Section: Research Paper

Assessment of organoleptic and physicochemical properties of herbal shampoos: Formulation considerations of fermentation method

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Abstract

Hair is recognized as the most important organ in the mammalian body that affects self-defense, gender differentiation, extreme temperature protection, and attractiveness. Most of the time, hair loss is permanent and causes alopecia. Due to intense worry and strain, many people who are experiencing hair loss are looking for several therapies, including mythology, conventional and therapeutic healing, and the use of minoxidil and finasteride. Hair root activation is necessary to promote healthy hair growth and stop hair loss. The goal of the current research was to create a herbal hair shampoo for various uses using various plants (hair application). The necessary plant pieces were crushed, boiled, and sieved to create the extract. A variety of criteria, including physical appearance, viscosity, pH, homogeneity, eye sensitivity (the Draize eye test), hair growth activity, hair weight, stability test, and others, were used to evaluate the herbal hair shampoo formulation. These criteria are listed in this text. A prepared herbal hair shampoo with pseudoplastic behavior was discovered to be light brown in hue. The formulation's texture was lubricious and silky, and its pH was within acceptable bounds. After application with little sensitivity the first time, herbal hair shampoo showed excellent hair growth as well as hair weight and was confirmed to be stable for seven days. Recent studies have shown that herbal preparations may improve hair consistency.

Keywords: *Citrus sinesis*, Flaxseed, *Nigella sativa*, *Zingiber officinale*, Castor oil, *Trigonella foenum-graceum*

Introduction

A dynamic and well controlled system behind the development of hair. It is a cyclical system that includes the synthesis, elongation, and eventual loss of hair shafts. Anagen, Catagen, and Telogen hair follicles typically make up human hair. The Anagen hair follicle actively collects cytochrome and produces the hair shaft with ease. The telogen hair follicle is unable to produce newborn hair shafts as the hair follicle evolves into the anagen hair follicle. [1]. Alopecia areata (AA) is an immune-mediated skin condition that often causes non-scarring hair loss. Its frequency is greater in young patients (aged 21 to 40) and there is no discernible difference in incidence between men and females in the general population, which has an incidence range of 1.7 percent to 2.1 percent. This disorder may negatively impact patients' quality of life in a way that is similar to how other skin conditions like psoriasis and dermatitis have been documented to do. The etiology of AA may be attributed to inflammatory cascades that lead to the immune privilege of the hair follicle being compromised, which is connected to T lymphocyte invasion, and an autoimmune process that results in the production of autoantigens from proteins related to melanogenesis. Moreover, a variety of factors, including oxidative stress, diseases, drugs, injuries, possible emotional stress, and family histories, genetic underpinnings, environmental factors, and AA pathogenesis have all been linked [2]. A number of inflammatory skin conditions, including AA, are thought to be significantly influenced by oxidative stress, which has been linked to impaired antioxidant defense or overexpressed reactive oxygen species (ROS). This is because several studies have shown that AA has a negative impact on the balance of oxidants and antioxidants in the blood and skin tissue. A number of medications, including topical and systemic steroids, phototherapy, and immune suppressants like cyclosporine and methotrexate, were used in the most recent clinical treatments for AA with a focus on immune regulation. Additionally, certain vitamins and micronutrients are anticipated to play a role in the treatment of AA and can reduce oxidative stress [3].

Orange peel, or Citrus sinensis, has amazingly beneficial effects for hair. With regular usage, these peels may make hair smoother and shinier. They are teeming with antioxidants and assist in treating the damage brought on by contaminants. Orange peels, which are rich in vitamin C, may be used regularly to make dry, dull hair more bouncy and lustrous. Orange peels, a rich source of vitamins B12 and E, aid in the growth of hair. Moreover, they slow down the onset of graying hair [4, 5].

The highest plant source of -3 fatty acids, or "-linolenic acid," is flaxseed (ALA). Low in saturated fatty acids (9%), high in monounsaturated fatty acids (18%), and high in polyunsaturated fatty acids (73%) are the fatty acid compositions of linseed oil [6]. The reduction of cardiovascular disease, atherosclerosis, asthma, cancer, obesity, osteoporosis, autoimmune and neurological diseases is one of the potential health benefits of flaxseed oil, fibers, and lignans. Moreover, flaxseed is a rich source of fatty acids and antioxidants that help to clear the scalp of impurities and dead skin cells. As a moisturizer for the scalp and hair, flaxseed gel may be used to encourage growth and improve the condition of existing

hair. Flaxseed gel has excellent conditioning properties, is quite moisturizing, and leaves hair feeling very fluffy [7, 8].

Black cumin is the popular name for the annual blooming plant Nigella sativa (NS), which is native to Pakistan, India, and the Mediterranean region. Thymoquinone (TQ), dithymoquinone (DTQ), thymohydroquinone (THQ), and thymol (THY) are the main pharmacologically active components of NS [9]. TQ, which makes up the majority (30% - 48%) of NS, has been shown to prevent inflammatory cell infiltration in the brain by inhibiting NF-kB activation. TQ has anti-inflammatory properties by reducing prostaglandin D2 (PGD2) production and cyclooxygenase 2 (COX-2) expression, which explains why NS has been used as a natural remedy for several diseases and inflammatory disorders [10]. Moreover, new research has shown that PGD2 has a key inhibitory role in the hair follicle; in particular, its interaction with the GPR44 receptor found there seems to result in follicle shrinking. This aids in the development and maintenance of vellus-like hair and is believed to prevent stem cells from developing into progenitor cells and vellus-like hair from turning into terminal hair that is naturally thick and pigmented. Hence, PDG2 activation and synthesis as well as NF-kB suppression by NS may help to control the hair cycle. Black seeds are found in the flower Nigella sativa, which is indigenous to Eastern Europe, Southwest Asia, and the Middle East. The spices kalonji, black caraway, black cumin, and black onion seeds are well known. For thousands of years, these seeds have been utilized in the treatment of gastrointestinal worms, arthritis, diabetes, headaches, and allergies. A 2016 research found that nigella sativa seed is a perfect component for pharmaceuticals and cosmetics. According to the study, black seed oil has antibacterial, antifungal, anti-inflammatory, and antioxidant activities. Black seed oil proponents assert that by addressing issues like dandruff while keeping the scalp hydrated, these qualities may assist maintain scalp protection. Black seed oil is ideal for calming scalp disorders that cause inflammation, flakiness, and pain since it has anti-inflammatory properties when mixed with a carrier oil [11]. Moreover, it eliminates viruses and fungi, thus it might benefit those with lice. Like lavender, lemon, and rosemary essential oils, black seed oil is an adjuvant, ensuring that it blends well with other components to have medical benefit. Black seed oil has anti-rheumatic properties, but it also works wonders to treat joint ailments brought on by aging and sports activity. As far as natural hair is concerned, black seed oil may assist in regrowing those thinning areas on your scalp. For thousands of years, Nigella sativa oil has been used often to cure hair loss [12].

In addition to micronutrients like B-vitamins, antioxidants, and trace elements that are found in the hair, fenugreek seed extract is a nutritional supplement. Leguminous fenugreek (Trigonella foenum-graceum L.) is a perennial plant. Southern Europe, Western Asia, and the Mediterranean are the original home regions of fenugreek. A large variety of active compounds, including saponins, particularly diosgenin, yamogenin, and gitogenin derivatives, trigonelline alkaloids, flavonoids, galactomannan vitamins, and fiber, are found in fenugreek seeds [13]. The seeds have a pleasant aroma and a tart taste. It has a long history in the ancient world as a food and medicine plant. Fenugreek seeds have attracted a lot of attention recently due to their medicinal potential, which includes their use as a hypoglycemic, antiulcerogenic, hypo-cholesterolemic, and antihypertensive drug [14]. Fenugreek is recognized to promote healthy hair development, although the exact mechanism

has not been identified. Fenugreek is said to interact physiologically with the production of DHT (dihydrotestosterone) by increasing the blood flow to hair follicles and steroid saponins. One possible cause of both male and female variant hair loss is the effect of DHT on genetically predisposed hair follicles. When DHT is bound to the hair follicle, the hair gradually shrinks and eventually falls off [15, 16].

The root of Zingiber officinale (L.) Rosc is ginger, one of the most widely used spices in the world. It has a number of active components, including beta bisabolene, shogaol, zingerone, and gingerol. As a folk remedy, it has been used for a number of illnesses including rheumatoid arthritis, neurological disorders, inflammation, and asthma. Several of its active ingredients have anti-diabetes, anti-cancer, and anti-inflammatory properties in addition to significant antioxidant activity and the decreased expression of a few pro-inflammatory indicators. Ginger also works well as a therapy for dandruff and an inflamed, itchy scalp. It naturally reduces swelling and is antibacterial, which helps to keep the skin clear and wholesome. In general, ginger promotes hair development, inhibits hair thinning, and makes hair shiny and smooth [17, 18].

Castor oil, sometimes referred to as Ricinus communis, is a nutrient-rich vegetable oil made from castor beans. While it comes from tropical East Africa, it is often used for aesthetic and therapeutic reasons in India and the West Indies. Except when it comes from roasting or boiling, castor oil, often known as black castor oil, is normally translucent or light yellow. At the molecular level, castor oil is a long-chained fatty acid. According to Bridgette Hill, "Fatty acids feed hair follicles vital proteins and nutrition and prevent inflammation of hair follicles." Castor oil is not only anti-inflammatory, but it also has a few other noteworthy properties. The advantages of using castor oil include: "increasing the gloss or shine of the hair, increasing the elasticity of the hair shaft by coating the hair shaft, and offering antibacterial qualities." Ricinoleic acid makes about 90% of the fat in castor oil. It is thought to stimulate the prostaglandin E2 receptor, which may help to dilate the blood vessels. The powerhouse of the follicle, the dermal papilla or hair root, receives more oxygen- and nutrient-rich blood when increased blood vessel dilatation is applied to the scalp [19].

The goal of the current research was to create a herbal hair shampoo for improving hair quality using extracts of several plants.

Material and methods

Collection of plant part

For the preparation of herbal hair oil various plant materials was collected viz., *Citrus sinensis*,

Linum usitatissimum, Nigella sativa, Zingiber officinale and *Trigonella foenum-graecum* from the Medicinal Plant Garden of Pranveer Singh Institute of Technology, Kanpur, Uttar Pradesh, India and was properly authenticated in the Department of Pharmacognosy.

Formulation of herbal hair shampoo

Table 1 presents the different ingredients used in the formulation of herbal hair shampoo. All fresh herbs, such as *Citrus sinensis peel, Zingiber officinale* roots and *Linum usitatissimum seeds, Nigella sativa, Trigonella foenum-graecum* were specifically weighed and dispensed in 500 ml of water. The contents referred to above were boiled for 15 min. It was permitted to

cool after 15 minutes of boiling and then filtered. Castor oil and vitamin E were added to filtrate. Prepared shampoo in a spray bottle.

Table 1: Ingredients used in preparation of herbal Hair Shampoo

Ingredients	**Plant part**	**Quantity (%)**
Citrus sinensis	Peel of fruit	70
Zingiber officinale	Root	5
Linum usitatissimum,	Seed	5
Nigella sativa,	Seed	8
Trigonella foenum-graecum	Seed	10
Castor oil	-	0.5
Vitamin E	-	1.5

Evaluation of The Hair shampoo

Physical appearance

The physical appearance, color and feel of the prepared herbal hair shampoo is visually tested for physical appearance. Table 2 reflects the outcomes.

Homogeneity Test

A clean and dry object glass was smeared with the hair shampoo and a cover glass was sealed. The appearance under light of some coarse particle/homogeneity was investigated. Herbal hair shampoo was tested by visual examination for homogeneity and tested for some lumps, flocculates or aggregates [20].

pH Test

The pH meter was calibrated using pH 4 and pH 7 buffer solutions. The electrode was soaked in the hair shampoo and left until the pH normalized after a few minutes [21].

Table 2: Evaluation parameters of herbal hair shampoo

Parameters	**Results**			
Physical appearance	Yellowish brown			
Homogeneity	Good			
pH*	6.7±0.022			
Viscosity (cps) *	**10 RPM**	**20 RPM**	**50 RPM**	**100 RPM**
	7800±0.011	5950±0.023	1860±0.009	2220±0.052
Spreadability	Good			
Draize eye test	Mild irritation			
Hair length (mm)*	7^{th} Day	14^{th} Day		21^{st} Day
Normal control	1.22 ±0.14	1.53±0.11		2.15 ±0.27
Negative control	1.46 ±1.93	1.59 ±0.07		2.89 ±0.16
Herbal hair shampoo	3.27 ±1.36	3.99 ±0.19		6.77 ±0.14
Weight of the hair (mg) on 3rd week of the treatment	224.98			

*the value is expressed as mean ±SD, n=3

Viscosity

On a Brookfield viscometer (RVDV-II+PRO), spindle number 6 was used to test viscosity. 50 ml of hair shampoo was poured in the beaker, and viscosity measurements were taken at 10, 20, 50, and 100 rpm [20].

Spreadability

The spreadability of preparations that are semi-solid was evaluated and assessed using a parallel plate method. A gram of hair shampoo was compressed between two 20 by 20 cm horizontal plates, the top of which weighted 125 g. After one minute, the spread diameter was measured. The formula used to determine spreadability was S = M L / T.

Where S is spreadability, M is weight in the pan (attached to the higher slide), L is distance traveled by the glass slide, and T is the amount of time (in seconds) needed to fully separate the slides from one another [21].

Check the eye's sensitivity (Draize eye test)

In physiological NaCl, one drop of hair shampoo was injected into the rabbit's left eye (as the control is the right eye). The following times were noted: 30 minutes, 60 minutes, 120 minutes, 240 minutes, 1 day, 2 days, 3 days, and 4 days. It established the cornea, iris, and conjunctiva scores [21].

Test of hair growth activity

Three 4x4 cm portions on each foot, right and left, of the rabbits' shaved backs were subjected to this test. After applying depilatory cream (Veet ® cream) for 3 to 5 minutes, the area was washed with water until it was hair-free. 70 percent ethanol was then added as an antiseptic. The rabbit was left behind for 24 hours before any action assessment was finished. Treatment 1 served as the standard control since there was no intervention, but Treatment 2 served as the positive control because the test substance was added to the hair shampoo. Treatment 1 served as the standard control since there was no intervention, whereas Treatment 2 served as the positive control by including the test substance into the hair shampoo. The rabbit was subsequently given 0.1 ml of each medication twice daily for three weeks. Day 0 of using the hair shampoo was designated as such [5, 6].

Evaluation of hair growth's quality

Visual inspection of two criteria, initial hair growth time (the shortest amount of time required for hair to grow on the shaved area, measured from the darkening of the skin color showing initial hair growth), and completion time for hair growth, was used to measure the quality of hair growth analysis (minimum time required for the entire shaved area to be covered with new hair) [22].

Observations of hair length growth

On the days of 7, 14, and 21, 10 randomly selected hair strands from each box were collected. The hair was dragged out, made straight, and adhered to the tape. Mitutoyo Digimatic wireless calipers were used to measure. To determine if the difference between the research area and the control was statistically significant, the average length gathered was examined [4, 21].

Hair measurements are made

The hair was taken on day 21, weighed, and statistically measured to determine the weight of each box [3, 20].

Microbiological contaminant

A thin loopful of hair shampoo was put over nutritional and Sabouraud agars, and the samples were then incubated for 48 hours at 37°C to detect any microbial contamination. One gram of the material was distributed in 4 ml of sterile Ringer solution containing 0.25% Tween 80 in order to assess the level of contamination. The required dilutions were prepared in the same dispersion vehicle, and 0.5 ml was mounted on the appropriate solid medium using the surface viable technique. Emergent colonies were counted after the necessary incubation period [23, 24].

Stability

The herbal hair shampoo was stored for three months at 65% RH at two different temperatures of 4°C and 30°C. After three months, the pH and viscosity of the herbal hair shampoo were measured and compared to their initial values [21].

Findings and analysis

Physical attributes

All of the herbal hair shampoos had a light brown hue with a transparent appearance, and upon application, they were discovered to be smooth.

Homogeneity

The manufactured herbal hair shampoo was visually inspected for the look and presence of any lumps, flocculates, or aggregates. It has been shown that produced shampoo homogeneity is satisfactory.

Rheological study

The rheological examination of prepared herbal hair shampoo was conducted and it was observed that viscosity was reduced as the shear rate increased (Figure 1).

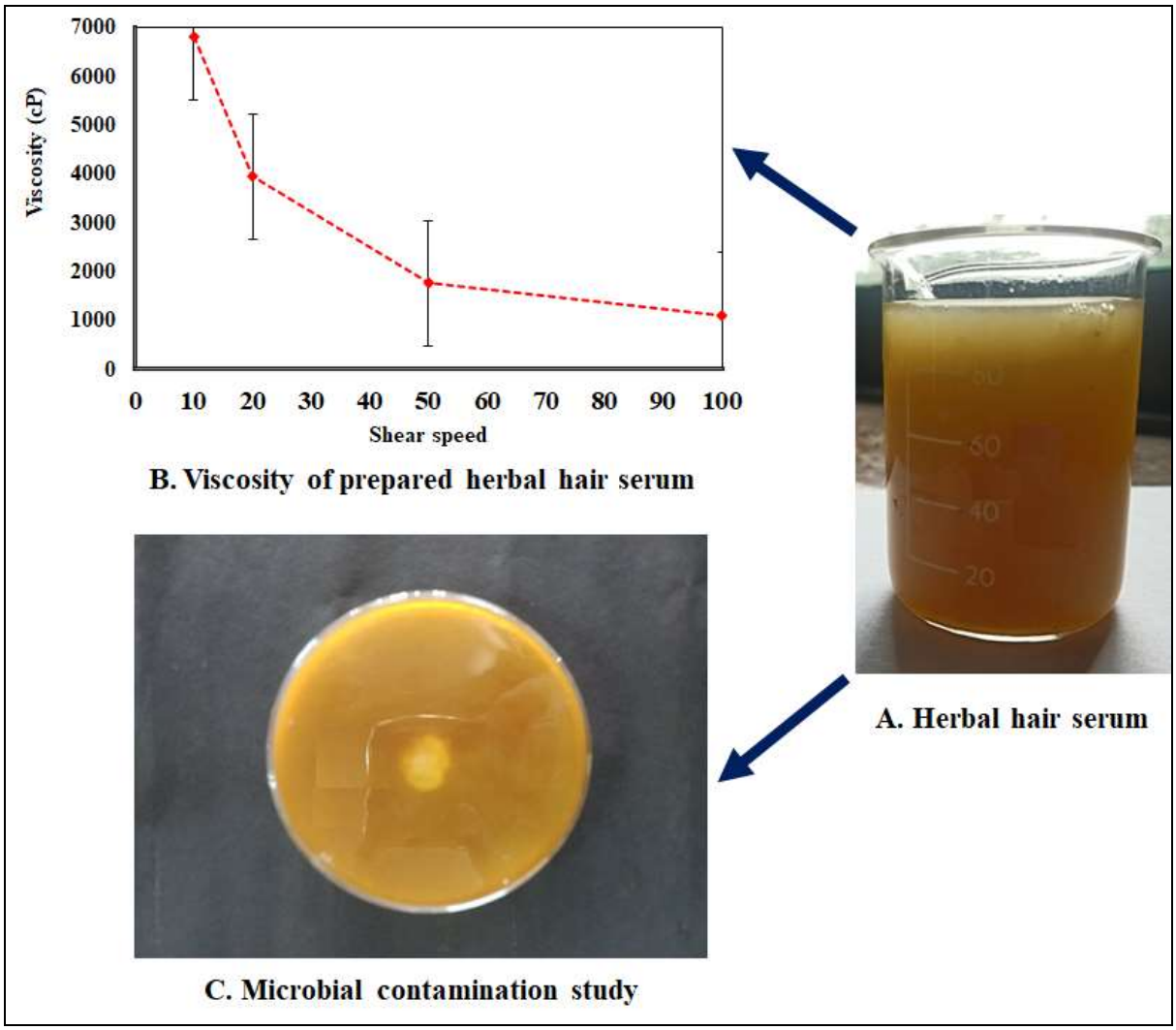

Figure 1: Figure showing A: Herbal hair shampoo; B: Rheological study; C: Microbial contamination study

Pseudoplastic flow was also described, which is a beneficial attribute for topical herbal hair shampoo since it guarantees optimum area coverage upon application. The mechanistic explanation for the flow behaviour found is as follows: the long-chain molecules of the polymers are spontaneously arranged in dispersion under standard storage conditions. These molecules tend to organize their long axes in the direction of the force exerted when applying shear stress. This stress induced orientation reduces the internal resistance of the material. In addition, the solvent molecules which was earlier associated with the polymer molecules will also be released. Thus, the effective concentration and size of the molecules are low. Now, the material allows greater shear rate on progressive increase in the shearing stress [19].

pH determination

The pH of the whole herbal hair shampoo was 7.3, which was sufficient for the hair, suggesting that the herbal hair shampoo was consistent with the hair [19].

Draize eye test

To evaluate the protection of the herbal hair shampoo, a sensitivity assessment test was performed. To determine the ocular sensitivity index, the test was conducted on 3 rabbits.

Tests on the left eye were conducted with controls on the right eye. In physiological saline, 3 drops of sterile 2.5 percent herbal extract were added to the left eye of the rabbit, with measurements of 30 minutes, 60 minutes, 120 minutes, 240 minutes, 1 day, 2 days, 3 days, 4 days. There was no image of opacity in the left eye, normal conjunctiva, no swollen eye lid, and the iris image appeared normal. It was seen that the eyes created tears. The index of ocular annoyance was 2 on the first day and 0 on the second, third and fourth days (maximum ocular irritation index of 110). Thus, it can be inferred that there was moderate inflammation in the herbal shampoo [21].

Hair growth activity test

Table 2 shows that normal control showed a very close outcome with negative control for the first week, while positive control showed little difference. However, in areas treated with positive regulation, there is a substantial hair length difference between both normal control and negative control and hair. However, a major difference was reported by comparing the growth behavior displayed by normal control, negative control and positive control. The discrepancy between the groups was found to be important ($p<0.05$). Thus, it was found that, although using a single formula, the hair shampoo used in this study displayed a more important hair growth activity of 6.98 mm. In Table 2 it can be seen that in the third week, herbal hair shampoo corresponded to a greater weight [21, 22].

Microbial Contamination

At the end of seven days, the microbial contamination of the herbal hair shampoo after 24 hours was observed to be 1.89 CFU for fungi [23].

Stability Studies

Table 3 revealed that the herbal hair shampoo was stable during the research time, as these shampoos showed no physical instability and there was no noticeable difference in the pH before and after the study [23, 24].

Table 3: Stability study of Herbal hair shampoo

pH*		**Viscosity at 100 rpm (centipoise) ***	
Initial	Final	Initial	Final
6.8±0.022	6.9±0.012	1112±0.002	1123±0.117

*the value is expressed as mean ±SD, n=3

Conclusion

According to research, herbal hair shampoo offers a range of vital nutrients required to maintain the sebaceous glands' healthy operation and promote the development of natural hair. The usage of herbal cosmetics evolved significantly in terms of personal hygiene and the healthcare system. As a result, there is a lot of commotion in the herbal cosmeceutical personal care or health care sector, which is genuinely focusing and paying particular attention to the manufacture of herbal-based cosmetics. As it is today, the industry is rapidly expanding in the future years and has an enormous potential for multiple boost. Bioactive chemicals are used in cosmetic formulations because they have a positive effect on bodily traits and provide nutrients necessary for maintaining healthy, attractive hair. It may be concluded that herbal hair shampoo that has been made has a positive impact on the mechanism of hair growth and consistency improvement**.**

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No Animals/Humans were used for studies that are base of this research.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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