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ORIGINAL ARTICLE

Mexican Mint (*Coleus amboinicus*) Emulgel: Natural Solution for Antidandruff Hair Care

Sakshi Shetty[1,*], Sonali Naik[2], Likhitha Suvarna[1]

[1] *Bachelor of Pharmacy, Mumbai Educational Trust's Institute of Pharmacy, Mumbai, Maharashtra, India*
[2] *Associate Professor, Mumbai Educational Trust's Institute of Pharmacy, Mumbai, Maharashtra, India*

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* *Corresponding author.*
Sakshi Shetty
sakshivijayashetty@gmail.com

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ABSTRACT

Today, the world is developing more and more in all sectors at a faster pace. But with all these developments, people are more prone to falling ill due to factors such as pollution, unhealthy lifestyles, busy work schedules, etc. Nowadays, even children suffer from the issue of dandruff and scalp infections which in later teenage years leads to unhealthy scalp and hair fall problems. Dandruff is a common scalp condition that affects individuals of all ages. It not only causes discomfort but also undermines hair health and aesthetics. Although scalp disorders are not among those that cause severe physical illness or morbidity yet are of great social concern. Scalp and hair conditions have more of a psychological impact on societies. Even minor changes in hair like graying of hair, affects the self-confidence and self-esteem of an individual. There are many types of medications and formulations to treat hair dandruff issues, one of which includes a novel drug delivery system such as "Emulgel" in the form of a mask. The anti-dandruff hair mask offers multiple benefits, including reduction in scalp inflammation and effective management of hair dandruff problems. Furthermore, it rejuvenates the scalp and nourishes hair follicles due to its natural vitamins and nutrient content.

Keywords: Dandruff; Pityriasis capitis; Seborrheic dermatitis; Mexican mint; Herbal hair mask; Hair mask

INTRODUCTION [1–4]

Dandruff or *Pityriasis capitis* is regarded as a mild non-inflammatory form of seborrheic dermatitis. The cause of seborrheic dermatitis is not well understood but appears to be related to the composition of the sebaceous gland secretions, the proliferation of *Malessezia* yeasts, and the host immune response. Excessive drying of skin and over-activity of oil glands is known as seborrhea.

Seborrheic dermatitis is a chronic, recurring, cutaneous condition that causes erythema and flaking, sometimes appearing as macules or plaques with dry white or moist oily scales as well as inflammation of the infected area. In adults, it commonly occurs in areas with high concentrations of sebaceous glands. The face and scalp are the most frequently affected areas, and involvement of multiple sites is common [4].

Types of Dandruff [4]

Dandruff can be classified as disorders of the sebaceous gland or skin scaling disorders.

Dandruff can be of two types, they are:

1. Oily dandruff (*Pityriasis steatoides*)
2. Dry dandruff (*Pityriasis sicca*)

Dandruff may have several causes, including [4]

- Irritated, oily skin
- Sensitivity to hair care products (contact dermatitis)
- Other skin conditions, such as psoriasis and eczema
- Poor hygiene
- Seasonal changes (the condition may worsen in the winter leading to dry skin)
- Age- Dandruff usually begins in young adulthood and continues through middle age. That doesn't mean older adults don't get dandruff. For some people, the problem can be lifelong.
- *Malessezia furfur* feeds on natural scalp oils called sebum - this oil is what keeps hair and scalp moisturized. As the sebum is broken down, it produces

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a by-product called oleic acid. 1 in 2 people are sensitive to oleic acid and the scalp responds by becoming irritated. In response to the irritation, the scalp starts to become inflamed, red and itchy.

- Other factors including[5]:

1. **Heat** - As temperatures rise, the dandruff-causing microbe *Malessezia furfur* is more likely to thrive, raising the prospect of a dandruff flare-up.
2. **Sweat**- This can also provide ideal conditions for *Malessezia furfur* to thrive, raising the prospect of a flare-up.
3. **Pollution**- In warmer weather we tend to be outside more, and this puts us in contact with more pollutants which can damage the scalp's natural defenses. With weakened defenses, there may be more at risk of dandruff.

Signs and Symptoms may include [4]

- Skin flakes on your scalp, hair, eyebrows, beard or mustache, and shoulders.
- Itchy scalp.
- Red and greasy patches of skin.
- Tingly feeling on the skin.

Mechanism causing Dandruff by Malessezia species [1]

The fungi *Malessezia* can lead to dandruff by either or both of the following mechanisms-

- *Malessezia* stimulates the enzyme called Lipase on the scalp. The enzyme causes oxidation of triglycerides of sebum to produce unsaturated and saturated fatty acids. Saturated fatty acids are consumed by the fungi for self-proliferation and growth. Unsaturated fatty acids include oleic acid and aracidonic acid. Oleic acid is an irritant for human skin while aracidonic acid is involved in potentiating the inflammatory responses. Hence the result of the degradation of the fatty acids is scalp skin irritation, inflammation, and fungal growth. These further cause dry flakes called dandruff. Supplementary **Fig. S2** represents this mechanism.
- The other mechanism by which these fungi cause dandruff is altering the normal shedding of dead skin cells. Few enzymes on the scalp eat up connections between dead skin cells to slough them individually. *Malessezia* modifies this function of enzymes and inhibits cutting of the connections. This leads to aggregation of corneocytes that shed off in clusters, leading to visible white flakes. This mechanism has been explained in Supplementary **Fig S3**.

Rationale behind the Formulation

Dandruff is an overall scalp disorder/disease. The treatment of dandruff includes application of topical, antifungal, or other products. Some of the courses of treatment using therapeutic mechanisms include the following in the diagram (Figure 1).[6]

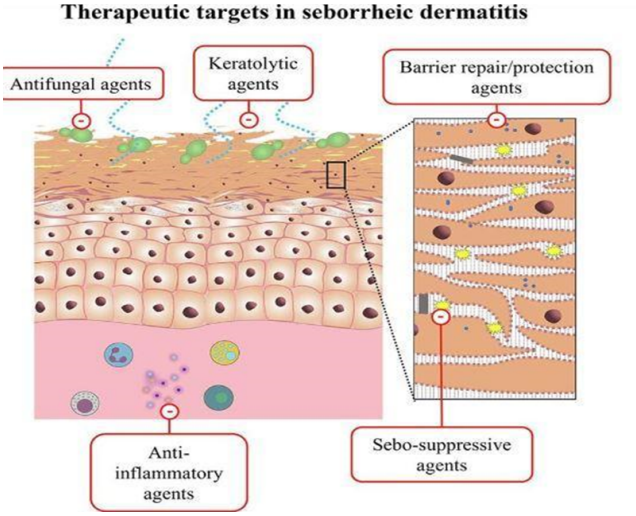


Fig. 1: Rationale for hair dandruff problems [6]

1. **Antifungal agents** which kill and reduce the growth of the species
2. **Anti-inflammatory agents** which reduce the inflamed scalp caused due to dandruff.
3. **A Keratolytic agent** which clears the oil plugs caused on the uppermost layer of the scalp and breaks the flakes thereby reducing the dandruff appearance.
4. **Sebo suppressive agents** which suppress over production of sebum from sebaceous glands and reduce their accumulation on the scalp.
5. **Barrier repair/Protection Agents** which forms a layer over the stratum corneum layer of the scalp in order to avoid dryness of the scalp.
6. **Combined therapy** which can be a combination of one or more of the above mechanisms.

Let us look at the target area of treatment by analyzing the main cause of dandruff and how to treat them-

- Our target area is the infected scalp of a person who suffers from the issue of dandruff.
- The fungi - *Malessezia* can lead to dandruff by either or both of the above mechanisms- either by increased secretions of lipase or by unwanted flakes formation.

Since there are enough synthetic anti dandruff products in the market such as salicylic acid, ketoconazole, coal tar and many more of them, one of the drawbacks of these synthetic products is that they have some side effects after prolonged use. So, in order to reduce these side effects, we as researchers are trying to find out natural alternatives that can help treat dandruff naturally.

Our aim is to prepare a medicated hair care product which could be easily applicable as well as it can be used on daily purpose even in their busy schedule. The idea of formulating the herbal anti-dandruff hair mask is to soothe the irritating, oily and flaky scalp by controlling dandruff thus giving you itchy free healthy scalp and nourished hair. So, in order to increase the contact time of the product with customers' usage, we also need to focus on the formulation of the product which will deliver the therapeutic effect even after less amount of contact time. The product should also be able to release the drug into the system efficiently in a short period of time as well. Our route of administration is topically or through the skin surface. So, we can use one of the aspects of formulating the product which includes the above criteria i.e. **an Emulgel.**

Preparation of an ideal Emulgel is illustrated in the Supplementary **Fig S4**.

Properties of an ideal Emulgel[5]

- Pleasing Appearance.
- Ease of Application & Easily Removable.
- Non-greasy feeling.

Herbs such as Moringa leaves (*Moringa oleifera*), Mexican mint leaves (*Coleus amboinicus*) and Hibiscus leaves (*Hibiscus rosa-sinensis*) were chosen carefully for testing their antifungal properties against dandruff-causing species[7–10].

The following three herbs were firstly extracted with various solvents such as DW, Ethanol, and results showed that most of the yield was extracted better with water compared to ethanol extracts. But ethanolic extracts were preferred over aqueous extract due to its enhanced activity.

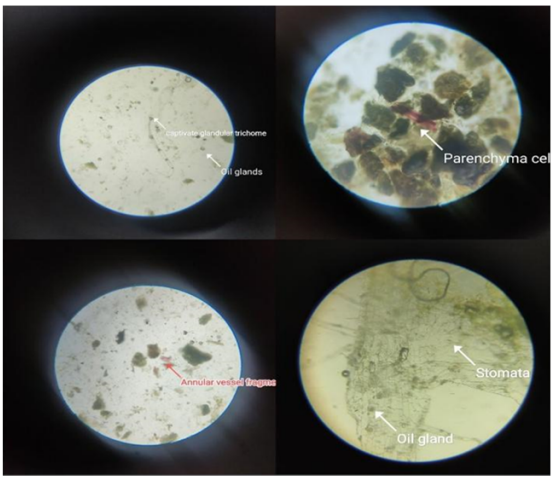
Later, an antifungal bioassay was performed using these extracts on culture *Malessezia* species to see whether the herbal extracts were able to inhibit the growth of organisms individually as well as combined where Mexican mint was found to be most effective against the species. So, Mexican Mint was further considered for future studies.

Plant Profile

Fig. 2: Mexican mint leaves [11]

1) Mexican Mint[12–17]

- **Synonyms:** Indian borage, French thyme, Indian mint, Cuban oregano, Ova paan
- **Biological Name:** *Plectranthus amboinicus* / *Coleus amboinicus*, belonging to the family Lamiaceae.
- **Geographical Source:** Native parts of Africa, the Arabian Peninsula, and India.
- **Description:** Mexican mint is a member of the mint family and has similar leaf-shape. It is semi-succulent, and thus its leaves and stems are fleshy. The plant appears almost entirely green, though some varieties have leaves with white edges.
- **Chemical Constituents:** Thymol[13], Carvacrol, α-Humulene, γ-terpinene, α-terpineol, 1, 8-cineole, eugenol, α-pinene, β-phellandrene, p-cymene, chlorogenic acid, DPPH, caffeic acid.
- **Uses:** Fragrance and flavorant, Anti-inflammatory, Antimicrobial properties.
- **Microscopic features**[15] **:**



Slide (a) shows captivative glandular trichome and oil glands, slide (b) shows parenchyma cells, slide (c) shows annular vessel fragments and slide (d) shows stomata.

Fig. 3: Microscopic features of Mexican Mint

2) Pumpkin seed oil[18]

- **Synonyms:** Pepita, little seed of squash, Semillas de Calabaza, Nano Verde Di Milano.
- **Biological Name**: *Cucurbita pepo*, belonging to the family Cucurbitaceae.
- **Geographical Source**: Native to the Americas (originating from northeastern Mexico and Texas).
- **Source of Collection**: Choose a mature fruit, extract the seeds, clean the seeds, dry the seeds, and store the seeds.

Fig. 4: Pumpkin seeds[19]

- **Chemical Constituents**: Cholesterol, Cholestanol, 24-Methylenecholesterol, Stigmasterol, 7Δ-Avenasterol, α-Tocopherol, γ-Tocopherol, Sitosterol, Δ5, 24-Stigmastadienol (24R)-24-Ethylcholesta-7, 25 (27)-dien-3β-ol.
- **Physical Identification:** The seeds are typically flat and oval with one axis of symmetry, have a white outer husk, and are light green in color after the husk is removed.
- **Uses:** It also promotes hair growth and strengthens the hair shaft and prevents breakage and provides nourishment to the hair.

Formulation of Emulgel

- **Materials used**

Pumpkin seed oil, Tween 80, Stearic acid, Mexican mint extract, Glycerine, Citric acid, Carbopol 940, Triethanolamine, Distilled water and Perfumery agents.

- **Preparation of Plant extract**

The leaves were collected from nearby surrounding garden areas with optimum climate conditions and dried under artificial drying conditions using a hot air oven at 70 degrees for 7.5 hours[13]. Then the dried leaves were powdered in a grinder and later a weighted quantity of powder was mixed along with ethanol (say 10 grams/100ml), and it was extracted using condenser for 8-10 hours. The extract was later collected by filtering the filtrate and drying it to consistent extract using a water bath.

- **Preparation of Emulgel**

1. Mix Pumpkin seed oil, Tween 80 & Stearic acid in a beaker while heating it in a water bath at 70°C. Add the extract solution to the oil phase.
2. In another beaker, mix all the water phase ingredients except citric acid & keep stirring continuously to avoid any lump formation. Maintain the water bath temperature at 70°C using a thermometer. Soak Carbopol 940 in little amounts of water and keep it for a while before adding it into formulation.
3. Now, slowly add water phase into the oil phase after cooling them down a bit but warm enough to form emulsion. Adjust the pH of the solution using citric acid and measure it using a digital pH meter. After adjustment, add presoaked Carbopol into the mixture and keep stirring using a magnetic stirrer machine.
4. Add Triethanolamine drop wise till it forms a proper consistent Emulgel mask. Add fragrance to the product and fill it in an airtight container bottle.

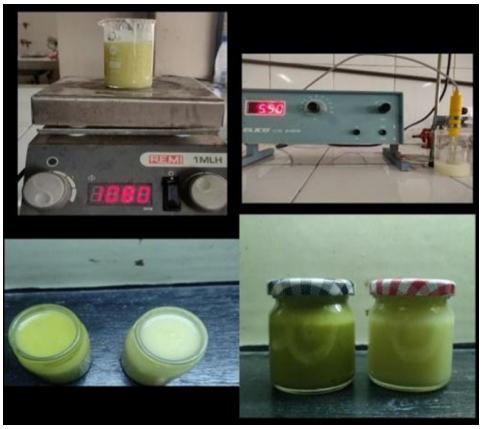


Fig. 5: Formulation of Herbal Anti Dandruff hair mask

Table 1: Formulation of Emulgel[20]

Sr. No	Name of Ingredient	Quantity Taken	Role of Ingredient
	A) Oil Phase	-	-
1	Pumpkin seed oil	20 ml	Oily base
2	Tween 80	30 ml	Non-ionic surfactant
3	Stearic Acid	7 gm	Stiffening agent, provides Pearlescent shine to product
4	Plant extract	0.5gm/1ml alcohol	API
	B) Water Phase	-	-
5	Glycerine	30 ml	Humectant
6	Citric acid	q.s to pH 5.5	pH adjuster
7	Carbopol 940	1 gm	Gelling agent
8	Triethanolamine	q.s	Thickening agent, viscosity builder
9	Distilled Water	19 ml	Vehicle
	C) Additives		
10	Bergamot citrus + Jasmine oil	q.s	Fragrance

MATERIALS AND METHODOLOGY

Preformulation Test

1. Extractive values[21]

Extractive values were performed in order to know which extract would give most of the plant soluble extraction in that particular solvent. So, about 50 ml of solvents like DW & Ethanol and 2.5 gm of dried herb extract were taken in a conical flask and continuous stirring by magnetic stirrer was provided for 6 hours and left for maceration for 18 hours. Then these solutions were filtered out and 12.5 ml of the respective filtrate was transferred into a pre weighed porcelain dish and the solvents were evaporated in a water bath according to the required temperatures. After evaporation, these extracts were placed in a desiccator and readings were taken until constant weights were achieved. Following formula was applied for extractive values:

$$\frac{\%\ of\ solvent}{soluble\ extract} = \frac{Weight\ of\ residue}{Weight\ of\ Herb} \times 100$$

2. Antifungal Bioassay[8–10]

Well Diffusion method was used wherein a mixture of Brain Heart Infusion broth (BHI) & Sabouraud Dextrose Agar (SDA) media were poured into sterile plates and solidified, the cultures were inoculated using swab & wells were created using cork borer. Into these wells, the standard & test samples were added using micropipettes & the plates were incubated for 24 hours outside the incubator and results were noted.

In order to conduct this assay, primary identification and determination of species *Malessezia furfur* (MTCC NO 1374) were done using the gram staining method which is shown in Supplementary **Fig S5** and Supplementary **Table S1**.

This assay method was performed in order to conclude the activity of selected herbal extracts against *Malessezia furfur* individually and combined.

- **Standard drug** - Ketoconazole.
- **Test substance** - (a) Individually- Moringa, Hibiscus, Mexican mint; (b) Combined - Mexican mint + Moringa, Hibiscus + Moringa, Mexican mint + Hibiscus and Combination of all the three.
- **Materials:** Saline solution (0.9% w/v), earbuds for swabbing, nichrome loop, plugged test tubes, Petri plates, micropipette tips, beaker, nonabsorbent cotton, conical flask, test and standard solution, solvents like DW and alcohol, disinfectant, test inoculum of *Malessezia furfur*.
- **Procedure:**

1. Sterilize all the required materials in autoclave at 121°C for 20 minutes after pressure of 15 psi is reached.
2. Create the sterile working field by cleaning the area with disinfectant and light the burners such that all the work takes place between the burners.
3. Pour the media into sterilized plates and wait for it to solidify.
4. Pour the sterilized saline into the sterile test tube & subculture the species/culture in saline. Make sure to compare and adjust its turbidity by comparison with Mc Farland tube no 1. Similarly, prepare test solutions in sterile test tubes only using solvent which also will be negative control.
5. Now, inoculate the prepared culture on the plate using sterile buds. After 5-10 minutes, create wells using cork borer & pour the standard and test solutions into their respective wells using micropipette.
6. Place these plates in a sterilized area for incubation. After 24 hours, note down the zone diameter and compare its efficacy.

3. Absorption Maxima (λ_{max})

The absorption maxima were performed to determine the optimum wavelength at which maximum energy or light is absorbed by the active constituents.

For determination of the spectra, PBS solution of pH 7.4 was prepared according to the method described in I.P 2022 and pH was adjusted to 7.4 using digital pH meter before any quantitative estimation[22]. Similarly, another solution of PBS was prepared using the above procedure and adjusted to pH 5.5[22]. This step is mandatory as we will determine in-vitro drug diffusion studies under pH 7.4 and 5.5 which are pH values for blood and scalp respectively. When the pH of the solution changes, there is ionization in some of the molecules of the solution, thus the structure of the molecule changes, which will affect the determination of absorbance. Hence absorption maxima will be measured in two different pH systems[23].

Mexican mint extract of accurately weighed amount (100mg) were dissolved in ethanol and then made up to 10 ml with PBS of each pH. Each ml of the stock solution contains 10 mg of extract containing active constituents (primary stock solution). From this primary stock solution, 1 ml was withdrawn and made up to 10 ml with PBS of each pH (secondary stock solution) was taken in standard cuvette and scanned in the range of 200-700 nm in a UV spectrophotometer. Therefore, further all measurements were taken according to requirements of the test.

4. Standard curve for drug diffusion studies and quantitative tests

A) For Drug Diffusion studies and drug content - Mexican mint of accurately weighed amount of extract (0.5 gm) was dissolved in 1 ml ethanol which becomes the primary stock solution. From this primary stock solution, concentrations of 30, 60, 90, 120 and 150 mg were prepared and made up to 1 ml with PBS of each pH (secondary

stock solution). From this stock solution, further 100 times dilution was done such as each test tube contains 10 ml for reading and was scanned at 672 and 677 nm in a Ultraviolet-Visible (UV) spectrophotometer.

B) For Phenol Content[24] - A set of standard solutions of gallic acid (20, 40, 40, 60, 80 and 100 μg/ml) were prepared from a stock solution of 0.01 gm/10 ml with each ml containing 10 mg of GAE. The Folin-Ciocalteu assay method was used for the determination of the total phenol content. The reaction mixture consists of 1 ml of prepared reading and 9 ml of distilled water taken in a volumetric flask (25ml). One milliliter of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. Incubated for 90 min at room temperature and 10 times dilutions were prepared. Record the standard solutions against the reagent blank at 550 nm with an Ultraviolet-Visible (UV) spectrophotometer.

C) For Alkaloid Content[24] - A set of reference standard solutions of atropine (20, 40, 60, 80 and 100 μg/ml) were prepared from a stock solution of 0.01 gm/10 ml with each ml containing 10 mg of atropine. 5 ml of bromocresol green solution and 5 ml of phosphate buffer (pH 4.7) were added in the above prepared solutions and were transferred to a separating funnel and yellowish chloroform layer collected in a 10-ml volumetric flask and diluted to the volume with chloroform. Further, 10-time dilution was done. The absorbance was determined against the blank reagent at 470 nm with an UV-Visible spectrophotometer.

5. TLC/Thin Layer chromatography

Thin layer chromatography (TLC) is an affinity-based method used to separate compounds in a mixture. So, following procedures were performed.

- **Stationary Phase:** TLC Plates Silica gel 60 F 254
- **Mobile Phase:** n-Butanol: Acetic acid: Water (10:1:1 v/v/v)
- **Saturation Time:** 20 min

Mobile phases were prepared according to the requirements and kept in the saturation chamber (beaker/measuring cylinder) for saturation. Meanwhile, spots of respective samples were spotted onto the stationary phase and left for a minute to dry off. After which, these plates were carefully inserted in the chamber and left for development of spots. Further derivatization of these spots was done using iodine vapors. Spots were visualized in TLC UV chamber and calculation of Retardation factor was done by using the formula-

$$Retardation\ factor = \frac{Distance\ travelled\ by\ the\ compound}{Distance\ travelled\ by\ the\ solvent\ front}$$

6. Chemical tests (Qualitative Analysis)

The alcoholic extract of Mexican mint was subjected to following preliminary phytochemical analysis:

Table 2: Chemical Test of Mexican mint [14,15]

Sr No	Experiment	Observation	Inference
1	Bromine water-sample + Br water	Brown to whitish buff precipitate	Acetyl Groups present
2	Phthalein Dye test-Sample + heat with phthalic anhydride + conc HCl	Colorless condensation which becomes pink for few seconds when dil NaOH is added	Phenols present
3	Ferric Chloride test- sample+ few drops of 5% $FeCl_3$	Blue, green, or violet color	Tannins present
4	Libermann buccards test-sample + acetic anhydride heat and cool the solution. Add 1 ml Conc H_2SO_4	Pink color or violet color	Steroids or triterpenoids present
5	Salkowski Test-Few drops of Conc H_2SO_4 to sample. Shake and allow standing without disturbing.	Reddish to brown layer formation	Terpenoids Present

7. Quantitative Analysis[24]

This analysis is done to quantify the presence of chemical constituents in the herbal extract. The procedure for standard curves is mentioned in point 3 of Preformulation studies while for sample preparation and estimation, similar procedure was followed as mentioned below-

A) Phenolic content- The reaction mixture consists of 1 ml of prepared sample (1gm/ml) and 9 ml of distilled water taken in a volumetric flask (25ml). One millilitre of Folin-Ciocalteu phenol reagent was treated to the mixture and shaken well. After 5 minutes, 10 ml of 7 % Sodium carbonate (Na_2CO_3) solution was treated to the mixture. The volume was made up to 25 ml. Incubated for 90 min at room temperature and 10 times dilutions were prepared. Record the standard solutions against the reagent blank at 550 nm with an Ultraviolet (UV) or Visible spectrophotometer. The concentrations were calculated using the standard graph plotted above.

B) Alkaloid content- Samples were initially extracted with 2N HCL and chloroform and separated the chloroform layer. 5 ml of bromocresol green solution and 5 ml of phosphate buffer (pH 4.7) were added in the sample solution (0.5 gm/ml) and were transferred to a separating funnel and yellowish chloroform layer collected in a 10-ml volumetric flask and diluted to the volume with chloroform. Further 10-time dilution was done. The absorbance was determined against the reagent blank at 470 nm with an

UV/Visible spectrophotometer. The concentrations were calculated using the standard graph plotted above.

C) Flavonoid content- The reaction mixture consists of 1 ml of sample solution (0.5gm/ml) preparation and 4 ml of distilled water was taken in a 10 ml volumetric flask. To the flask, 0.30 ml of 5 % sodium nitrite was treated and after 5 minutes, 0.3 ml of 10 % aluminum chloride was mixed. After 5 minutes, 2 ml of 1M Sodium hydroxide was treated and diluted to 10 ml with distilled water. Further 100-time dilution was done. The absorbance for standard solutions was determined against the reagent blank at 510 nm with an UV/Visible spectrophotometer. The concentrations were calculated using the standard graph.

8. Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

Minimum Inhibitory concentration (MIC) of fungicide is the minimum concentration required to inhibit/arrest the growth of all microorganisms in the culture and Minimum fungicidal concentration (MFC) is the minimum concentration required for the complete death of fungal strains present in the culture. This test was done to determine the exact strength required to be used in the effective formulation of the mask.

Materials: Saline solution (0.9% w/v), nichrome loop, plugged test tubes, Petri plates, nonabsorbent cotton, micropipette tips, beaker, conical flask, test solution, solvents like DW and alcohol, disinfectant, SDA Broth, BHI Broth, agar-agar, inoculum of culture, Mexican Mint extract.

1. Prepare broth solution of SDA and BHI using standard values, then fill the solution in a burette & fill 1 ml of solution to each test tube. Plug the test tubes using nonabsorbent cotton and sterilize with other requirements in an autoclave.
2. Inside Sterile conditions, prepare serial dilutions of each set such that test tube 1 has initial stock concentration and each test tube contains 1 ml of solution in the end.
3. Stock solution/Test tube 1 has following concentrations - Mexican Mint (1 gm/ml).
4. Now add 30 ug of prepared inoculum solution to each test tube. Incubate these test tubes for 24 hours, note down the MIC reading and then streak each reading into a prepared sterile agar plate using nichrome loop.
5. Further incubate those plates for 24 hours and note down the MFC readings after 24 hours. Whether the sample solution is Fungistatic or Fungicidal can be determined by following formula- **MFC/MIC ratio**.[25]

- Condition 1- MFC/MIC ratio < 4, extract is Fungicidal.
- Condition 2- MFC/MIC ratio $\geq$ 4, extract is Fungistatic.

9. Anti- Inflammatory Assay[26]

The principle objective behind the Egg Albumin Denaturation Assay is to determine whether compounds can stop or hinder egg albumin from becoming denatured under particular circumstances. Denaturation is the term used to describe how a protein changes in structure and loses its biological activity. The egg albumin denaturation assay is based on the idea that substances with anti-inflammatory qualities may be able to stabilize protein structures and prevent denaturation, which is frequently linked to inflammation and tissue damage. It measures a drug or compound's capacity to prevent or lessen egg albumin denaturation to evaluate its anti-inflammatory effects. Mexican mint is an anti-inflammatory herb due to its thymol content present in the herb.

Preparation of 1% egg Albumin: 1 gm of egg albumin powder was dissolved in 100 ml of DW and mixed slowly to avoid any lump formation. DW should not be warm otherwise egg albumin may coagulate. Filter through the cotton filter into a beaker.

Prepare a stock solution of Mexican mint alcoholic extract of 0.1 gm/100 ml alcohol. From this, solutions were further diluted for readings in range from 50,100,150,200 & 500 ug with alcohol and made up to 2 ml each for following set of each reading-

- **For test solution** = 2 ml std/sample solution + 0.2 ml of 1% egg albumin solution + 2.8 ml PBS solution.
- **For Blank solution** = 0.2 ml alcohol + 2 ml std/sample solution + 2.8 ml PBS solution.
- **For Control solution** = 0.2 ml of 1% egg albumin solution + 2 ml alcohol + 2.8 ml PBS solution.

Similarly, a reading of standard diclofenac solution (500 ug/ml) was prepared by following the above procedure.

These solutions were incubated at 37°C for 30 mins and later heated in a water bath for 15 mins at 70°C. Readings were taken at absorbance 600 nm.

% inhibition can be calculated by the formula-

$$\%\ inhibition = \frac{Absorbance\ of\ control - \left(Absorbance\ of\ Test - Absorbance\ of\ Blank\right)}{Absorbance\ of\ Control} \times 100$$

Post Formulation Test

1. **Homogeneity**[4]

It can be defined as a formulation which has its smooth consistency, no coarse particles and has uniform color. The product was taken in a beaker and was observed for these properties and results were noted.

2. **Spreadability**[4,27]

The spreadability of the product was measured by spreading 0.5 g of the gel on a glass plate and then a second glass plate was employed. 100 gm of weight was permitted to rest on the upper glass plate for 5 min. The diameter of

the circle after spreading of the Emulgel was determined by formula-

$$S = M*L/T$$

Where S = spreadability, M = Weight tied to upper slide, L = Length of glass slides, T = Time required to separate the slides.

3. **Viscosity**[4]

100 gm of product were taken in 250 ml beaker. The prepared solution is placed in the Brookfield Viscometer and a proper speed is selected for the spindle according to the expected viscosity of the sample. The Brookfield Viscometer determines viscosity by measuring the force to turn the spindle in the solution at a given rate.

4. ***In vitro* diffusion studies**[4]

The objective of *in vitro* dissolution testing is to evaluate the variables that affect the rate and extent of release of a drug substance from the finished dosage form, and in turn, the in vivo performance of the drug product. Franz cell diffusion apparatus was used for 6 hours study while parchment membrane/cellulose membrane was used as a passing membrane. Prepare PBS solution of pH 5.5 and 7.4 according to the procedure in IP 2022[22]. At an interval of 30 minutes, take the reading of 2 ml with appropriate measures and take the absorbance at 672 and 677 nm for pH 5.5 & 7.4 respectively.

5. **Drug content**[4]

This test was done in order to determine the strength of API in the product. So, 0.05 gm formulation was mixed in 25 ml of PBS buffer of pH 7.4 on a conical flask, then kept stirring using a magnetic stirrer for about 2 hrs and occasionally shook the flask with mild action. Record absorbance at 677 nm and calculate the drug content using standard curve equation.

6. **Anti-fungal bioassay of product**

The antifungal efficacy of both the products in two conditions were determined-

A) Storage under normal conditions (25°C).

B) Storage at higher temperature (45°C).

Similar procedures as mentioned in Preformulation studies- point 7C was followed and zones of inhibitions were noted after 24 hrs.

7. **Stability studies**

Both the products were stored at accelerated stability studies conditions (45°C, 60% RH) for 30 days and were observed after each 10 days to determine its consistency, its appearance, color and presence or absence of phase separation were noted.

RESULT AND DISCUSSION

Following results were obtained for various parameters of product.

Pre-formulation Studies

1. **Extractive values**

It was found to be 5.2 % and 4% for water and ethanol extracts respectively. Further, only ethanol extracts were taken into consideration due to degradation of water extracts after some time and presence of key activity. Refer to Supplementary **Table S2** for exact values.

2. **Antifungal Bioassay**

Refer to Supplementary **Fig S6** for images.

Table 3: Pre-Formulation antifungal bioassay

Herb Extract Individually	Zone of Inhibition (mm)	Activity Index (A.I)[28]	Herb Extract Combined	Zone of Inhibition (mm)	Activity Index (A.I)[28]
1) Moringa	10	0.71	1) Mexican mint + Moringa	12	0.85
2) Hibiscus	7	0.5	2) Mexican mint + Hibiscus	11	0.78
3) Mexican Mint	14	1	3) Hibiscus + Moringa	9	0.64
4) Positive control (KTZ)	14	1	4) Hibiscus + Moringa + Mexican Mint	10	0.71

Following observations were made

1. When compared with positive control (Ketoconazole/KTZ), it was found that individually these herbs show moderate antifungal property amongst which Mexican mint showed better activity which is close to positive control.
2. Whereas in mixtures, their overall activity gets reduced due to hindrance of other chemicals present in the other herb when combined.
3. Hence, it was decided to further use Mexican mint for its favorable activity.

3. **Absorption maxima** (λ_{max})

Following graphs were noted for extracts (See Supplementary **Fig S7**)-

At pH 5.5, it was found to be 677 nm and at pH 7.4, it was found to be 672 nm.

4. **Standard curve for drug diffusion studies and quantitative tests:**

Refer to Supplementary **Fig. S8, S9 and S10** for graphical interpretations.

5. **TLC/Thin Layer Chromatography**

It was found that thymol (0.74) was present and identified in the Mexican Mint. Refer to Supplementary **Fig. S11** and **Table S3** for more.

6. **Chemical test (Qualitative analysis)**
Refer to Supplementary **Fig. S11** and **Table S4** for more.

Table 4: Chemical Test

Sr. no .	Chemical Constituent	Result
1	Phenols	+
2	Tannins	+
3	Acetyl groups	+
4	Terpenoids	+
5	Triterpenoids	+

7. **Quantitative analysis- Flavonoid content, Phenolic content and alkaloid content** [24]

In qualitative estimation, it was found that there was no detection of alkaloids because of which it was also not detected while performing alkaloid content test for the herb. However, Liquid chromatography - mass spectrometry (LC-MS) can be used as a possible method for evaluation of alkaloids of this herb. Refer to Supplementary **Table S5** for values.

8. **Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)**

When calculated for MIC/MFC ratio, it was found to have Fungicidal action with an index of 2 [25]. Thus, Mexican Mint alone can be used for treating dandruff which is harsh and causes inflammation of scalp due to dandruff as this herb also shows anti-inflammatory action in further tests. Refer to Supplementary **Fig S13** and **Table S6** for more.

9. **Anti- Inflammatory Assay**

While comparing our sample (Mexican mint) with standard (Diclofenac) we found that at the same concentration (500 ug/ml) where standard shows 95% inhibition, the sample shows 53.12% inhibition. But at lower concentration i.e. 100 ug/ml, it was found to show 99.3% inhibition. Refer to Supplementary **Fig S14** for image.

Post Formulation [4,20,27]

1. **Homogeneity:** Hair mask was found to have smooth consistency, no coarse particles and have uniform color throughout the formulation.
2. **Spreadability:** Spreadability was found to be 7.32 g·cm/sec.
3. **Viscosity:** Spindle no 5 was used for viscosity determination and following observations were made in Supplementary **Table S7**. The formulation has higher viscosity which resists its flowability and at the same time allows it to form a paste like texture for easy application.
4. **In vitro diffusion studies:** % Drug release was found to be 37.2% & 38.67% for pH 5.5 & pH 7.4 respectively at the end of 6 hours. Refer to Supplementary **Fig. S15** and **Table S7** for more.
5. **Drug content:** The permissible limit for drug content is 95% to 98%. [29] Refer to Supplementary **Table S8** for values.
6. **Anti-fungal bioassay of product:** It was observed that Mexican mint had the same efficacy at both the temperatures indicating it can be stored at low temperatures or room temperature to maintain its efficacy. Refer to Supplementary **Fig. S16** and **Table S9** for more.
7. **Stability studies:** At the end of 30 days, the Mexican Mint hair mask was found to have the same consistency, smooth appearance, same color, and absence of phase separation. However, at 20^{h} day, the color of mask turned from green to pale green. It was further tested for its antifungal efficacy and found to show the same efficacy as compared to products stored at normal conditions. Refer to Supplementary **Table S10** for values.

CONCLUSION

Emulgel is a newer approach to treating dandruff issue through tropical application as it will act as a depot of drugs which releases drugs in a sustained manner. It is emerging as one of the novel methods in pharmaceutical formulations particularly in cosmetics due to its aesthetic appearance and easiness of application. As it can be used as a cosmetic as well as a therapeutic formulation, this hair mask prepared above with a natural herb can be used as safer and effective means to tackle daily dandruff problems and inflammation caused by it. This type of hair product for treating dandruff can be used for daily purpose as applying a hair mask seems efficient and effective while having busy lifestyle. Due to anti-inflammatory and antifungal properties of Mexican mint derived through our studies; we can say that the hair mask is effective enough to treat dandruff problems for daily purpose to some extent. However, severe forms of dandruff such as seborrheic dermatitis needs medical attention and cannot be fully cured using herbal means alone.

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