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SYNERGISTIC FUSION: UNITING NATURE AND SCIENCE IN ANTI-FUNGAL SHAMPOO FORMULATION FOR ENHANCED HAIR AND SCALP WELLNESS

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ABSTRACT
The objective of this research is to create, combine, and enhance a natural anti-fungal shampoo with herbal constituents that are well-known for their therapeutic benefits. The main components, which include flaxseeds, neem extract, onion extract, shikakai extract, reetha extract, and beetroot extract, were chosen for their ability to work in concert to improve the health of the hair and scalp. A careful assessment of the shampoo's physical attributes, such as its viscosity, pH level, and organoleptic qualities, was part of the development process. In addition, the cleaning action and filth dispersion were evaluated together with the foaming ability and foam stability. This study highlights the potential of natural ingredients in developing safe, effective, and consumer-friendly anti-dandruff shampoos, promising a novel addition to the market that aligns with current wellness trends. The ultimate goal is to provide a superior solution for managing dandruff, thereby meeting consumer preferences and needs through a holistic approach that combines the best of nature and science.

KEYWORDS: Anti-dandruff, herbal shampoo, pH, foaming ability, viscosity.

INTRODUCTION
Shampoo is a substance containing surfactants, available in various forms like liquid, solid, or powder. When applied correctly, it effectively eliminates grease, dirt, and dead skin cells from the hair, scalp, and shaft without causing harm to the user.[1-4] Currently, traditional herbal shampoos are among the most popular hair care products due to their perceived safety and lack of side effects. While chemical shampoos might initially enhance the appearance of hair, they can eventually harm the roots and lead to issues such as:

- Dryness and itchiness of the scalp
- Premature aging and graying of hair
- Split ends and excessive hair loss

To address these problems, switching to a herbal shampoo is recommended, as it helps restore lost nutrients and counteract damage.[5-10]

Herbal anti-dandruff shampoos are cosmetic products formulated with herbal ingredients like plant extracts and essential oils. They are used to address various hair and scalp issues, including:

- Eliminating dandruff
- Adding natural colour to the hair
- Reducing excess oil
- Promoting healthy hair growth
- Removing dust, dirt, and scalp flakes
- Preventing hair loss
- Enhancing softness and smoothness of the hair

These shampoos are believed to penetrate hair roots, stimulate sebaceous glands, improve blood circulation, and strengthen both hair roots and shafts. They are also utilized to combat conditions such as alopecia, thinning hair, clubbing, graying, roughness, and breakage. Numerous plants with beneficial effects on hair are commonly included in these shampoos.[11-13] The significance of natural products for dandruff treatment lies in their ability to address the root causes of dandruff, such as fungal infections or dry scalp, while also providing nourishment and hydration to the scalp and hair . Unlike harsh chemical-based products, natural remedies tend to be gentler on the scalp, reducing the risk of irritation or allergic reactions. Natural products for dandruff treatment are gaining popularity due to their effectiveness and minimal side effects compared to synthetic alternatives.[14-18]

These products typically utilize ingredients derived from plants, herbs, or other natural sources known for their anti-inflammatory, anti-fungal, anti-moisturizing properties. Some common natural ingredients used in

dandruff treatment include tea tree oil, coconut oil, aloe vera, apple cider vinegar and neem oil.

MATERIALS AND METHODS

Materials

All the raw materials for obtaining extract were collected from local areas and farms. Rest of the chemicals used were of research grade.

Table 1: Formulation Table.

INGREDIENTS	FORMULATION CODE				
	F1	F2	F3	F4	F5
Guar gum	2.75mg	2.75mg	2.75mg	2.75mg	2.75mg
Beetroot powder	1mg	1mg	1mg	1mg	1mg
Shikakai extract	1ml	2ml	1ml	1ml	1ml
Neem extract	2ml	1ml	2ml	2ml	1ml
Reetha extract	1ml	1ml	1ml	2ml	1ml
Onion extract	1ml	1ml	1ml	2ml	2ml
Flax seed extract	3 ml	3ml	3ml	3ml	3ml
Glycerine	4mg	4mg	4mg	4mg	4mg
Citric acid 0.5%	4 ml	5ml	5.5ml	6ml	6ml
Apple cider vinegar (ACV)	2 ml of 1:1 (ACV-WATER)				
Lavender oil	q.s.				
Water	q.s. 25ml				

Procedure

i. Guar gum was dispersed in glycerine and shaken until a homogeneous suspension was obtained.
ii. To this mixture, the beetroot powder was added until a reddish-maroon colour obtained.
iii. Citric acid was added to the mixture in drop wise until a mixture having required viscosity was obtained.
iv. To this mixture, the extracts of Shikakai, Neem, Reetha, Onion was added in predetermined amount respectively
v. The mixture was stirred continuously inorder to mix all the components.
vi. To the solution, the flax seed extract was added followed by apple cider vinegar and distilled water.
vii. Finally the Lavender oil was added to the homogenous solution and mix thoroughly to obtain the shampoo in required consistency.

Evaluation Tests of Shampoo

a) Physical appearance of drug: The drug was visually inspected for the physical appearance.

b) Determination of pH: The pH of the prepared herbal shampoo in distilled water (10%v/v) was evaluated by means of pH meter at room temperature.

c) Determination of solid content: In this experiment, we began by measuring and noting the weight of an empty, dry, clean evaporating dish. We then added approximately 4 grams of prepared herbal shampoo to the dish and recorded the combined weight to establish the initial weight of the shampoo. To find the dried weight of the shampoo, we placed the dish on a hot plate and allowed the shampoo to fully evaporate. We calculated the percentage of solids using the following formula:

$$\text{Percentage of solid} = \frac{\text{Dried weight of shampoo}}{\text{Initial weight of shampoo}} \times 100$$

d) Dirt dispersion test: In a wide-mouthed test tube, we added two drops of cleanser to 10 ml of purified water. Next, we introduced one drop of Indian ink into the formulated shampoo, sealed the test tube with a stopper, and shook it for 10 minutes. We then measured the volume of ink in the foam and rated the result as none, slight, medium, or heavy.

e) Form stability and form ability: The foaming ability was assessed using the cylinder shake method. A 50 ml solution of 1% shampoo was placed in a 250 ml graduated cylinder, which was then covered with a hand and shaken 10 times. The volume of foam was measured 1 minute after shaking, and this process was repeated at 1-minute intervals for a total of 4 minutes. Only the volume of the foam was recorded.

f) Determination of Surface tension: Surface tension measurements were taken for shampoo solution. The stalagmometer was fixed perpendicularly with a clamp and stand and then filled with water by sucking. The flow of water was controlled at the rate of 10-15 drops per minute by keeping a clamped rubber tube at the top. The number of drops is counted as they fall when the meniscus passes between the mark A and mark B (fixed volume). The process is repeated to collect at least three readings. The stalagmometer is then dried and the experiment was repeated using the formulated shampoo.

Methods

Preparation of Anti-Fungal Shampoo

The formulation of Anti-fungal Shampoo was made according to the formula presented in table 1.

$$\frac{\gamma 1}{\gamma 2} = \frac{\frac{V\rho 1}{n1}}{\frac{V\rho 2}{n2}}$$

Where, V is the volume of liquid delivered, n is the number of drops, ρ is density

g) Detergent stability: The Thompson method was employed to assess the detergency ability of the samples. In brief, a bundle of hair was washed with a 5% sodium lauryl sulfate (SLS) solution, dried, and then divided into 3-gram portions. These samples were immersed into n-hexane solution containing 10% artificial sebum and shaken for 15 minutes at room temperature. After removal, the solvent was evaporated at room temperature, and the sebum content was measured. Each sample was then split into two parts: one part was washed with 0.1 ml of a 10% test shampoo, while the other served as the negative control. After drying, the remaining sebum on each sample was extracted with 20 ml of n-hexane and weighed again. The detergency power percentage was calculated using the following formula:

DP = 100 (1 -T/C)

Where, DP represents the percentage of detergency power, C is the sebum weight in the control sample, and T is the sebum weight in the test sample.

h) Cleaning action: Five grams of wool yarn were first immersed in grease, then placed into a flask containing 200 ml of water with 1 gram of shampoo. The water temperature was maintained at 35°C. The flask was shaken for 4 minutes at a rate of 50 shakes per minute. Afterward, the solution was removed, and the yarn was taken out, dried, and weighed.

i) Rheology evaluation: The viscosity of the shampoos was assessed using a Brookfield Viscometer (Model DV-l Plus, LV, USA), which was operated at spindle speeds ranging from 0.3 to 10 rpm. Viscosity measurements were taken with spindle T95. Throughout the study, both the temperature and the size of the sample container were kept constant.

j) Anti-fungal Study: In order to test the plant extracts' anti-fungal activity against the microorganism Malasezzia furfur, microbial tests were conducted on the extracts of reetha (Sapindus mukorossi), shikakai (Acacia concinna), onions (Allium cepa), flax seed (Linum usitatissimum), and neem (Azadirachta indica). Sabouraud Dextrose Agar (SDA) was the culture medium used. The SDA medium was transferred in 30ml quantity into 6 petri plate under laminar air flow arrangement. After that, the petri plates were cooled to solidify the medium. The medium on individual petri plates was then layered with 1ml of the inoculum suspension using spread plate technique. The microbial culture was allowed to diffuse into agar. Using a sterile cork borer, wells measuring 3 mm in diameter were punched into the agar medium. Then, the prepared formulations F1, F2, F3, F4, F5, and the standard Ketoconazole anti-dandruff shampoo (positive control) were added to the wells, which were then incubated for 72 hours at 37 °C. Another set of agar petri plates were prepared and plant extracts of neem, onion, flaxseed, reetha and shikakai were added to the wells. Zone of inhibition was measured in millimetres following incubation. These were compared with a typical zone of inhibition derived from positive control.

RESULTS AND DISCUSSION

a) Physical properties and appearance of formulated shampoo

Table 2: Physical appearance of drug.

Formulation Code	Physical Properties
F1	Formation of agglomerates, red colour, pleasant smell, less foamability
F2	Formation of agglomerates, red colour, pleasant smell, less foamability
F3	Less agglomerates, red colour, pleasant smell, slight foamability
F4	No agglomerates, red colour, pleasant smell, moderate foamability
F5	No agglomerates, red colour, pleasant smell, more foamability

Figure 1: Formulated Anti-fungal shampoo (F5).

b) Determination of pH

The pH of the shampoo solution prepared with 10% distilled water was measured at 25°C. The pH level is crucial for enhancing hair quality, stabilizing the scalp, and reducing eye irritation. To minimize hair damage, it is currently common practice to develop shampoos with a lower pH value. A lower pH (mild acidity) helps to tighten the hair cuticles and prevent swelling, which contributes to a shinier appearance. The results are shown in Table 3. The optimal pH range of shampoo was found be 4-9. Hence the formulation F5 was found to be within this pH range.

Table 3: Determination of pH.

Formulation Code	*pH*
F1	*4.21*
F2	*5.72*
F3	*5.37*
F4	*4.06*
F5	*4.28*

c) Percentage solid content

Research indicates that shampoos with higher solid percentages are more difficult to rinse out of hair, while those with lower solid percentages remain fluid and are removed more quickly. For effective cleaning, an ideal shampoo should contain 8-12% solids. The given formulation F1, F2, F3, F4 has a percentage solid content below the standard range. The percentage solid content of the formulation F5 was found to be 11 % which belongs to the given standard range.

Table 4: Determination of Percentage solid content.

Formulation Code	Percentage Solids
F1	34.50%
F2	18.25%
F3	15.30%
F4	13.25%
F5	11%

d) Dirt dispersion test

In the dirt dispersion test with Indian ink, the volume of ink present in the foam was measured, and the result was categorized as none, light, moderate, or heavy.

Table 5: Determination of Dirt dispersion.

Temperature	Formulation Code	The Loaded Shampoo
At 25°C	F1	VERY HIGH
	F2	HIGH
	F3	MODERATE
	F4	NONE
	F5	NONE

e) Foam stability test

Although the development of foam has little effect on how effectively shampoos clean hair, its value cannot be overstated to the user, making a crucial factor in the assessment of shampoo. Shampoos made using distilled water can facilitate foam. As demonstrated in table 6 below, all shampoos that were tested to determine the foam height at different time intervals. It indicates the high foam stability.

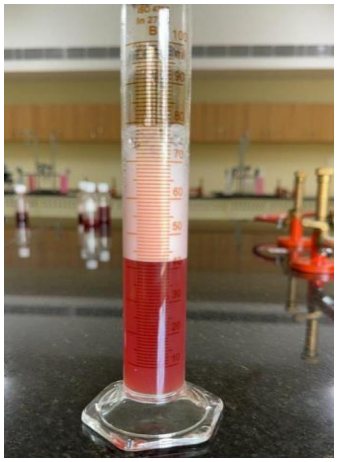


Figure 2: Foam stability of batch F5.

Table 6: Determination of Foam stability.

Temperature	Formulation Code	Foam Height
At 25°C	F1	60 ml
	F2	62 ml
	F3	65 ml
	F4	70 ml
	F5	80 ml

f) Determination of Surface tension

The prepared shampoo was observed to reduce surface tension by approximately 35.18 dynes/cm. Lowering surface tension is a key mechanism contributing to the detergent properties of the shampoo. Effective detergency is often marked by reducing the surface tension of water from its natural level of 72.8 dynes/cm to a range of 32–37 dynes/cm.

Table 7: Determination of Surface Tension.

Sl.no	Formulation Code	Surface tension (dynes/cm)
1	F1	30.15±0.13
2	F2	32.24±0.51
3	F3	31.23±0.43
4	F4	33.43±0.34
5	F5	34.67±0.65

g) Determination of Detergent ability

Though cleaning or soil/sebum removal is the primary aim of shampoo, the experimental estimation of detergency has been difficult since there is no agreement on standard soil. There is no agreeable value for detergency ability of a shampoo. As seen from the results the various concentration of shampoo has varying detergency ability. Shampoo F5 has shown the maximum detergency.

Table 8: Determination of Detergent ability.

Sl.no	Formulation Code	Detergency (%)
1	F1	61.23±0.32
2	F2	63.21±0.04
3	F3	62.07±0.03
4	F4	63.11±0.02
5	F5	65.12±0.12

h) Determination of Cleaning action

Cleaning action was tested on cotton yarn in grease. Although cleaning is primary aim of shampoo, experimental determination has been difficult since there is no real agreement on standard soil. A shampoo should ideally have cleaning ability of 11. As seen from result there has been significant cleaning action for the formulations. The results are shown in table 9.

Table 9: Determination of Cleaning action.

Sl.no	Formulation Code	Cleaning (%)
1	F1	18.81±0.07
2	F2	24.21±0.04
3	F3	28.07±0.03
4	F4	31.11±0.02
5	F5	33.61±0.05

i) Rheological evaluation

The rheological evaluation shows that the viscosity of samples changes gradually with increase in rpm and therefore the shampoo formulations are time dependent. At low rpm the formulation shows the presence of clumps and high viscosity. As the rpm increase the formulations with low viscosity, which increases the flowability and spreadability on hair is obtained.

Table 10: Determination of rheology.

Sl. no	Formulation Code	Viscosity (P)
1	F1	28.11±0.02
2	F2	27.43±0.02
3	F3	25.21±0.02
4	F4	24.21±0.02
5	F5	22.11±0.02

j) Anti-fungal Study

Neem's anti-dandruff activity is attributed to its chemical constituents, including azadirachtin, nimbin, salannin, quercetin, and gedunin, which synergistically control fungal and bacterial growth, reduce inflammation, and soothe the scalp, thereby inhibiting Malassezia growth and improving scalp health, supporting neem's traditional use in treating dandruff and its potential as a natural scalp remedy. Onions contain several chemical constituents that contribute to their anti-dandruff activity, including quercetin, kaempferol, allicin, sulforaphane, and fatty acids. These compounds work together to control fungal and bacterial growth, reduce inflammation, soothe the scalp, prevent flaking, and improve scalp health. Their antifungal, antibacterial, anti-inflammatory, and moisturizing properties make onions a potential natural remedy for dandruff.

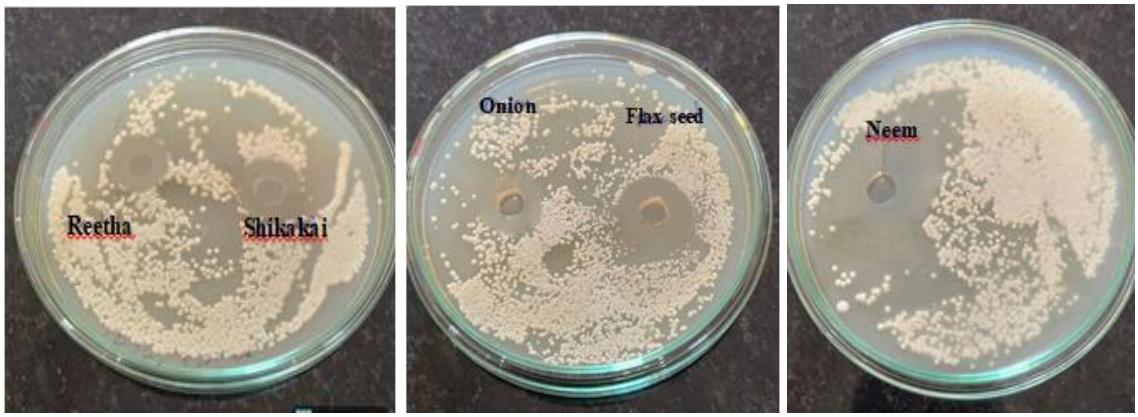
Flaxseed's anti-dandruff and scalp wellness properties are attributed to its chemical constituents, including omega-3 fatty acids, lignans, flavonoids, mucilage, and phenolic acids. These compounds work together to reduce inflammation, control fungal and bacterial growth, moisturize and nourish the scalp, and promote healthy hair growth, making flaxseed a potential natural remedy for dandruff and scalp issues.

Reetha and Shikakai, traditional Ayurvedic herbs, effectively combat dandruff through their active constituents. Reetha's saponins, flavonoids, and tannins, and Shikakai's saponins, flavonoids, and alkaloids, work together to control fungal and bacterial growth, reduce inflammation, remove dead skin cells, and promote healthy hair growth, making their combination a potent natural remedy for dandruff.

Table 11: Zone of Inhibition obtained for different samples.

Formulation code	Mean diameter of zone of inhibition (mm)
Neem extract	26
Shikakai extract	10
Flax seed extract	14
Onion extract	12
Reetha extract	10
F1	8
F2	14
F3	20
F4	28
F5	33
Ketoconazole shampoo (Positive control)	28

The results indicate that all plant extracts and formulations exhibited antimicrobial activity against Malassezia furfur, with varying degrees of efficacy. Neem extract and F5 showed the highest activity among the plant extracts and formulations, respectively.



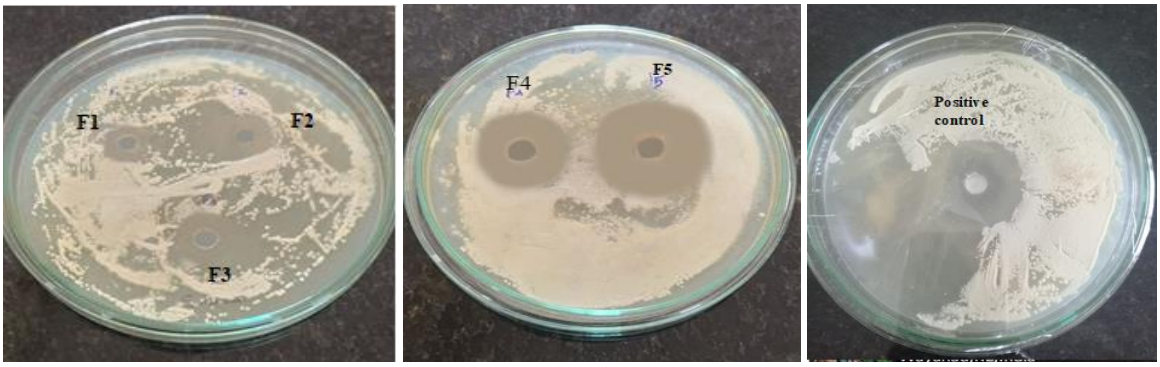


Figure 3: Zone of inhibitions obtained for different samples.

CONCLUSIONS

This study was aimed to formulate an anti-dandruff shampoo. The effect of onion, flax seed and neem on dandruff was also studied. The formulation F5 containing neem, flaxseed and onion extract was chosen as the optimized batch. During the antimicrobial activity the concentration of reetha extract was modified to 2ml for greater zone if inhibition. The batch F5 was chosen as optimized batch. The batch F5 shows required detergency, agreeable viscosity, easy spredability. The results also demonstrate that the variation in concentration of neem and onion extracts effect the physicochemical characteristics and the anti-dandruff activity of shampoo significantly. The optimized batch has shown characteristics activity against dandruff causing agents. Neem has been found to have potential action against dandruff. The fungal pathogens were shown to grow less when exposed to aqueous leaf extract of neem. Onions contain antioxidants and enzymes that protect hair from prematurely graying and also antiseptic and antibacterial properties, which can help with hair loss. Flax seed helps to increase the elasticity of hair, strengthen the hair from root, stop premature hair greying, reduce hair thinning and also reduces the dandruff and itchy scalp. The reetha extract act as a conditioning agent by reducing the frizz and making hair manageable It has also been found that the reetha extract may inhibit the growth of Malassezia furfur which causes dandruff. Shikakai is a Ayurvedic herb that has been used for years as a cleanser to promote healthy, long hair, control dandruff, and treat skin ailments. Glycerin supports the formulation by acting as a humectants and skin protectant. Due to its smooth texture and ease of application, guar gum powder is a natural component used in shampoos. The citric acid used as the pH adjusting agent also plays an important role in controlling dandruff. The chelating capabilities of citric acid is very helpful in soap products. Apple cidar vinegar has been used as the preservative agent. Along with preservation, it also exhibits cleansing action, antifungal properties and also balances the scalp pH.

The limitations of this research point towards topics to be addressed in the future. Irritant test on animals were not studied. Neem, flax seed and onion have been used as antifungal agent in this study. In future, this can be replaced with other natural antifungal extracts.

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CONFLICT OF INTEREST

Authors declare no conflicts of interest in this research work.

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