

## FORMULATION AND EVALUATION OF POLYHERBAL ANTI DANDRUFF HAIR GEL

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## ABSTRACT

Dandruff is a prevalent scalp condition that remains challenging to completely eliminate with conventional chemical treatments. In an effort to address this, a polyherbal anti-dandruff hair gel was formulated and systematically evaluated for stability and quality. The study focused on synthesizing an herbal gel using ethanolic extracts of *Zingiber officinale* (ginger) rhizomes and *Moringa oleifera* (moringa) leaves, carefully combined in predetermined proportions with suitable excipients. The formulation was designed to provide effective anti-dandruff treatment with improved safety and minimal side effects compared to synthetic alternatives. The gel was subjected to a comprehensive evaluation, including phytochemical screening to confirm the presence of bioactive compounds, physicochemical characterization to assess consistency, spreadability, pH, and viscosity, as well as antimicrobial and anti-dandruff activity assays. The antidandruff efficacy was assessed against *Malassezia furfur*, the primary causative agent of dandruff. Additionally, antimicrobial studies were conducted to evaluate the gel's activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*, two common pathogenic microorganisms. Stability testing was performed over a 30-day period under controlled conditions to assess the gel's ability to retain its physical properties and therapeutic efficacy. The results indicated that the polyherbal gel exhibited favorable physicochemical properties, maintained its structural integrity, and remained stable throughout the study period. Furthermore, the gel showed significant anti-dandruff activity, with notable inhibition of *Malassezia furfur* growth, alongside promising antimicrobial effects against *K. pneumoniae* and *S. aureus*. In conclusion, the polyherbal anti-dandruff gel demonstrated both effective anti-dandruff and antimicrobial properties, making it a viable natural alternative to conventional treatments. Its stable formulation and beneficial therapeutic properties suggest its potential for further development and use in managing dandruff and associated scalp conditions.

**KEYWORDS:** Polyherbal hair gel, *ZingiberOfficinales*, *Moringa Oleifera*, *Malassezia furfur*, Antidandruff activity, Anti-microbial activity.

## INTRODUCTION

Hair, an integral part of the integumentary system, extends into the dermal layer through hair follicles and is a distinguishing characteristic of mammals. It plays various roles, including sensory perception, protection against cold and UV radiation, and its appearance can have a profound psychological impact. The unique features of hair, such as length, color, diameter, and cross-sectional shape, contribute to the distinct profiles observed among different ethnic groups and individuals.<sup>[1]</sup>

Dandruff is a common scalp condition characterized by flaky and itchy skin. It can affect individuals of all age groups, but is more commonly observed in adults. The main cause of dandruff is a fungal infection caused by a fungus called *Malassezia*. This fungus is naturally present on the scalp and feeds on the oils produced by the hair follicles. The outermost layer of the scalp, known as the epidermal layer, continuously undergoes a

process of renewal. New skin cells are formed in the lower layers of the epidermis and gradually push their way to the surface. In the case of dandruff, this process is accelerated, causing the skin cells to mature and shed off within a shorter time period of 2-7 days, as opposed to the normal cycle of around a month.

Herbal hair gel is a natural and nourishing alternative to conventional hair styling products. Made with carefully selected botanical ingredients, it provides a gentle and effective way to achieve desired hairstyles while promoting the health of your hair. Packed with herbal extracts, hair gel not only holds your hair in place but also delivers nourishment and hydration to each strand. The herbal ingredients work in harmony to strengthen hair, reduce frizz, and add a healthy shine, leaving your locks looking and feeling their best. Free from harsh chemicals and synthetic additives, herbal hair gel is a great choice for those seeking a more eco-friendly and sustainable hair care option. Its delightful aroma and

lightweight texture make styling your hair an enjoyable experience while knowing you are using a product that is gentle on both your hair and the environment.<sup>[2]</sup>

Cold maceration is favoured for its ability to extract a broad spectrum of compounds from plant materials while minimizing the degradation of heat-sensitive constituents. This gentle extraction method helps preserve the plant's natural characteristics, aroma, and therapeutic properties, making it a popular choice for those seeking a more holistic approach to extracting beneficial compounds from plants.<sup>[3]</sup>

## MATERIALS AND METHODS

### Collection of Selected Herbs

The rhizomes of *Zingiber Officinales* and *Moringa Oleifera* leaves was collected from the partly shaded moist tropic regions of punnayarkulam – a village in the district of Thrissur, Kerala. The specimens were authenticated by Dr. M Bheemalingappa Scientist- Bof KSCSTE Forest Botany Department of Kerala Forest Research Institute.

### Preparation of ethanolic extract of selected Herbs

Collected and selected parts of herbs such as *Zingiber Officinales* and *Moringa Oleifera* were washed with distilled water and grinded individually by simple grinding. Then powder was extracted with ethanol and was filtered, centrifuged and used for further studies.<sup>[4]</sup>

### Qualitative phytochemical analysis

The ethanolic extracts of *Zingiber Officinales* and *Moringa Oleifera* were subjected to standard phytochemical screening tests for establishing different constituents present in it.

#### 1. Test for alkaloids

Small amount of EEPA was mixed with few ml of dilute Hydrochloric acid. Shaken well and filtered. Following tests were performed with the obtained filtrate.

- Mayer's test: A few drops of Mayer's reagent (Potassium mercuric iodide solution) were added to 2-3 ml of filtrate. Cream (dull white) precipitate indicates the presence of alkaloids.
- Dragendorff's test: A few drops of Dragendorff's reagent (Potassium bismuth iodide solution) were added to 2-3 ml of filtrate. Orange red precipitate indicates the presence of alkaloids.
- Hager's test: A few drops of Hager's reagent (Picric acid) were added to 2-3 ml of filtrate. Yellow precipitate indicates the presence of alkaloids.
- Wagner's test: A few drops of Wagner's reagent (solution of Iodine in Potassium iodide) were added to 2-3 ml of filtrate. Reddish brown precipitate indicates the presence of alkaloids.

#### 2. Test for carbohydrates

- Molisch's test: Few drops of Molisch's reagent were added to 2-3 ml of filtrate, followed by addition of concentrated Sulphuric acid along the sides of the

test tube. Formation of violet colour at the junction of two liquids indicates the presence of carbohydrates.

- Benedict's test: Few ml of filtrate was mixed with equal volume of Benedict's reagent (alkaline solution containing cupric citrate complex) and heated in boiling water bath for 5 minutes. Formation of reddish-brown precipitate infers the presence of reducing sugars. □ Fehling's test: 1 ml Fehling's-A (Copper sulphate in Distilled water) was added to 1 ml of Fehling's-B (Potassium tartarate and Sodium hydroxide in Distilled water) solution, boiled for one minute. To this added 1 ml of filtrate and heated gently. Formation of brick red precipitate indicates the presence of reducing sugars.

#### 3. Test for steroids and sterol

- Salkowski reaction: A small quantity of the EEPA was mixed with 2 ml Chloroform and 2 ml concentrated Sulphuric acid. Shake it well. Chloroform layer appears red and acid layer shows greenish yellow fluorescence.
- Liebermann- Burchard reaction: A small quantity of the EEPA was mixed with Chloroform. To that mixture added 1-2 ml of acetic anhydride and 2 drops of concentrated Sulphuric acid along the sides of the test tube. The solution becomes red, then blue and finally bluish green colour.

#### 4. Test for saponins

- Froth test: 5 ml of test sample was added to Sodium bicarbonate solution. After vigorous shaking the mixture, kept it for 3 minutes. A honey comb like froth formation indicates the presence of saponins.
- Foam Test: A small quantity of the extract was diluted with 20 ml of distilled water and shaken it in a graduated cylinder for 3 minutes. Foam of 1cm after 10 minutes indicates the presence of saponins.

#### 5. Test for glycosides

- Keller-Killiani test: Glacial acetic acid was added to 2 ml extract, followed by the addition of trace quantity of Ferric chloride and 2 to 3 drops of concentrated Sulphuric acid. Reddish brown colour appears at the junction of two liquid indicates the presence of cardiac glycosides.
- Legal's test: 1 ml of Pyridine and 1 ml of Sodium nitroprusside was added to a small quantity of the extract. Pink to red color indicates the presence of glycosides.
- Baljet test: A small quantity of the extract was added to Sodium picrate solution. Yellow to orange colour formation indicates the presence of glycosides.

#### 6. Test for flavonoids

- Alkaline reagent test: A few drops of Sodium hydroxide solution was supplemented to the extract. Development of an intense yellow color, which turns to

colorless on addition of few drops of dilute Hydrochloric acid, indicates the presence of flavonoids.

#### 7. Test for proteins and aminoacids

- Ninhydrin test: A mixture of 3 ml test solution and 3 drops of 5% Ninhydrin solution was heated in a boiling water bath for 10 minutes. Formation of purple or bluish color indicates the presence of free amino acids.
- Biuret test: 3 ml of test solution was added to 4% Sodium hydroxide and few drops of 1% Copper

sulphate solution. Formation of violet color indicates the presence of proteins.

#### 8. Test for tannins

- Lead acetate test: A few drops of Lead acetate was added to 5 ml of aqueous extract. Formation of yellow or red colour precipitate indicates the presence of tannins.<sup>[5,6,7,8,9]</sup>

### Formulation of polyherbal hair gel<sup>[10,11,12]</sup>

**Table 1: formulation of polyherbal hair gel.**

S.No	Ingredient	Formulation		
		F1	F2	F3
1.	Moringa extract	150 mg	100 mg	100 mg
2.	Ginger extract	100 mg	50 mg	25 mg
3	Carbopol 934	1 gm	1 gm	1 gm
4	Methyl Paraben	0.1 gm	0.1 gm	0.1 gm
5	Propylene glycol	1 ml	1 ml	1 ml
6	Triethanolamine	0.5 ml	0.5 ml	0.5 ml
7	Water	Q. S	Q. S	Q. S

#### Preparation of Polyherbal antidandruff Gel

About 0.4 gm of carbapol 934 is dissolved in 18 ml distilled water. Stir the mixture for 2 hours with the help of magnetic stirrer. After the mentioned time add the drug extract of Moringa (2.67 mg) and Ginger (1.67 mg). Again, stir the mixture with the help of magnetic stirrer until it gets dissolved. 0.1 mg of Methyl paraben is dissolved in water and mixed with 1ml of propylene glycol. Both the mixture is stirred for 20 minutes with the help of magnetic stirrer. Add triethanolamine drop by drop until the gel is obtained.<sup>[13]</sup>

#### Physiochemical evaluation of herbal Anti-dandruff gel

**Clarity:** The clarity of the gel formulations is determined by visual inspection under black and white backgrounds. The clarity is graded as turbid, clear, or very clear based on the appearance of the gel.<sup>[14]</sup>

**pH:** A known amount (2.5 g) of gel is weighed and dispersed in 25 ml of distilled water. The pH of the resulting dispersion is measured using a digital pH meter (Elico pH meter).<sup>[15]</sup>

**Spreadability:** The spreadability of the gel is determined using the parallel plate method. Two glass slides are used for this purpose. One slide is fixed on a wooden block, and 1.0g of the gel sample is placed on it. The second slide is placed on top, and a weight of 1 kg is applied for 5 minutes to remove air bubbles and create a uniform film of the gel. Excess gel is scraped off from the edges. Then, a string is attached to the top slide, and a weight of 20 g is used to pull the top slide, separating it from the bottom slide. The distance covered (7.5 cm) is noted.

The spreadability is calculated using the formula.

Spreadability = Weight tied to upper slide sample\* Length moved in the glass slide / Time taken to separate both slides

**Viscosity:** The viscosity of the gels is determined using a Brookfield viscometer, specifically the Brookfield DV-II + Pro viscometer, equipped with a small sample adapter and spindle number SC4-18/13R. The gel is subjected to a torque ranging from 10 to 100%, and the viscometer measures the resistance to flow, providing information about the gel's viscosity.<sup>[16]</sup>

#### Procedure for Anti-bacterial activity Clinical microbial cultures and culture media

The antimicrobial property of hair gel was examined against clinical *K. pneumoniae*, *S. aureus*. Clinical Microbial cultures were procured from microbiology lab Coimbatore, Tamil Nadu. Muller-Hinton agar media of Himedia Pvt. Bombay, India used for the media for the microbial test. The antibacterial activity evaluated by using the Himedia zone reader.

#### Inoculum preparation

100µl clinical *K. pneumoniae* and *S. aureus* microorganisms were inoculated individually in 5.0 ml of sterile nutrient broth (NB) media, and incubated at 37°C for 24h. 200µl from the organisms' 24 h fresh culture was dispensed into 30ml sterile nutrient broth and set 2-4 h to standardize the bacterial culture to 10<sup>8</sup> CFU/ml (colony forming units).

#### Kirby-Bauer method - well diffusion method

Sample and standard drugs (antibacterial-Ciprofloxacin 200mg/100ml) antimicrobial activity conducted initially using agar well plate method. *K. pneumoniae* and *S. aureus* inoculums prepared using sterile nutrients broth

media. Mueller Hinton agar double strength media were made by autoclaving 0.760 g in 100 ml. Fresh inoculum inoculate on the Mueller Hinton agar plates by using sterile cotton swabs. Agar wells prepared using sterile cork-borer, 101 and 104 (100µg) and Ciprofloxacin 50µl (50µg) were placed agar well using micropipette under aseptic conditions. Agar plates incubated for 30 min at the refrigerator to diffuse the formulation into the agar, and finally, plates incubated at 37°C for 24h. Antibacterial activity evaluated by using the Himedia zone reader.<sup>[17,18]</sup>

#### Procedure for Anti-dandruff activity Antimicrobial activity of hair gel against to clinical microorganism Clinical microbial cultures and culture media

The antimicrobial property of the sample examined against clinical *Malassezia furfur*. Clinical Microbial cultures were procured from microbiology lab Coimbatore, Tamil Nadu. Potato dextrose agar media of Himedia Pvt. Bombay, India used for the media for the microbial test. The antibacterial activity evaluated by using the Himedia zone reader.

### RESULT AND DISCUSSION

**Table 2: Extractive Yield of ethanolic extracts of ginger and moringa.**

Name of plant	Extract	Method of extraction	Physical nature	Yield% w/w
Ginger	Ethanol	Cold maceration	Solid	2.67
Moringa	Ethanol	Cold maceration	solid	1.47

#### Determination of Extractive yield Moringa

Weight of empty beaker= 101.36 gm  
 Weight of extract + beaker = 104.03 gm  
 weight of the extract (Moringa) = 104.03 gm - 101.36 gm  
 = 2.67 gm

#### □ Ginger

Weight of empty beaker= 101.36 gm  
 Weight of extract + beaker = 102.83 gm  
 weight of the extract (Ginger) = 102.83 gm - 101.36 gm  
 = 1.47 gm

□ **Extractive value of Moringa:**  $\frac{\text{Weight of extract} \times 100}{\text{Weight of sample}}$

=  $\frac{2.67}{50} \times 100$   
 = 5.34 %

#### Inoculum preparation

100µl clinical *Malassezia furfur* microorganisms were inoculated individually in 5.0 ml of sterile potato dextrose broth (PDB) media, and incubated at 37°C for 24h. 200µl from the organisms' 24 h fresh culture was dispensed into 30ml sterile nutrient broth and set 2-4 h to standardize the bacterial culture to 10<sup>8</sup> CFU/ml (colony forming units).

#### Kirby-Bauer method - well diffusion method

Sample and standard drugs (antibacterial-Fluconazole 2mg/ml) antimicrobial activity conducted initially using agar well plate method. *Malassezia furfur* inoculums prepared using sterile PDB. PDB media were made by autoclaving 0.760 g in 100 ml. Fresh inoculum inoculate on the PDB plates by using sterile cotton swabs. Agar wells prepared using sterile cork-borer, sample and fluconazole 10µl (10µg) were placed agar well using micropipette under aseptic conditions. Agar plates incubated for 30 min at the refrigerator to diffuse the formulation into the agar, and finally, plates incubated at 37°C for 24h. Antidandruff activity evaluated by using the Himedia zone reader.<sup>[19,20]</sup>

□ **Extractive value of Ginger:**  $\frac{\text{Weight of extract} \times 100}{\text{Weight of sample}}$   
 =  $\frac{1.47}{50} \times 100$   
 = 2.94 %

#### Phytochemical studies

The phytochemical studies of *Moringa oleifera*, *Zingiber officinale* done. The presence and absence of Phytoconstituents in the ethanolic extract of the above sample was shown in the table.

**Table 3: Inference of Phytochemical Test.**

SI No	Phytoconstituents	Ethanolic extract	
		<i>Moringa oleifera</i>	<i>Zingiber officinale</i>
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Steroids	+	-
4	Tannins	+	+
5	Glycosides	+	+
6	Flavonoids	+	+
7	Amino acids	+	+

### Characterization of poly herbal gel formulations

Table 4: Characterization of poly herbal gel formulations.

Formulations	Clarity	pH	Homogeneity	Spreadability (g.cm/sec)	Viscosity (cps)
F1	Turbid	6.2	Not Good	4.4	14400
F2	Turbid	6.5	Not Good	4.7	27000
F3	Clear	7.3	Good	6.8	25620

#### Clarity test

The clarity test of poly herbal antidandruff gel was done by clarity test apparatus. The result shows that gel was clear. Formulation 3 is clearer than others.

#### Spreadability

The spreadability test was done using parallel plate method. The result shows formulation 3 pass the test and having spreadability value 6.8.

#### pH Test

The pH of the optimized formulation f3 shows 7.3, which is suitable for antidandruff gel. pH of the gel was identified by digital pH meter (Elico pHmeter).

#### Optimized formula of polyherbal Antidandruff Gel<sup>[49]</sup>

Table 5: formula for optimized polyherbal hair gel (F3).

S No	Ingredients	F3
1	<i>Moringa oleifera</i> extract	100mg
2	<i>Zingiber officinale</i> extract	25mg
3	Carbapol 934	1g
4	Methyl paraben	0.1g
5	Propylene glycol	1ml
6	Triethanolamine	q.s



Fig 1: Optimized polyherbal antidandruff hair gel (F3).

#### Screening of Antimicrobial activity of Gel Formulation For Bacteria

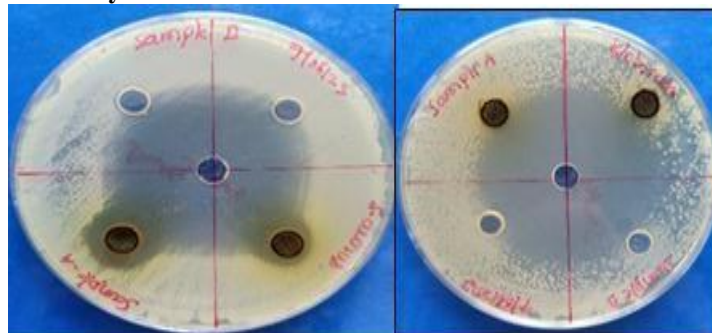


Fig 2: Antimicrobial activity of optimized Gel Formulation on *K. pneumoniae* and *S. aureus*.

**Table 6: Antimicrobial activity of optimized Gel Formulation (F3) on *K. pneumoniae* and *S.***

S.NO	Test organism	Zone of Inhibition (mm) n=2	
		<i>K. pneumoniae</i>	<i>S. aureus</i>
1	F3	R	14
3	CIP	32	30

The antimicrobial property of the sample examined against clinical *K. pneumoniae*, *S. aureus*. Sample and standard drugs (antibacterial-Ciprofloxacin 200mg/100ml) antimicrobial activity conducted initially

using agar well plate method. *K. pneumoniae* and *S.aureus* inoculums prepared using sterile nutrients broth media. The result shows gel formulation have antimicrobial activity.

**Fig 3. Antidandruff activity of optimized Gel Formulation (F3) against clinical microorganism (*Malassezia furfur*).**

S.NO	Test organism	Zone of Inhibition (mm) n=2
		<i>Malassezia furfur</i>
2	F3	10
3	Fluconazole	20

Sample(F3) and standard drugs (antifungal-Fluconazole 2mg/ml) antifungal activity conducted initially using agar well plate method. *Malassezia furfur* inoculums prepared using sterile PDB. Agar plates incubated for 30 min at the refrigerator to diffuse the formulation into the agar, and finally, plates incubated at 37°C for 24h. Antidandruff activity evaluated by using the Himedia zone reader. It shows gel having antidandruff activity.

In conclusion, the formulation F3 of the Herbal Antidandruff gel showed positive results in terms of its physicochemical properties, presence of bioactive compounds, antimicrobial activity, antidandruff activity and stability. These findings indicate that formulation F3 has the potential to be an effective antidandruff product. These findings will provide more evidence regarding the effectiveness and safety profile of the F3 formulation, ensuring its suitability for use in managing dandruff.

## CONCLUSION

Three batches of Polyherbal Antidandruff gel (formulations F1, F2, and F3) were formulated and subjected to physicochemical evaluations, including clarity, pH, spreadability, and viscosity. Based on these evaluations, formulation F3 was selected as the optimized gel formulation.

The ethanolic extracts of *Zingiber Officinale* (ginger) and *Moringa Oleifera* were found to be rich in bioactive compounds through phytochemical screening. The antimicrobial screening results indicating significant inhibition of fungal and bacterial growth in the tested samples for formulation F3 are promising. This suggests that the gel has potential antimicrobial activity, which can be beneficial in combating the microorganisms associated with dandruff, such as *Malassezia furfur* fungi. The ability to inhibit the growth of these microorganisms is important in the management of dandruff and maintaining a healthy scalp. Further investigations and studies can help elucidate the specific mechanisms and effectiveness of the antimicrobial activity of formulation F3, providing valuable insights into its potential as an antidandruff product. The selected formulation F3 underwent stability studies according to ICH guidelines. The gel was subjected to storage at room temperature and refrigeration temperature for one month. The results indicated that the formulation remained stable throughout the study period without any significant physical changes.

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