Research Article

# The Formulation and Evaluation of Herbal Hair Cream

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**ABSTRACT**

Herbal cosmetics are increasingly recognized globally for their safety, efficacy, and minimal side effects compared to synthetic products. This study formulated and evaluated an herbal hair cream using *Wrightia tinctoria* leaves (oil infusion in coconut oil for antifungal effects), *Hibiscus rosa-sinensis* flowers (warm water mucilage for conditioning), and *Plectranthus amboinicus* leaves (hydro-distillation via Clevenger apparatus for volatile oil). Preliminary phytochemical screening of *Wrightia tinctoria* confirmed alkaloids, flavonoids, glycosides, saponins, tannins, steroids, carbohydrates, and proteins. The cream, prepared with suitable excipients, exhibited excellent organoleptic properties: good consistency, pleasant odour, smooth texture, and scalp-compatible pH (6–7). Physicochemical evaluations showed low acid value, optimal spreadability, easy washability, and stability. Antifungal activity against *Candida albicans* via agar well diffusion demonstrated significant zone inhibition, highlighting potent antifungal potential. HPTLC fingerprinting of the infused oil, mucilage, and volatile oil revealed multiple phytoconstituents for quality assurance. GC-MS analysis identified carvacrol as the dominant compound in *Plectranthus* oil, responsible for antifungal action and fragrance. Overall, the formulation offers a stable, effective, natural alternative for managing fungal scalp conditions while nourishing and conditioning hair.

**Keywords:** *Wrightia tinctoria*, *Hibiscus rosa-sinensis*, *Plectranthus amboinicus*, Anti-fungal effect, High Performance Thin Layer Chromatography (HPTLC), Gas Chromatography-Mass Spectroscopy (GC-MS).

## INTRODUCTION

The global recognition of Indian herbs in herbal cosmetics underscores their timeless value. Since ancient times, beauty practices have been integral to human societies, with communities relying on natural elements for enhancement. Women, in particular, have turned to various herbs in beauty regimens to enhance their allure, drawing in younger generations eager to achieve an attractive look. These herbal skincare items, crafted from plant-based components, provide tangible benefits like healing, calming, revitalization, and conditioning. As gifts from nature, herbal cosmetics are surging in popularity worldwide. A major appeal lies in their formulations, which deliver strong therapeutic effects with minimal side effects compared to synthetic options.

For millennia, herbs and spices have supported human wellness. Generations of Indian women have harnessed these plants to care for their skin, hair, hands, feet, and overall fragrance. In royal Indian courts, diverse botanicals were key to boosting physical appeal and hygiene. Today, enduring herbal products such as face washes, shampoos, conditioners, soaps, and more form part of everyday routines. Herbal cosmetics, by contrast, are products made from natural botanicals that offer physiological perks like beautifying, skin smoothing, appearance improvement, healing promotion, and conditioning thanks to their plant-derived active agents.[1,2,3]

Hair stands as an essential body feature, prone to troubles like shedding, frizz, unruliness, low density, poor conditioning, early greying, dandruff, thinning, dullness, and more. Variations occur in shape, length, thickness, texture, and hue, with cross-sections possibly round, triangular, oval, or flattened—influencing curl patterns. All mammals possess hair, mainly for temperature regulation. It profoundly shapes personal identity and confidence across genders. People universally aspire to lustrous, thick, black, healthy locks, prioritizing their upkeep regardless of length. Hair comprises two main parts: the follicle and the shaft.[4]

Herbal hair cream are semi-solid scalp/hair applications rich in plant actives like extracts and oils, aimed at nourishment, growth stimulation, and tackling scalp issues with few synthetics or side effects. They protect, style, and refine hair texture/appearance via emulsions (oil-in-water or water-in-oil) that spread easily. Often 10-25% water-based, they blend gels with oils or use polymer-stabilized dispersions of fats, esters, and waxes.

Shear-thinning polymers ease application, while oil/wax viscosity dictates greasiness post-drying. Scalp fungal issues like dandruff and seborrheic dermatitis stem from Malassezia or dermatophyte overgrowth, causing itch, flakes, and health decline with cosmetic/psychological tolls. Standard treatments like ketoconazole or azoles work topically but risk irritation, resistance, and side effects, spurring natural alternatives. Plant blends shine with antifungals—phenolics, flavonoids, terpenoids, oils—from neem, garlic, turmeric, aloe vera—disrupting membranes, blocking ergosterol, and halting growth. Studies affirm their prowess against ringworm, athlete's foot, and candidiasis.[4,5,6,7]

**Plant profile**

***Wrightia tinctoria***

*Wrightia tinctoria*, obtained from the dried leaves, bark, and seeds of *Wrightia tinctoria* (Roxb.) R.Br. of the Apocynaceae family, stands as a versatile medicinal plant rich in bioactive compounds including alkaloids, saponins, phenolics, flavonoids, isatin, tryptanthrin, stigmasterol, rutin, tryptophan, and indigotin. Known vernacularly across languages as Sweet Indrajao or Pala indigo plant in English, Dudhi, Mitha-indrajau, or Karayaja in Hindi, Danthappala, Ayyappala, or Kambippala in Malayalam, Vetpalai in Tamil, it belongs to the Plantae kingdom, Gentianales order, Apocynaceae family, Wrightia genus, and tinctoria species, originating from India and Burma.[8,9,10] This petite deciduous tree reaches 5-8 meters tall with smooth scaly bark, elliptic-lanceolate or oblong-lanceolate leaves measuring 6-15 cm long and 3-6 cm wide that taper smoothly or bear slight hairs on young undersides with sharp or rounded bases, 6-12 pairs of primary leaves, and 3-4 mm petioles; its pale aromatic blossoms form loose terminal clusters with small oval bracts, a smooth calyx bearing internal glands and 2.2-5.0 mm oval segments rounded at the apex with thin margins, a brief 2.5-3.0 mm white corolla tube, 6-8 mm elongated dull lobes, linear scales on filaments and lobes, stamens at the tube apex with short expanded filaments and sagittate anthers, a two-carpel ovary with numerous ovules, and 20-40 cm long tube-shaped ridged follicles narrowing at ends with 1.2-2.0 cm seeds topped by a 3.0-3.7 cm deciduous coma.

Widely distributed across Asia, Africa, and Australia—native to Australia, India, Myanmar, Nepal, and Vietnam—it thrives in Western, Central, and Peninsular India within arid, semi-arid, and moist regions across diverse soils.[17,18] Traditionally revered in Karnataka and Tamil Nadu as the "jaundice curative tree" for its leaf juice treating icterus, crushed fresh young leaves packed into decayed teeth or chewed ease dental pain with their strong odor, leaf extracts serve as snake bite antidotes, febrifuges, digestives, and restoratives; Siddha medicine employs it for psoriasis and skin disorders, while bark acts as a tonic, anthelmintic, antidiarrheal, febrifuge for intestinal pain, diarrhea, tuberculosis, dermatitis, hemorrhoids, thirst, digestion, cleansing, antipyretic effects, and edema; seeds function as remedies, digestives, and anti-parasitics known in Unani as "Lisanul-e-Asafir," Inderjao Shireen, or Meetha inderajao, with root and leaf extracts lowering blood pressure.[19,20]

**Figure 1:** *Wrightia tinctoria*

***Plectranthus amboinicus***

*Plectranthus amboinicus*, derived from the fresh or dried leaves of *Plectranthus amboinicus* (Lour.) Spreng. of the Lamiaceae family, boasts chemical constituents such as flavonoids, glycosides, tannins, carvacrol, and thymol.[11,12,13] Called Indian borage in English, Panikoorka in Malayalam, Patharchur in Hindi, Pan ova in Marathi, Doddapatre in Kannada, and Amalkuchi in Bengali, it falls under the Plantae kingdom, Lamiales order, Lamiaceae family, *Plectranthus* genus, and amboinicus species, originating from Southern and Eastern Africa.[21] This aromatic perennial herb thrives in tropical and subtropical zones with thick soft fleshy stems creeping along the ground, opposite broad ovate to circular leaves with jagged edges that are juicy, fuzzy, and emit a potent delightful fragrance from essential oils, surfaces dotted with tiny hairs and prominent veins for a soft feel, diminutive pale purple to lavender bilabiate flowers in terminal or axillary whorls, and primarily vegetative propagation in well-drained warm soils; its hispidly villous or tomentose fleshy stalk spans 30-90 cm, with simple broad oval dense leaves densely haired—glandular hairs thickest below creating a frosted appearance—and a leaf flavor of invigorating pleasant scent prized for medicinal, culinary, and ornamental value due to rich phytochemicals. Cultivated widely in India, Malaysia, various Asian and American countries, and gardens, it flourishes in ancient and modern tropical regions, also known as Cuban oregano, Spanish thyme, Oregano Brujo in Puerto Rico, or Indian basil. Traditionally, it manages headaches, ear pain, anorexia, indigestion, swelling, cramps, diarrhoea, cholera, gum issues, convulsions, asthma, cough, chronic bronchitis, fever, skin problems, flu, colds, liver disease, malaria, and bladder stones.[21,22]

**Figure 2:** *Plectranthus amboinicus*

***Hibiscus rosa-sinensis***

*Hibiscus rosa-sinensis,* obtained from the fresh or dried flowers and leaves of *Hibiscus rosa-sinensis* Linn. of the Malvaceae family, contains flavonoids, tannins, saponins, phenolics, alkaloids, terpenoids, and vitamins. Known as Gurhal or Jasum in Hindi, Chebarathi in Malayalam, Jaswand in Marathi, Shoe flower in English, Joba in Bengali, and Semparutti in Tamil, it belongs to the Plantae kingdom, Malvales order, Malvaceae family, Hibiscus genus, and rosa-sinensis species, originating from the Asian continent.[14,15,16] This persistent evergreen shrub, widespread in tropical and

subtropical areas, grows 1-3 m tall as a short perennial with medium-textured oval lobed glossy dark green leaves 4-6 inches wide and up to 8 inches long, short showy red flowers of 5-5.5 cm; leaves show biconvex transverse sections with cuticularized upper and lower epidermis bearing glandular and non-glandular trichomes over polygonal cells, dorsi-ventral lamina with single-row upper palisade discontinuous at the central rib revealing cortical tissues, large central collateral vascular bundle, and sub-epidermal collenchyma on both surfaces; flowers are large showy axillary on long pedicels with five petals, numerous stamens forming a prominent staminal column around the style, superior penta-locular ovary, typically red but with cultivated varieties in yellow, rose, white, or orange.[23] Likely originating from India with ancient Moors attributing it to Espana and others viewing it as artificial hybrids rather than wild the name derives from Greek "hibiskos" meaning large white or marshmallow-like mallow. Traditionally used as pain relievers, fever reducers for asthma, wounds, diabetes, laxatives, and inflammation suppressors, it exhibits anti-inflammatory, analgesic, antifungal, antioxidant, and antimicrobial properties.[24,25]

**Figure 3:** *Hibiscus rosa-sinensis*

## MATERIALS AND METHODS

### Collection and Authentication

The plants used in the study, *Wrightia tinctoria* (Roxb.) R. Br, *Hibiscus rosa-sinensis* and *Plectranthus amboinicus* (Lour.) Spreng were collected from a local area located in Kottayam district and Idukki district.

The identification and authentication of the medicinal plants were carried out by Dr.Varghese M.C, Head of Department of Botany at Deva Matha College, Kuravilangad.

### Extraction of *Wrightia tinctoria* by oil infusion method

Oil infusion is a traditional extraction technique employed to derive bioactive phytoconstituents from plant materials into a carrier oil, leveraging mild heat, sunlight, or time-mediated diffusion to solubilize lipophilic compounds such as flavonoids, phenolics, essential oils, and terpenoids without the need for sophisticated equipment or harsh solvents.

The oil infusion procedure for *Wrightia tinctoria* involved collecting fresh, healthy leaves, which were washed, air-dried briefly to remove surface moisture, and then finely chopped into small fragments to maximize surface area for efficient extraction.

**Figure 4:** *Wrightia tinctoria* leaves are infused in coconut oil

**Figure 5:** *Wrightia tinctoria* oil

Approximately 35g of chopped leaves were uniformly blended into 80 mL of virgin coconut oil within a clean borosilicate glass beaker to ensure even dispersion of plant material. The beaker was subsequently positioned in direct sunlight for 8 hours daily, with gentle stirring using a sterile glass rod to promote uniform infusion and prevent settling. This solar maceration process was sustained over 21 consecutive days, during which daily visual and olfactory assessments tracked progressive changes, including intensification of the oil's amber hue and development of a characteristic herbal fragrance indicative of active compound transfer. On the 21st day, post-final stirring, the mixture was allowed to settle overnight; the following morning, the infused oil was carefully decanted and filtered through a double-layered muslin cloth to eliminate residual leaf debris, yielding a clear, potent extract. The filtered oil was then transferred to a sterilized, amber-colored airtight glass container to shield it from light and air oxidation, ensuring long-term stability and readiness for incorporation into herbal hair cream formulations.[26]

### Hydro Distillation method for the collection of volatile oil from *Plectranthus amboinicus*

Hydrodistillation is a classical pharmacognostic technique for extracting essential oils from aromatic plant materials, involving submersion of biomass in water, boiling to generate steam that carries volatile lipophilic compounds, condensation of the vapor mixture, and subsequent phase separation to isolate the lighter oil fraction from hydrosol.[27]

Fresh leaves of *Plectranthus amboinicus* (150-200 g) were collected, thoroughly washed under running tap water to remove debris, and chopped into small uniform pieces to enhance surface area for efficient extraction.

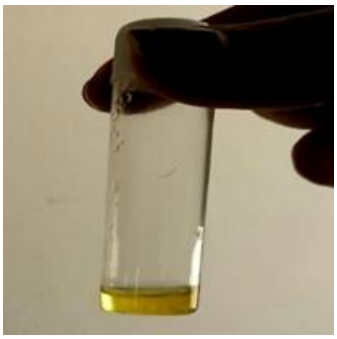

**Figure 6:** *Plectranthus amboinicus* oil

**Figure 7:** Clevenger apparatus

The chopped material was transferred to a 1000 mL round-bottom flask along with 500 mL distilled water, assembled within a standard Clevenger-type hydrodistillation apparatus comprising the boiling flask, a condenser connected via a swivel joint, and an integrated graduated separating trap for oil-water phase collection. The setup was heated on a mantle or electric heater with continuous water flow through the condenser to maintain efficient cooling. Gentle boiling was sustained for 5-6 hours until no further oil droplets appeared in the trap, indicating distillation completion. The oil was carefully drained from the trap. The purified essential oil was then decanted into a pre-sterilized glass vial, sealed airtight, and stored at -4°C in a freezer to preserve volatile constituents, aroma profile, and bioactivity for incorporation into herbal hair cream formulations.[28]

**Isolation of Mucilage from Hibiscus Flower**

Fresh flowers of *Hibiscus rosa-sinensis* were collected and washed thoroughly with distilled water to remove adhering dirt and impurities. The petals were separated manually and cut into small pieces. The fresh petals were then soaked in freshly boiled water and allowed to stand for 1 hours. After soaking, the mixture was gently stirred and filtered through a clean muslin cloth to separate the viscous mucilage extract from the residual petal material. The filtrate obtained was a clear to slightly turbid, viscous mucilaginous solution.[29]

**Figure 8:** *Hibiscus rosa-sinensis* soaked in boiled water

**Figure 9:** *Hibiscus rosa-sinensis* mucilage

**Preliminary phytochemical screening of extracts** [30,31]

**High Performance Thin Layer Chromatography (HPTLC)**

High Performance Thin Layer Chromatography (HPTLC) analysis was carried out to develop an HPTLC fingerprint profile of the given sample using silica gel 60 $F_{254}$ TLC plates and toluene–ethyl acetate as the mobile phase. HPTLC analysis was performed using a CAMAG HPTLC system consisting of CAMAG Linomat V sample applicator, CAMAG Twin Trough Development Chamber (20 × 10 cm), CAMAG TLC Scanner, and WINCATS software for data acquisition and processing. The chromatographic conditions included precoated silica gel 60 $F_{254}$ TLC plates as stationary phase with plate size 7 × 10 cm manufactured by E. Merck KGaA, Darmstadt, Germany.

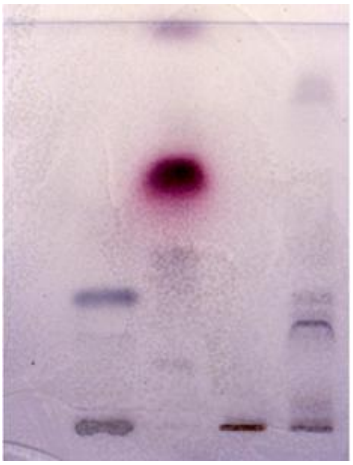

**Figure 10:** Spot detection by HPTLC

**Table 1:** Preliminary phytochemical screening of extracts

| Phytochemical Class | Tests | Procedure | Observation |
|---|---|---|---|
| **Alkaloids** | Dragendorff's test | Add 2-3 drops of Dragendorff's reagent to 2 ml of extract. | Orange-red precipitate. |
| | Mayer's test | Add 2 drops of Mayer's reagent to 2 ml of extract. | White precipitate. |
| | Hager's test | Add 2 drops of Hager's reagent to 2 ml of extract. | Yellow precipitate. |
| | Wagner's test | Add 2 drops of Wagner's reagent to 2 ml of extract. | Brown precipitate. |
| **Carbohydrates** | Molisch's test | Add 2 drops of alcoholic α-naphthol to 2 ml of extract, shake, then add 1 ml concentrated $H_2SO_4$. | Violet colour ring at liquid junction. |
| | Fehling's test | Add 2 ml of filtrate to 2 ml each of Fehling's solutions A and B. | Red precipitate. |
| **Flavonoids** | Aqueous NaOH test | Add aqueous NaOH solution to a few ml of extract. | Yellowish-orange colour. |
| | Lead acetate test | Dissolve extract in 5 ml distilled water + 3 ml 10% lead acetate solution. | Green precipitate. |
| | Shinoda test | Add Mg turnings to extract, followed by dropwise concentrated HCl. | Pink colour. |
| **Glycosides** | Keller-Kiliani test | To 2 ml extract, add 1 ml glacial acetic acid with $FeCl_3$, then concentrated $H_2SO_4$. | Blue colour. |
| **Proteins/ Amino acids** | Ninhydrin test | Add 2 drops of ninhydrin solution to 2 ml of extract. | Dark blue colour. |
| | Biuret test | Add biuret reagent (1% $CuSO_4$ in 10% NaOH) to 2 ml of extract. | Purple color. |
| **Steroids** | Salkowski test | To 2 ml extract, add 2 ml chloroform and equal volume concentrated $H_2SO_4$; shake gently. | Brown ring at junction; violet color in supernatant. |
| **Saponins** | Foam test | Add 1 ml extract to 5 ml distilled water; shake vigorously. | Stable foam for 15 min. |
| **Mucilage** | Ruthenium Red test | Add a few drops of Ruthenium Red solution to 2 ml of extract on a slide. | Pink-red stain or coloration. |

Sample application parameters were sample solvent methanol, application mode band, applicator CAMAG Linomat V, dosage speed 150 nL/s, number of tracks 4, application position 10 mm from bottom edge, band width 8.0 mm, sample volume applied 10 µL per track, and application positions 13 mm, 26 mm, 39 mm, and 52 mm. The mobile phase composition was toluene: ethyl acetate (7.3: 0.7 v/v) with total volume 10 mL.

Chamber saturation and development used twin trough chamber (20 × 10 cm) with chamber saturation time 30 minutes, development technique ascending, and solvent front distance 70 mm. Plate drying after development was oven drying at 60°C for 5 minutes. Post-chromatographic derivatization used vanillin–sulphuric acid reagent by spraying method followed by post-derivatization heating with oven drying at 120°C for 20 minutes. Detection and scanning were conducted in absorbance mode using CAMAG TLC Scanner at detection wavelength 254 nm.[32]

### Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The sample was diluted with hexane, filtered through a nylon syringe filter (13 mm, 0.2 µm), transferred into a vial, and injected into the GC-MS system. GC-MS analysis was performed using an Agilent Technologies 7890 A GC coupled with a 5975C triple axis detector. The column used was DB-5MS (30 m length × 0.250 mm diameter × 0.25 µm film thickness). Analysis involved injecting 2 µL of the sample in split mode (50:1 ratio) with helium gas (99.9995% purity) as the carrier gas at a flow rate of 0.6 mL/min. The analysis was conducted in electron impact (EI) mode with 70 eV ionization energy and the injector temperature maintained constant at 280°C.[12]

### Formulation of herbal hair cream

The formulation involves incorporating bioactive extracts into a hair cream matrix for enhanced scalp bioavailability and consumer appeal. Hair creams combine herbal extracts with food-grade excipients to deliver antioxidants in a luxurious, spreadable form.

- *Wrightia tinctoria* oil: Active ingredient widely used in treating psoriasis, eczema, and other skin diseases.[33,34,35] Prepared by soaking leaves in coconut oil under hot sunlight. Exhibits analgesic, anti-inflammatory, and antipyretic activities.[36,37,38]
- *Hibiscus rosa-sinensis* mucilage: Rich antioxidant source that acts as a natural conditioner, improves hair growth, and provides softness and shine to hair.[39,40]
- *Plectranthus amboinicus* oil: Provides volatile oil with antifungal and antibacterial activity for controlling dandruff and maintaining scalp hygiene. Imparts pleasant fragrance to the formulation .[40,41]
- Glyceryl monostearate: Emulsifying agent and improves formulation consistency.[41,42]
- Glycerin: Humectant that retains moisture in the scalp and prevents dryness .[41,43]

- Sodium benzoate: Preservative that inhibits microbial growth and enhances shelf life of preparations .[42,44]
- Vitamin E: Antioxidant that protects oils from oxidation and improves scalp health.[41,45]

**Table 2:** Formulation of hair cream

| Sl. No | INGREDIENTS | FORMULA 1 | FORMULA 2 | FORMULA 3 |
|---|---|---|---|---|
| 1. | *Wrightia tinctoria* oil | 3g | 3g | 3g |
| 2. | *Hibiscus rosa-sinensis* mucilage | 5g | 5g | 5g |
| 3. | *Plectranthus amboinicus* oil | 2 drops | 2 drops | 2 drops |
| 4. | Glyceryl monostearate | 0.5g | 0.3g | 1g |
| 5. | Glycerin | 0.1g | 0.3g | 0.3g |
| 6. | Sodium benzoate | 0.5g | 0.5g | 0.5g |
| 7. | Vitamin E | 0.2g | 0.2g | 0.2g |

**Procedure**

Preparation of oil phase:

- Oil phase is prepared by adding *Wrightia tinctoria* oil, glyceryl monostearate (GMS) and cetyl alcohol into a clean and dried china dish.
- Heat this mixture in a water bath up to 70°C until it melted.
- Stir gently to obtain a uniform oil phase.

Preparation of aqueous phase:

- Aqueous phase is prepared by adding hibiscus mucilage, glycerin and sodium benzoate into another china dish.
- Heat this mixture in a water bath up to 70°C.
- when both the phases reach the same temperature, slowly transfer the aqueous phase into oil phase with continuous stirring.
- Allow the cream to cool to room temperature and add vitamin E and volatile oil to achieve a smooth uniform cream.
- Transfer the cream into well closed container.[46]

**Anti-fungal Assay**

**Microorganism used:** *Candida albicans*

Antifungal effect of *Wrightia tinctoria* oil sample on the mycelial growth of the fungi *Candida albicans* was carried out using agar well diffusion method. Petri dish was selected with flat bottom which are previously sterilized at 160-170°C for 1 hour. Petri dish was filled up to depth of 4-5mm with PDA (Potato dextrose agar) culture medium and was then solidified and inoculated with *Candida albicans.* A hole of 6mm diameter is punched with sterile work area aseptically. A volume of 100µl/ml concentration of *Wrightia tinctoria* oil and antifungal agent i.e., Clotrimazole (10 mg/ml) was introduced into *Candida albicans* inoculated plate containing agar well. Plate was incubated at 28**°**C for 24 hours. The antifungal effect of *Wrightia tinctoria* oil sample was determined by measuring the diameter of the colonies.[47]

**Evaluation**

Evaluation of herbal hair cream is a crucial step to ensure their quality, safety, efficacy and consumer acceptability, especially in pharmaceutical formulations.

**Determination of Physical Appearance**

Organoleptic properties such as Color, Odor, State, Appearance are done by visual inspection.[48]

**Determination of Acid Value**

Acid value is determined for both olive oil and coconut oil. Exactly 2 g of oil is ethanol (previously neutralized with potassium hydroxide). The solution is then titrated against standardized potassium hydroxide using phenolphthalein as the indicator. The endpoint is the appearance of a pale pink color.[49]

$$\text{Acid Value} = \frac{estimated\ normality \times titre\ value \times 5.61}{2}$$

**Determination of pH**

pH of the cream was measured using pH meter. The cream is mixed with distilled water and the pH. electrode was immersed into the cream.[50]

**Spreadability test**

Exactly 1 g of the sample is placed between two 20 × 20 cm glass plates. A 1000 g weight is then placed on top for 1 minute, after which the diameter of the spread sample is measured.[51]

$$S=\frac{(M \times L)}{t}$$

**Washability test**

To ensure effective application and evaluation, first clean and dry the hair thoroughly to remove any residues or styling products. Divide the hair into sections for even coverage, then gently massage a pea-sized amount of cream

per section from roots to ends, focusing on areas needing conditioning or treatment. After the 4–5-hour dwell time (ideally under controlled conditions like room temperature away from direct sunlight), rinse thoroughly with lukewarm water followed by a mild shampoo if needed. Pat dry and assess hair texture, shine, manageability, and any residue.[52]

### Stability Studies

The sample is stored for 2 months at room temperature and periodically observed for changes in color, odour, and appearance.

## RESULTS AND DISCUSSION

### Phytochemical screening

The preliminary phytochemical screening of extract *Wrightia tinctoria* leaves and *Hibiscus rosa-sinensis* mucilage was performed.

**Table 3:** Chemical test of extracts

| Constituents | *Wrightia tinctoria* extract | *Hibiscus rosa-sinensis* mucilage |
|---|---|---|
| Alkaloids | + | - |
| Carbohydrates | + | - |
| Flavonoids | + | - |
| Glycosides | + | - |
| Protein and amino acids | + | - |
| Steroids | + | - |
| Saponins | + | - |
| Mucilage | - | + |

### High Performance Thin Layer Chromatography

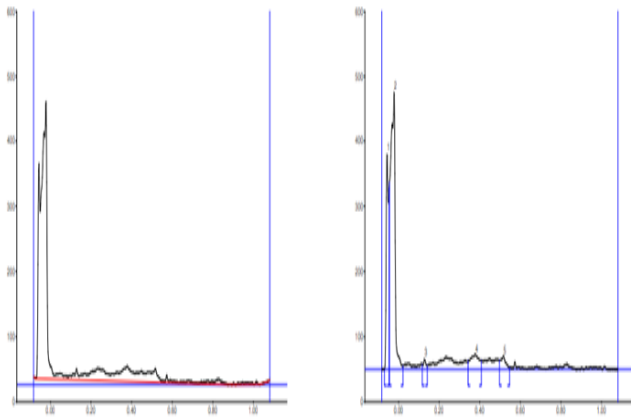

**Figure 11:** HPTLC study of volatile oil *Plectranthus amboinicus*

**Table 4:** HPTLC study of volatile oil *Plectranthus amboinicus*

| Peak | Rf | Area % |
|---|---|---|
| 1 | -0.07 | 26.38 |
| 2 | -0.05 | 62.22 |
| 3 | 0.12 | 1.44 |
| 4 | 0.34 | 6.51 |
| 5 | 0.50 | 3.45 |

**Table 5:** HPTLC study of *Hibiscus rosa -sinensis* mucilage

| Peak | Rf | Area % |
|---|---|---|
| 1 | -0.05 | 4.29 |
| 2 | 0.49 | 41.53 |
| 3 | 0.65 | 14.55 |
| 4 | 0.79 | 39.63 |

**Table 6:** HPTLC study of coconut oil infused *Wrightia tinctoria*

| peak | Rf | Area % |
|---|---|---|
| 1 | -0.07 | 2.64 |
| 2 | -0.04 | 28.88 |
| 3 | 0.05 | 8.58 |
| 4 | 0.17 | 10.00 |
| 5 | 0.47 | 21.22 |
| 6 | 0.78 | 4.69 |
| 7 | 0.81 | 20.72 |
| 8 | 0.91 | 3.27 |

### Gas Chromatography-Mass Spectroscopy analysis

The compounds were identified after comparing the spectral configurations obtained with that of available mass spectral database (NIST -08 SPECTRAL DATA)

**Table 7:** GC-MS study of volatile oil *Plectranthus amboinicus*

| SL NO | RT | MOLECULES | AREA % |
|---|---|---|---|
| 1 | 9.74 | Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro deriv. | 0.13% |
| 2 | 9.93 | 1R-α-Pinene | 0.09% |
| 3 | 11.5 | β-Myrcene | 0.69% |
| 4 | 11.91 | α-Phellandrene | 0.07% |
| 5 | 12.17 | α-Terpinen | 0.63% |
| 6 | 12.4 | o-Cymene | 4.57% |
| 7 | 13.17 | γ-Terpinen | 3.57% |
| 8 | 15.78 | Borneol | 0.11% |
| 9 | 15.92 | p-Menth-1-en-4-ol | 1.32% |
| 10 | 18.45 | Carvacrol | 75.08% |
| 11 | 20.27 | Caryophyllene | 6.81% |
| 12 | 20.88 | α-Caryophyllene | 2.20% |
| 13 | 21.44 | β-Eudesmene | 2.18% |
| 14 | 21.54 | α-Selinene | 0.57% |
| 15 | 22.95 | Caryophyllene oxide | 1.55% |
| 16 | 23.38 | 1,5,5,8-Tetramethyl-12-oxabicyclo[9.1.0]dodeca-3,7-diene | 0.40% |
| 17 | 28.28 | Cinerone | 0.05% |

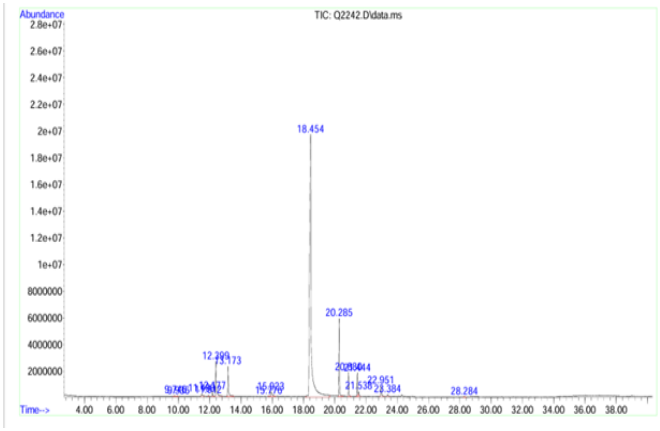


**Figure 12:** GC-MS study of volatile oil *Plectranthus amboinicus*

**Antifungal Assay**

Sample: *Wrightia tinctoria* oil.

Organisms tested: *Candida albicans*

Concentrations used: 100µl

Positive control (PC) used: Clotrimazole(10mg/ml)

Negative control (NC) used: Distilled water

**Table 8:** Antifungal test

| Organism | *Wrightia tinctoria* oil | Positive Control |
|---|---|---|
| *Candida albicans* | 10mm | 18mm |

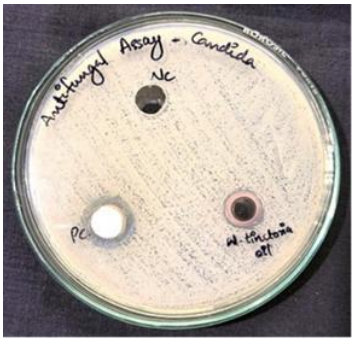


**Figure 13:** Antifungal assay

**Formulation**

The optimal formulation for preparing herbal hair cream was determined through trial and error.

**F1** demonstrated inadequate consistency, compromised stability, discoloration, and structural failure resulting from phase separation.

**F2** exhibited discoloration, an unpleasant odour, and inconsistent texture.

The formulation was adjusted using an emulsifier while maintaining the oil content. **F3** exhibited good appearance and consistent texture across all preparations.

**Evaluation**

**Physical evaluation**

**Table 9:** Physical evaluation

| Property | Inference |
|---|---|
| Colour | Dusty purple-grey |
| Odour | Pleasant odour |
| State | Semisolid |
| Appearance | Smooth texture |

**Determination of Acid value**

The Acid value of the prepared cream was found to be 0.4..

**Table 10:** Acid value

| Oil type | Acid value (mg KOH/g) | Titre value (ml) |
|---|---|---|
| Coconut oil | 0.4 | 1.1 |

**Determination of pH**

The pH of the cream was determined to be in the optimum range of **5-6**, rendering it slightly acidic and closely matching the natural pH of the hair scalp (typically acidic), which promotes scalp health and minimizes irritation.

**Spreadability**

Spreadability of the cream was observed.

**Table 11:** Spreadability

| Sample | Length (cm) | Time taken(s) | Spreadability (gcm/s) |
|---|---|---|---|
| Cream | 5 | 14 | 35.71 |

### Washability

The formulated cream was applied to the skin, followed by an assessment of its ease and extent of washability using water.

### Stability study

No visible changes in colour, odour, or physical state were observed upon 2-month storage at room temperature, confirming the optimized formulation's excellent stability under ambient conditions.

## CONCLUSION

The present study successfully developed and comprehensively evaluated a novel herbal hair cream formulation incorporating *Wrightia tinctoria* oil, *Hibiscus rosa-sinensis* mucilage, and *Plectranthus amboinicus* volatile oil, demonstrating its potential as a safe, effective natural alternative for managing fungal scalp disorders and promoting hair health. Preliminary phytochemical screening confirmed the presence of key bioactive constituents including alkaloids, flavonoids, glycosides, saponins, tannins, steroids, and proteins in *Wrightia tinctoria* extract, while HPTLC fingerprinting established consistent phytoconstituent profiles across extracts, infused oil, mucilage, and the final cream, ensuring quality standardization. GC-MS analysis of *Plectranthus amboinicus* oil identified carvacrol (75.08%) as the predominant antifungal compound, corroborated by significant zone of inhibition against *Candida albicans* in agar well diffusion assays, comparable to standard clotrimazole. The optimized F3 formulation exhibited superior physicochemical properties dusty purple-grey color, pleasant odor, smooth semisolid texture, scalp-compatible pH (5-6), low acid value (0.4 mg KOH/g), excellent spreadability (35.71 g·cm/s), washability, and 2-month stability at room temperature without phase separation or degradation. These findings validate the synergistic therapeutic potential of the selected herbal trio for antifungal activity, conditioning, and scalp nourishment with minimal synthetic excipients, positioning this cream as a promising phytocosmeceutical for commercial development in addressing dandruff, seborrheic dermatitis, and related scalp conditions while meeting consumer demand for natural, side-effect-free hair care solutions.

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