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Development And Assessment of Anti- Dandruff Hairmask Containing Nigella Sativaoil and Annona Squamosa Seed Powder

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ABSTRACT

This research outlines the creation and assessment of herbal hair mask aimed at combating dandruff, featuring *Nigella sativa* (black seed oil) and *Annona squamosa* seed powder. Dandruff is a common scalp issue often associated with flaking, irritation, and hair loss, and may worsen due to the use of chemical hair treatments. To offer a more natural and safer option, a hair mask was produced using ingredients recognized for their antifungal, antibacterial, and anti-inflammatory effects. *Nigella sativa* contains a significant amount of thymoquinone, which aids in promoting scalp health, while *Annona squamosa* seed powder provides both antioxidant and calming properties. The hair mask was created through an emulsification technique, incorporating additional ingredients such as emulsifiers and stabilizers. Preliminary studies were conducted to evaluate the physical and chemical compatibility of the components. The final product was analyzed for various characteristics, including pH, spreadability, foam formation, solid content, and dirt removal. The optimized formulation exhibited desirable consistency, effective cleansing properties, an appropriate pH level, and easy rinsing. In summary, the findings indicate that this herbal hair mask is beneficial in alleviating dandruff while enhancing the health of the scalp and hair. It presents a safe and natural substitute for synthetic anti-dandruff solutions, with few adverse effects. This formulation shows significant promise for further advancement in the realm of herbal cosmetic products.

Keywords: *Herbal hair mask, Hair, Nigella sativa, Annona squamosa, Antidandruff*

1. INTRODUCTION

Transdermal drug delivery systems (TDDS) have become a promising method for administering medications through the skin, providing several benefits compared to conventional oral and intravenous methods. These systems bypass first-pass metabolism, lessen gastrointestinal side effects, and ensure a controlled release of medications. The skin, especially its uppermost layer—the stratum corneum—and hair follicles, plays a vital role in the absorption of drugs through the skin. Hair follicles particularly act as natural reservoirs that improve drug penetration, making them ideal targets for addressing hair and scalp-related issues.[1]

Dandruff is a common scalp condition marked by flaking, itching, and irritation. It often arises from fungal infections, excess oil production, and inadequate scalp hygiene. The excessive use of chemical-laden hair care products can exacerbate the issue by leading to dryness, split ends, and hair thinning. Consequently, there is a growing interest in herbal formulations that are both safe and effective, devoid of harmful side effects.[2]

Herbal cosmetics have become favored fortheir therapeutic benefits and low risk of adverse reactions. Hair masks among these products are commonly utilized to enhance scalp health and improve hair appearance. They offer deep conditioning, fortify hair follicles, and help eliminate dirt and dandruff. This research concentrates on creating an herbal anti-dandruff hair mask using *Nigella sativa* (black seed oil) and *Annona squamosa* seed powder. Both components are recognized for their antimicrobial, anti-inflammatory, and antioxidant properties.[3] The purpose of this formulation is to diminish dandruff, nourish the scalp, and improve overall hair quality, providing a natural and holistic alternative to synthetic options.[4]

Hair :

Hair is a protein structure that grows from follicles found in the dermis. It is one of the key characteristics of mammals. The human body, except for areas of hairless skin, is covered in follicles that produce thick terminal hair and fine vellus hair. Although a lot of focus on hair is placed on its growth, types, and care, it also plays an important role as a biomaterial mainly made up of protein, especially alpha-keratin. [4]

Structure of hair fiber :

Hair consists of keratinized protein strands formed from tightly packed dead epithelial cells.Hair is made up of three primary layers:

1. Cuticle[5]
2. Cortex[6]
3. Medulla[2]

Hair Growth Cycle :

The cycle of hair growth involves hair follicles undergoing various phases of development, resting, and falling out.

- Stages of hair cycle-

I. Anagen Phase
II. Catagen Phase
III. Telogen Phase

Exogen Phase

Figure.1 Stages of Hair Cycle

I. Anagen Phase (Growth Phase)

This phase represents the active growth of hair.

Duration: 2 to 7 years, influenced by genetic and nutritional elements.

Approximately 85-90% of hair on the scalp is in this stage at any moment.

In the hair bulb, matrix cells rapidly divide to create the hair shaft.

II. Catagen Phase (Transition Phase)

A brief involution phase indicating the conclusion of active growth.

Duration: 2 to 3 weeks.

The hair follicle diminishes in size and disconnects from the dermal papilla.[7]

III. Telogen Phase (Resting Phase)

The follicle is not active; the hair stays in position without growing.

Duration: 3 to 4 months.

Approximately 10-15% of hair on the scalp is in this stage.[2]

IV. Exogen Phase (Shedding Phase)

Hair is naturally shed from the follicle and falls away.

This process frequently coincides with the early anagen phase as new hair begins to grow.

Losing 50-100 strands of hair each day is regarded as typical.[6]

Issues associated with hair :

- Dandruff.
- Dry hair.
- Split ends.
- Frizzy hair.
- Hair thinning.
- Damage from heat.
- Graying hair.[8]

2. MATERIALS AND METHODS

Use of herbal drugs

1. *Nigella sativa*

Annona squamosa

1.*NIGELLA SATIVA* (BLACK SEED OIL)

Nigella sativa, also referred to as black seed or Kalonji, is a medicinal herb recognized for its abundant phytochemical content, including thymoquinone, nigellone, and a range of essential fatty acids. These components demonstrate antimicrobial, antioxidant, and

anti-inflammatory properties, which contribute to diminishing dandruff, alleviating scalp irritation, and fostering healthier hair growth. When applied as a hair mask, *Nigella sativa* helps cleanse the scalp, manage fungal infections such as dandruff, and nourish hair follicles, ultimately enhancing overall scalp health and hair texture.[9][10]

- Chemical constituents :

Thymoquinone, Nigellone, Essential fatty acids (linoleic and oleic acid), Vitamins (notably Vitamin E), Proteins and alkaloids, Saponins.[11]

- Uses :
    1. Reduces dandruff
    2. Prevents hair fall
    3. Moisturizes scalp
    4. Soothes scalp irritation
    5. Improves hair texture[12]

Figure.2 *Nigella sativa* seed

2.*ANNONA SQUAMOSA* (SEED POWDER)

Annona squamosa seed powder is derived from the dried seeds of the custard apple fruit. These seeds are abundant in acetogenins, alkaloids, saponins, and flavonoids, all of which exhibit potent insecticidal, antimicrobial, and antifungal properties. Historically, the seed powder has been utilized in herbal remedies for scalp issues, especially for removing lice, managing dandruff, and purifying the scalp. Its inherent pesticidal characteristics make it beneficial for maintaining scalp cleanliness and promoting overall hair health when incorporated into hair masks and herbal products.[13][14]

- CHEMICAL CONSTITUENTS

Acetogenins , Alkaloids (such as Anonaine), Flavonoids, SaponinsTannins[15]

- USES
    1. Treats dandruff
    2. Eliminates head lice
    3. Cleanses the scalp
    4. Reduces scalp infections

Soothes scalp inflammation[15]

3. EXPERIMENTAL WORK

Pre-formulation study :

1. Selection of drug :

Nigella sativa oil and Seeds of *Annona squamosa*.[16][17]

2.Characteristics :

Preformulation studies evaluate the physical, chemical, and mechanical characteristics of drugs and excipients to guarantee the stability of the formulation and its bioavailability. Important aspects include:

Physical description: Color, Odor, Appearance, Melting and boiling points.

- Solubility Analysis-

The solubility of *Nigella sativa* seed oil and *Annona squamosa* seed powder was assessed using the shake flask technique.

3.Organoleptic Properties :

- Color: Noted under bright lighting on a sheet of butter paper.
- Odor: Sensed in a limited amount.

4. Physiochemical Properties :

- pH

To evaluate the pH of the herbal hair mask and ensure it is within a suitable range for scalp application, a 1% aqueous dispersion was prepared. Accurately, 1 gram of the formulated hair mask was weighed and transferred into a clean 250 mL beaker containing 100 mL of distilled water. The mixture was stirred continuously for 30 minutes using a clean glass rod to achieve uniform dispersion. After stirring, the dispersion was allowed to stand and equilibrate for 1 hour at room temperature (25 ± 2°C).Prior to use, the digital pH meter was calibrated using standard buffer solutions of pH 4.0, 7.0, and 9.0. The pH electrode was then dipped into the prepared sample solution without touching the beaker's bottom or sides. Once the reading stabilized, the pH value was recorded.

- Melting point

The melting point of Annona squamosa seed powder was determined to assess its purity and physical properties. A small quantity of the powder was packed into a capillary tube sealed at one end. The tube was gently tapped to ensure uniform packing of the powder at the bottom. It was then placed in a digital melting point apparatus alongside a thermometer. The temperature was gradually increased, and the point at which the powder started to liquefy was noted as the onset of melting. The complete melting point range (from first melt to complete liquefaction) was recorded.[18]

Procedure of hair mask formulation :

1. Weighing of ingredients- All the required ingredients for hair mask preparation were Accurately weighed individually by using digital balance.
2. Preparation of Non-aqueous Phase (Beaker A)- *Nigella sativa*seed Oil, olive oil, stearic Acid, ceto- stearyl Alcohol,

Span 80

3. Preparation of Aqueous Phase (Beaker B)- *Annona squamosa* seed powder, Tween 80, purified water
4. Heating of Both Phases- Slowly add aqueous phase (B) into non-aqueous phase (A) with continuous stirring
5. Emulsification- Slowly add aqueous phase (B) into non-aqueous phase (A) with continuous stirring
6. Addition of Additive- Add perfume andpreservative to theemulsion
7. Homogenization- Use a blender or Mechanical stirrer to mix until creamy and Uniform consistency is achieved
8. Cooling and Evaluation- Allow to cool at room Temperature and Observe for any phase Separation.

Formulation table :

Batches	Annona Squamosa seed powder (gm)	Nigella Sativa Seed oil (ml)	Olive oil (ml)	Stearic acid (gm)	Ceto-stearyl alcohol (gm)	Span 80 (ml)	Tween 80 (ml)	Sodium benzoate (gm)	Distilled water (ml)
F1	3	3	5	2.5	3.5	0.6	1.5	0.5	Up to 30ml
F2	3	3	3	2	2.5	1	1	0.5	Upto 30ml
F3	3	2.5	3.3	2	2.5	1	1	0.5	Upto 30ml
F4	2	2	2	2	2	0.5	0.5	0.5	Upto 30ml
F5	2.5	2.5	2.5	2	2.5	1.5	1.5	0.5	Upto 30ml

Table no. 1 Formulation table

4. EVALUATION OF ANTI -DANDRUFF HAIR MASK

1. Organoleptic evaluation :

a) In this evaluation study, parameters such as color, odor, texture, and appearance of the Product were assessed.

b) Color: A small sample of the drug was placed on butter paper and examined under well-lit conditions to observe its color.

c) Odor: A very small amount of the drug was sniffed to detect the odor.

d) Boiling point and Melting point: The capillary tube method was used to determine these. A capillary tube with one end sealed was filled with a tiny amount of the drug.

2. Solubility methods :

The solubility of *Annona squamosa* seeds powder and nigella seeds oil was assessed in both distilled water and organic solvents. The solubility of these substances in various solvents was evaluated using the shake flask method, where the compound was added in excess to 10 ml of the respective solvent. The mixtures were stirred for 24 hours using a magnetic stirrer at 37°C and allowed to reach equilibrium after this period.

3. Pharmacognostic tests :

a) Carbohydrate test: Conduct the Benedict's test by mixing equal volumes of the Benedict reagent and the test solution, then heat the mixture for 5 minutes. Look for color changes and the formation of precipitate.

b) Amino acid test: For the Ninhydrin test, heat 3 ml of the test solution together with 3 drops of Ninhydrin solution in a boiling water bath for 10 minutes. Monitor the resulting changes.

c) Alkaloid test: Wagner's test – Add a few drops of Wagner's reagent to 2-3 ml of the test solution. The appearance of a red-brown precipitate indicates the presence of alkaloids. Dragendorff's test – To 2-3 ml of the test solution, introduce a few drops of Dragendorff's reagent. If alkaloids are present, a brick-red precipitate or orange-red coloration will occur.[19]

4. pH:

The pH of the created herbal hair mask was measured to confirm its suitability for the scalpand hair. A precise amount of one gram of the hair mask formulation was weighed and mixed with 100 mL of distilled water to make an aqueous solution with a strength of 1% w/v. A glass rod was used to continuously stir the contents togetherfor 30 minutes to ensure thorough mixing and was then left to sit for one hour at room temperature (25±2°C) to reach equilibrium.Before the measurement was taken, a digital pH meter was calibrated using standard buffer solutions with pH values of 4.0, 7.0, and 9.0.The pH meter's electrode was placed into the sample solution, making sure it did not contact the sides or bottom of the beaker. After the reading stabilized, the pH value was noted. Following the measurement, the electrode was rinsed with distilled water and properly dried to guarantee accuracy in subsequent tests.[13]

5. Solid content (%) :

4 grams of hair mask was added to an evaporating dish that had been refined and dried. The hair mask's liquid component was evaporated; its proper weightwas set up and the heat plate was placed into the evaporating dish. The weight of the hair mask's solid content was accurately determined after it had dried.

6. Dispersion of dirt :

2 drops of hair mask were added to a test tube that had been halfway filled with purified water. Upon adding a drop of India ink, the test tube was shaken ten times and sealed with a stopper. The foam's ink content was designated as None, Light, Moderate, or Heavy.

7. Foaming ability :

The cylinder shaking technique was used to evaluate the hair mask's foaming capacity. A 250 mL graduated cylinder containing 50 mL of a 1% hair mask solution was shaken ten times while being held in the hand. The entire quantity of foam generated was measured after a minute of shaking. For a total of 5 mins, the foam volume was shaken as well as recorded at one-minute intervalsin which the foam volume determined.

8. Spreadability :

A tool made of a glass slide and a wooden block was used to measure the hair mask's spreadability. A carefully weighed 5 g sample was placed on the underside of the block, and then the movable upper slide was placed onto the hair mask. The period that it took for the top slide to separate 5 cm from the assembly was then recorded. The formulaS = mxl /t was used for assessing spreadability, where S stands for spreadability, m for the weight fastened to the upper slide, l for the distance crossed by the upper slide, and t for the time required to separate the slides.

9. Washability :

For washability testing, 1–2 grams of the formulation were applied to a clean and dry hair strand. After allowing it to sit for 15–20 minutes, the strand was rinsed with lukewarm water without the use of shampoo. The formulation was deemed easily washable if it was completely removed without leaving any residue or greasy sensation.

10. Nature of hair after washes :

The condition of the hair post-wash was evaluated based on factors such as texture, smoothness, softness, shine, and manageability. The hair strand was allowed to air dry, and observations were made through visual and tactile assessment. A favorable outcome was indicated by soft, non-greasy, tangle-free hair that exhibited improved shine and texture.

11. Patch test :

Patch test was conducted by applying a small amount of the hair mask to a 1 cm² area on the inner forearm. The site was left exposed and monitored for 24 hours. If there was no redness, itching, or swelling, it suggested that there was no allergic response.

12.Homogenecity :

The homogenecity of the formulated mask was assessed through visual inspection once it wasplaced in the container. The hair mask was examined for its appearance and checked for any aggregates or flocculates.[21]

5. RESULTS AND DISCUSSION

1. PREFORMULATION STUDY OF THE DRUGS :

- **Organoleptic properties :**

Table 1. displays the findings related to the characteristics of drug samples, such as appearance, color, and odor.

Drug	**Identification test**	**Observed result**	**Standard result**
Nigella sativa oil	Appearance	Dark brown liquid	Amber colored liquid
	Colour	Dark brown	Pale yellow to amber colored
	Odour	Characteristic and spicy aroma	Characteristic mild peppery, bitter aroma
Annona squamosa seed powder	Appearance	Hygroscopic powder	Hygroscopic powder
	Colour	Dark brown	Dark brown
	Odour	Strong, fruity	Sweet and Fruity

Table no.2 Organoleptic properties of drugs

- **Boiling point and melting point :**

The boiling and melting points were measured utilizing the capillary tube technique. The results acquired are presented in Table 2, alongside a comparison with standard values.

Drug	**Observed result**	**Standard result**
Boiling point *Nigella sativa* oil	293 to 297°C	Around 300°C
Melting point *Annona squamosa* Seed Powder	31 to 34°C	Around 35°C

Table no. 3 Boiling point and melting point

- **Solubility profiles :**

Solubilty	**Solubility profile for *Nigella sativa***	**Solubility profile for *Annona squamosa***
Freely Soluble	Ethanol, Methanol	Ethanol, Methanol, DMSO
Sparingly soluble	-	Water
Soluble	Acetone, Vegetable oils (Olive oil)	Acetone
Practically Insoluble	Water	-

Table no.4 Solubility profile of *Nigella sativa* seed oil and *Annona squamosa* powder

UV SPECTROSCOPY:

Sample name- BSO (*Nigella sativa* oil).

Solvent- DMSO (Dimethyl sulfoxide)

Scan from- 800.0 nm

Scan to- 200.0 nm

Scan step- 1.0 nm

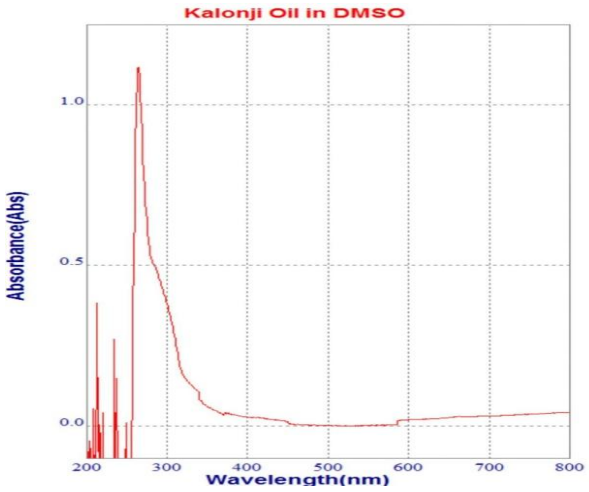

Figure.4 Wavelength vs Absorbance

Observation:

WL (nm)	Abs	T%
250	0.009	97.94
262	0.9938	10.14
268	0.9758	10.57
285	0.498	31.77
298	0.399	39.91

Table no.5 UV spectra wavelength and absorbance

The UV spectrum of the pure oil sample in DMSO shows an absorbance maximum at 262 nm, which closely aligns with the standard lambda max. The absorption spectrum for the BSO was recorded across a wavelength range of 200-500 nm (refer to figure). It is important to note that the absorption in the visible range

trongly indicates the presence of long conjugated double bonds in the substance. Therefore, from the gathered data, the wavelength exhibiting the highest absorbance (0.9938) was identified to be 262 nm.

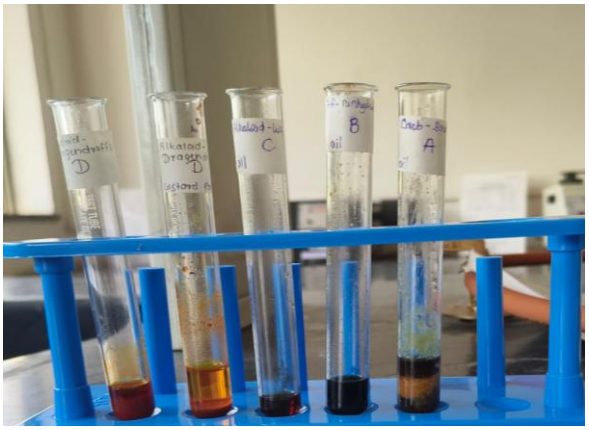

Figure .5Pharmacognostic tests

- PHARMACOGNOSTIC TESTS :

Sr no.	Test	Purpose for detection	Observation	Result
1.	Ninhydrin test (Oil)	Presence of Amino acid	Purple solution	Positive
2.	Wagner's test (Oil)	Presence of Alkaloids	Red ppt	Positive
3.	Dragendorff's test (Oil)	Presence of Alkaloids	Brown ppt	Positive
4.	Dragendorff's test (Powder)	Presence of Alkaloids	Orange- brown ppt	Positive

Table no.6 Pharmacognostic tests

6. EVALUATION PARAMETERS

PRELIMINARY BATCH EXAMINATION:

Batches	***Annona squamosa* seed powder (gm)**	***Nigella sativa* seed oil (ml)**	**Olive oil (ml)**	**Stearic acid (gm)**	**Ceto-stearyl alcohol (gm)**	**Span-80 (ml)**	**Tween-80 (ml)**	**Sodium benzoate (gm)**	**Distilled water (ml)**	**Description**
F1	3	3	5	2.5	3.5	0.6	1.5	0.5	Upto 30 ml	Particle size reduction required
F2	3	3	3	2	2.5	1	1	0.5	Upto 30 ml	Consistency not achieved
F3	3	2.5	3.3	2	2.5	1	1	0.5	Upto 30 ml	Phase separation occurred
F4	2	2	2	2	2	0.5	0.5	0.5	Upto 30 ml	Blending should be proper
F5	2.5	2.5	2.5	2	2.5	1.5	1.5	0.5	Upto 30 ml	Perfect formulation obtained

Table no.7 Preliminary Batches

EVALUATION PARAMETERS (F1-F5) :

Sr.no	**Test**	**Observations**				
		F1	**F2**	**F3**	**F4**	**F5**
1	Colour	Dark brown	Dark brown	Dark brown	Light brown	Brown
2	Nature	Semi-solid paste	Semi-solid paste	Semi-solid paste	Semi-solid paste	Semi-solid paste
3	Odour	Unpleasant	Unpleasant	Unpleasant	Pleasant	Pleasant

4	Texture	Rough	Smooth	Rough	Smooth	Smooth
5	Washability	Good	Good	Good	Not good	Good
6	Homogenecity	Floccules present	No floccules	Floccules present	No floccules	No floccules
7	Patch test	No redness, swelling and itching	No redness, swelling and itching	No redness, swelling and itching	No redness, swelling and itching	No redness, swelling and itching

Table no.8 Evaluation parameters

CHARACTERIZATION OF HAIR MASK

1. pH :

The optimized batches with proper texture F1, F2, F3, F4, F5 are shown in the Table.

Sr.no	Batches	pH
1	F1	6
2	F2	3.9
3	F3	5
4	F4	4
5	F5	4.7

Table no.9 pH determination

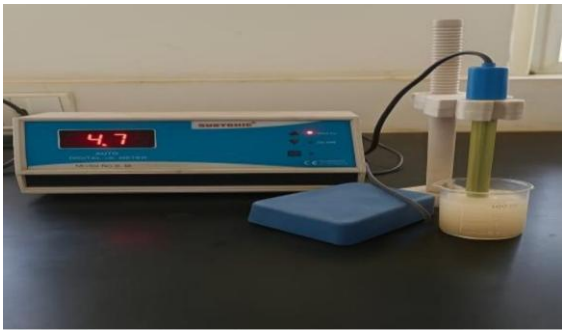

Figure.6 pH determination

2. Dirt dispersion :

The optimized batches demonstrated Light ink dispersion in foam,demonstrating that there probably will not be any dirt left in the foam; Consequently, the formulation that was created is satisfactory and is outlined in the table:

Test tube	Batches	Observation	Ink in foam
1	F1	Most ink collected in the foam	Heavy
2	F2	Noticeable ink in foam, less in solution	Heavy
3	F3	Moderate dispersion, some ink visible in foam	Moderate
4	F4	Good dispersion of ink in solution and foam	Mild
5	F5	Ink is well dispersed in the solution, minimal in foam	Mild

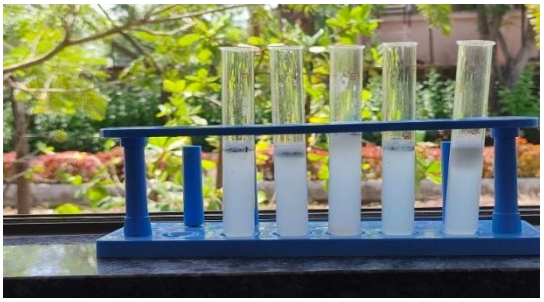

Figure .7 Dirt dispersion

3. Solid content :

The solid content in the hair mask makes the rinsing of hair a difficult procedure. The solid content of optimized batches is noted in the table :

Sr.no	Batches	Solid content (%)
1	F1	3
2	F2	6.7
3	F3	4
4	F4	5
5	F5	2

Table no.11 Solid content determination

Figure.8 Solid content

Foamability of hair mask:

Foam formation helps for removal of product, making it easier for use of formulation. The observations are noted in table:

Sr.no	Batches	Foam volume (ml)
1	F1	64
2	F2	57
3	F3	55
4	F4	52
5	F5	78

Table no.12 Foamability determination

5.Spreadability:

By using the formula, $S = mxl$ /t the spreadability is calculated and observations are made in table by comparison with standard value for Hair mask,

Good = 20-30 g.cm/sec

Moderate = 10-20 g.cm/sec

Poor = <10 g.cm/ sec

Sr.no	Batches	Spreadability
1	F1	21
2	F2	10
3	F3	13.1
4	F4	20
5	F5	23

Table no.13 Spreadability determination

7. SUMMARY AND CONCLUSION

The present study successfully achieved its primary objective of developing an herbal antidandruff hair mask utilizing the therapeutic properties of two potent natural ingredients —Kalonji oil, often referred as *Nigella sativa* oil,and *Annona squamosa* seed powder (Custard Apple seed powder). These ingredients were chosen based on traditional knowledge and modern pharmacological evidence supporting their antifungal, antibacterial, anti-inflammatory, and antioxidant activities, all of which are essential in managing dandruff and promoting overall scalp and hair health.

Out of all the formulations, Batch F5 stood out as the optimal product due to its desirable physical characteristics such as:

• Smooth and homogeneous texture

• Mild, pleasant fragrance

• Appropriate pH (around 4.7), suitable for scalp application

• Good spreadability, making it easy to apply ,effective washability, ensuring no residue remainson hair

• Mild dirt dispersion and foaming capacity, indicating efficient cleansing action.

Most importantly, the inclusion of *Nigella sativa* oil, rich in thymoquinone, and *Annona squamosa* seed powder, known for its acetogenins and antioxidants, led to a synergistic effect in combating dandruff, soothing the scalp, and nourishing hair follicles. This research validates that the formulated herbal hair mask is not only effective in treating dandruff, but also contributes to overall scalp health, hair nourishment, and strengthening of hair strands. Moreover, it offers a chemical-free, eco-friendly, and cost-effective alternative to synthetic anti-dandruff products, which often come with potential side effects.

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