 9 (Curry Ethyl acetate extract)	4	0.3, 0.42, 0.45, 0.6	2	0.39, 0.43	2	0.29, 0.35

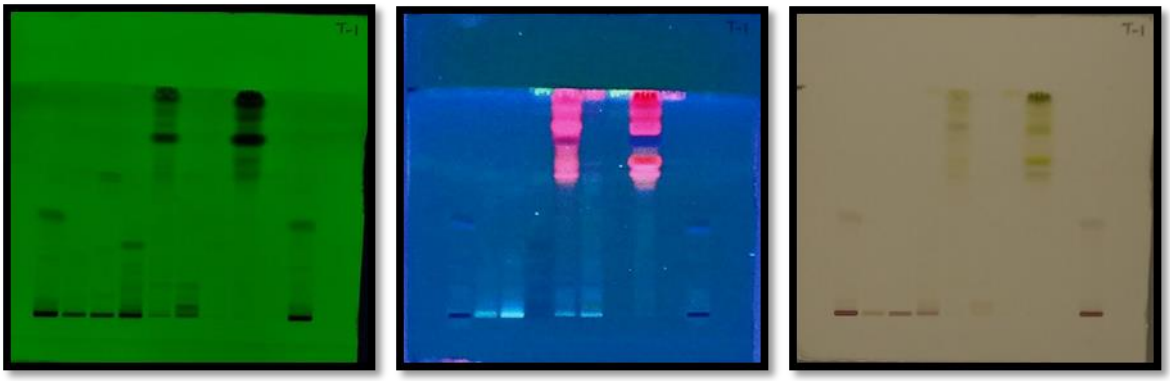

Figure No. 2: TLC plate under UV light at 254 nm and 366 nm, and under visible light.

3.9 UV SPECTRAL DATA ANALYSIS

UV spectral data analysis was performed on extracts from *Murraya koenigii* to identify their absorbance characteristics. the analysis involved preparing stock solutions from each sample, followed by a 1:100 dilution. The absorbance of the samples was measured across a wavelength range of 190 nm to 800 nm using a UV-visible spectrophotometer. The results are summarized in **Table No. 11.**

Table No. 11: UV Spectral Data of Extracts showing $\lambda_{max.}$

Samples	λ_{Max}	Wavelength
Curry water extract	0.153	200 nm
Curry ethanol extract	0.088	238.5 nm
Curry ethyl acetate extract	0.116	249 nm

3.10 DYEING OF COTTON AND HAIR

i. Extracts for dyeing properties on the cotton fabric

The fabric was soaked, dyed with extracts, rinsed, mordanted, washed, and dried. This process yielded vibrant, evenly distributed, and durable colors on the cotton fabric. The resulting colors are listed below in **Table No. 12.**

ii. Extracts for dyeing properties on the sheep hair and human hair

Hair samples were cleaned and air-dried for quality. They were dyed in a bath for six hours, rinsed, and air-dried again, resulting in superior, evenly colored hair. The colored dyes obtained are listed and detailed in **Table No. 12**

Table No. 12: Dyeing of cotton cloth with *M. koenigii* extracts.

Extracts	Cotton fabric	Sheep hair	Human Hair
Curry water	Off-white	-	-
Curry ethyl acetate	Dark green	Light green	Green
Curry ethanol	Olive green	Light green	Golden
1% $feso_4$ curry water	Light grey	-	-
1% $feso_4$ curry ethyl acetate	Olive green	Light green	Olive green
1% $feso_4$ curry ethanol	Dark green	Dark green	Green
1% pas curry water	Light yellow	-	-
1% pas curry ethyl acetate	Dark green	Green	Dark green
1% pas curry ethanol	Dark green	Olive green	Black

3.11 Evaluation of Dyes

i. Color fastness by grey scale evaluation

The dyed sheep and human hair were subjected to Color fastness through washing and assessed for color retention using the Grey Scale. The results are tabulated in **Tables No. 13.**

ii. Measurement of color intensity

The dyed sheep and human hair were subjected to the color intensity measured using the CIE method, results are recorded in **Table No. 13.**

iii. Light fading test Using Xenon Arc Lamp 2454.

The dyed sheep and human hair were evaluated with a Xenon Arc Lamp for 24 hours. The results are tabulated in **Table No. 13.**

Table No. 13: Evaluation of dyes by color fastness, color intensity and light fading.

Extracts	Color fastness				Color intensity				Light fading	
	Sheep hair		Human Hair		Sheep hair		Human Hair		Sheep hair	Human Hair
	Day 0	Day 7	Day 0	Day 7	H	CIE DE	H	CIE DE		
Curry ethanol extract	3	2	3	3	-	-	-	-		
1% $feso_4$ curry ethanol extract	4	4	4	4	92.19	0.12	94.62	4.88	5/6	5/6
1% pas curry ethanol extract	4	4	5	4	86.32	12.91	88.62	3.65	5/6	6/6
Curry ethyl acetate extract	3	3	4	3	-	-	-	-	-	-
1% $feso_4$ curry ethyl acetate extract	2	2	4	3	-	-	-	-	-	-
1% pas curry ethyl acetate extract	3	3	4	2	-	-	-	-	-	-

3.12 FORMULATION OF HERBAL HAIR DYE GEL

The method given in Table No.1 was followed to prepare an herbal gel using guar gum, aloe vera, and plant extracts, aiming to create a natural hair dye formulation with beneficial properties which gave a black color in appearance and also upon dyeing on human white hair.

3.13 EVALUATION OF FORMULATED HERBAL HAIR DYE GEL

The herbal hair gel was assessed for appearance, pH, homogeneity, extrudability, spreadability, skin irritation, viscosity, washability, and stability, ensuring product quality and effectiveness under various conditions. The readings and observations have been recorded in **Table No. 14.**

Table No. 14: Assessment metric for formulated herbal hair dye gel.

Parameters	Results
Color	Black
Odor	Characteristic
Appearance	Gel- smooth and consistent
Consistency	Thick
Foreign particles	None
Ph-1%	6.4
Homogeneity	Smooth, homogenous, and no aggregates

Extrudability	Good
Spreadability	6
Washability	Good washability
Open-patch test	Non-irritant
Viscosity	3928cps
Stability	Stable
Color	Black
Odor	Characteristic
Appearance	Gel consistency
Consistency	Thick

3.14 BRINE SHRIMP LETHALITY BIOASSAY

The brine shrimp lethality test uses Artemia salina to evaluate toxic substances. Test samples at varying concentrations are added, and survival rates are assessed to calculate the LC_{50} value.

This was carried out for both the dyes and the herbal hair dye gel formulation and the same is depicted in the given **Table No. 15** for the extracts, mordants, and formulation respectively.

Table No. 15: Toxicity Analysis of Extracts, Mordants, and Formulation Using Brine Shrimp Lethality Bioassay.

Concentration of the extracts	Total no. Of alive shrimps (out of 30)	Inhibition (%)
Control	23	23.3%
Curry ethanol extract		
50 µg/ml	10	56.7%
100 µg/ml	7	80%
150 µg/ml	5	83.3%
Curry ethyl acetate extract		
50 µg/ml	13	56.7%
100 µg/ml	6	80%
150 µg/ml	4	86.7%
1% ferrous sulfate		
50 µg/ml	17	43.3%
100 µg/ml	15	50%
150 µg/ml	13	56.7%
1% potassium aluminium sulfate		
50 µg/ml	21	30%
100 µg/ml	14	53.3%
150 µg/ml	12	60%
Formulation		
50 µg/ml	21	30%
100 µg/ml	18	40%
150 µg/ml	16	46.7%
200 µg/ml	14	53.3%
250 µg/ml	11	63.3%
300 µg/ml	9	70%

3.15 ANIMAL ACTIVITY

Albino Wistar rats were used in a skin irritation study under controlled conditions, applying the formulations via Draize's method. Erythema was scored on a 1-5 scale to assess skin reactions. All groups recorded a score of 1, indicating no reaction, signifying that both dyes were well-tolerated with no irritation or erythema and oedema observed. The scoring has been tabulated in **Table No. 16.**

Table No. 16: Scoring of Skin Irritation via Draize Method for Control, Standard, and Formulated Hair Dye.

Groups	No. of Animals	Results					
		A-1	A-2	A-3	A-4	A-5	A-6
Group-I Normal	6 Animals	1	1	1	1	1	1
Group-II Standard Hair Dye	6 Animals	1	1	1	1	1	1
Group-III Sample Hair Dye	6 Animals	1	1	1	1	1	1

1. Point- No reaction;
2. Points- Slight, patchy erythema;
3. Points- Slight but confluent or moderate but patchy erythema;
4. Points- Moderate erythema;
5. Points- Severe erythema with or without edema.

4. DISCUSSION AND CONCLUSION

The leaves of *Murraya koenigii Linn Spreng* (Rutaceae) were collected from a house garden in Sullia, Karnataka, and authenticated by a qualified expert. Pharmacognostical and physicochemical evaluations, including morphological and powder microscopy, confirmed the plant's genuineness, showing no signs of adulteration. Rheological studies revealed favorable bulk and tapped densities, Carr's index, and Hausner's ratio, indicating good flow properties of the powder. Proximate analysis, as per Indian Pharmacopoeia methods, demonstrated low moisture and ash content, suggesting minimal impurities and high stability. Phytochemical analysis identified alkaloids, flavonoids, phenols, and saponins, with notable anthocyanin and phenolic content contributing to antioxidant and dyeing properties. Thin-layer chromatography confirmed distinct chemical entities in the extracts. UV spectral data further validated the chemical profile. Dyeing studies on cotton and hair showed vibrant, durable colors, with mordants enhancing color intensity and fastness. Toxicity studies using the brine shrimp assay demonstrated the safety of the extracts and formulations. Skin irritation tests on rats confirmed that the herbal hair dye gel is non-irritant and safe for topical use. These findings validate *Murraya koenigii* as a potent, natural, and eco-friendly source for herbal dye formulations, supporting its utility in cosmetic applications while ensuring safety and efficacy.

SUMMARY

This study evaluates *Murraya koenigii* (curry leaves) as a potential natural hair colorant. Extracted using Soxhlet and maceration methods with solvents like ethyl acetate, *M. koenigii* yielded the highest extraction rate (11.6%). Phytochemical screening revealed the presence of phenols, flavonoids, and anthocyanins contributing to its antioxidant and pigmentation properties. Dyeing tests on cotton fabric, sheep hair, and human hair demonstrated that *M. koenigii* extracts produced vibrant and long-lasting colors, especially on hair substrates. The darker shades exhibited higher color fastness, indicating the extract's durability and effectiveness as a natural dye. Additionally, the formulation of an herbal gel containing *M. koenigii* extract maintained a stable pH of 6.4, suitable for hair and scalp applications, minimizing the risk of irritation. Toxicity assessments through the brine shrimp lethality assay (LC_{50} value of 138.95 µg/mL) and skin irritation tests confirmed the safety of the extract and its gel formulation for personal care use, with no adverse reactions observed in animal models.

These findings highlight *M. koenigii* is a promising natural hair dye source, with excellent dyeing properties, high pigment content, and antioxidant potential. Its safe application and environmental benefits position it as a viable alternative to synthetic dyes in the cosmetic industry, contributing to the growing demand for eco-friendly beauty products.

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