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Sustainable Use of Traditional Plant Extracts for the Formulation of Herbal Shampoos

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Abstract: Skin and hair care products described with natural bioactive compounds have been considered as a better alternative to synthetic ones. Therefore, this study aimed to develop a natural shampoo using traditional plant extracts. With an emphasis on safety and efficacy, its physicochemical properties were evaluated and compared with the marketed shampoo. Each plant material was ground and extracted using 70% hydro-alcoholic solvent for 5-7 days. Shampoo formulation was prepared using sprouted fenugreek seeds extract and pea fruit extract as key ingredient along with other natural ingredients including: reetha, amla, marigold, orange peel, castor oil, glycerine and distilled water. The physicochemical parameters and antioxidant activity of both formulations were ascertained to ensure the safety and efficacy of the designed formulation. According to the present study, the developed shampoo (F1 & F2) was yellowish green and brown in color, had a good odor and optimum pH value of 5.32–5.96. The viscosity of F1 and F2 was found to be 2.3 to 2.08 poise; both formulations reduced the surface tension to 49.08 and 50.7dynes/cm respectively. While the solid content of F1 & F2 shampoos was found to be 20.57 and -21.87%. The developed shampoo (F1 & F2) exhibited significant antioxidant activity with IC_{50} values of 53.46 (F1) & 69.4 µ/ml (F2), respectively. The findings of this study demonstrated that shampoo formulations were found to have functionality comparable to physicochemical features of marketed shampoo. However, further studies are necessary to validate the performance and quality of the shampoo.

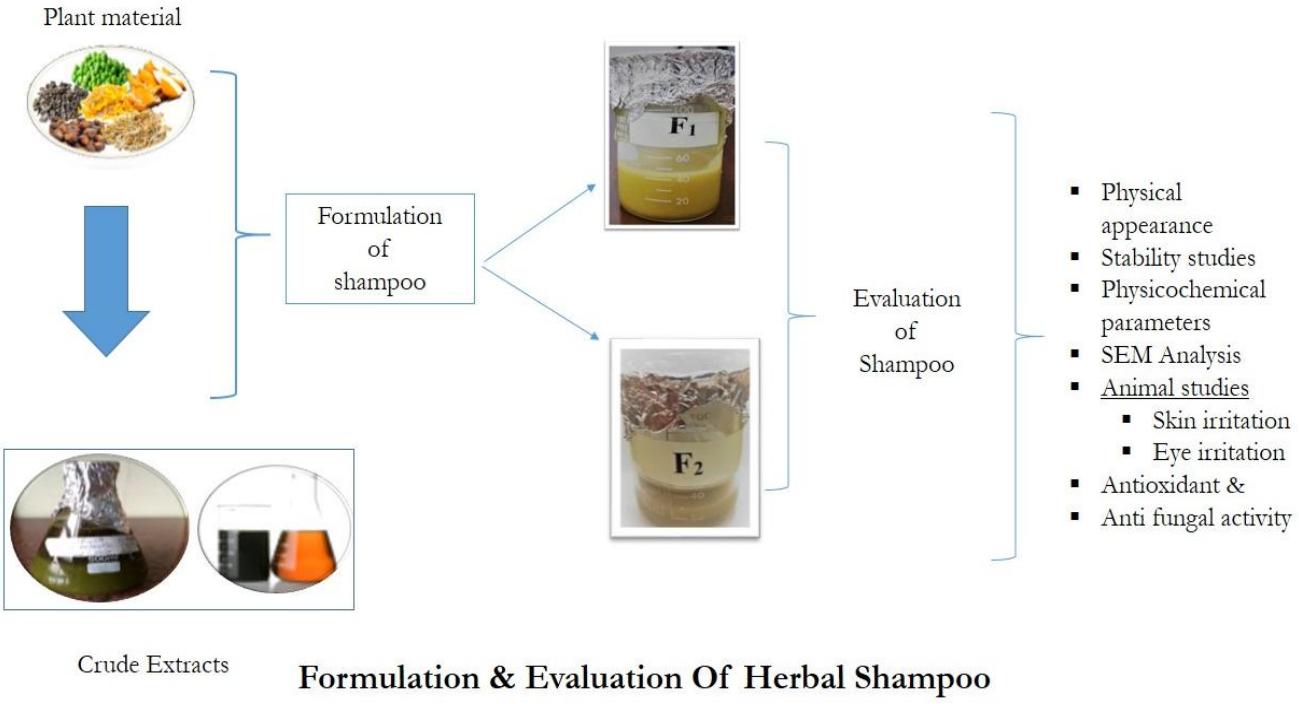
Keywords: herbal shampoo, shampoo formulation, traditional plant extracts.

可持续使用传统植物提取物配制草本洗发水

摘要：用天然生物活性化合物描述的皮肤和头发护理产品被认为是合成产品的更好替代品。因此，本研究旨在开发一种使用传统植物提取物的天然洗发水。着重于安全性和有效性，对其理化特性进行了评估，并与市售洗发水进行了比较。将每种植物材料研磨并使用70%

的水醇溶剂提取5-
7天。洗发水配方使用发芽胡芦巴种子提取物和豌豆果实提取物作为主要成分，以及其他天然成分,包括:雷萨、反洗钱法、万寿菊、橙皮、撒麻油、甘油和蒸馏水。确定了两种制剂的理化参数和抗氧化活性，以确保设计制剂的安全性和有效性。根据目前的研究，开发的洗发水（F1和F2）呈黄绿色和棕色，具有良好的气味和5.32-
5.96的最佳酸碱度值。发现F1和F2的粘度为2.3至2.08泊；两种配方均将表面张力分别降低至49.08和50.7达因/厘米。而F1和F2洗发水的固体含量被发现为20.57和-
21.87%。开发的洗发水（F1和F2）表现出了显着的抗氧化活性，半数最大抑菌浓度值分别为53.46(F1)和69.4微/毫升(F2)。这项研究的结果表明，洗发水配方的功能与市售洗发水的理化特性相当。然而，还需要进一步的研究来验证洗发水的性能和质量.

关键词：草本洗发水，洗发水配方，传统植物提取物.



Graphical abstract

Introduction

Shampoo is one of the most popular hair care products extensively used to remove dandruff, dirt, surface grease, sebum, or other fatty substancesfrom hair and scalp [1]. The word *shampoo* derives from the Sanskrit and Hindi word "*chapayati*" *or* "*champoo"* meaning "massage", "to soothe", "to push" or "to mold" [2]. Herbal shampoo was introduced in India and individuals used various Ayurvedic extracts for cleaning and beautifying their hair. Consequently, the concept of "*champooi*" or "*shampooing*" was presented by a Bengali traveller and entrepreneur in the 1800s. The first synthetic shampoo was introduced in 1930 by Drene. The shampoo contains water, a mixture of surfactants, foaming agents, viscosity modifiers, preservatives, fragrances, dyes, antioxi-dants, conditioning agents, and active ingredients [3]. An ideal shampoo should generate good foam content to fulfill the psychological state of the consumer. In addition, it should be easily removed from hairs upon rinsing with water, leaving the hair non-dry, soft, and minimum fly away. Additionally, it must be non-toxic and non-irritant to skin, eyes, and scalp and impart a pleasant aroma to the hair [4]. Although several synthetic, non-medicated, and medicated hair care products are extensively available in the local market, they are proven to be beneficial for hair growth. Conventional synthetic shampoos contain various chemical ingredients that potentially have detrimental effects, including dryness of hair, split ends, reduction

in the size or weakening of hair follicles, premature graying of hair, irritation to eyes, and skin [5].

Accordingly, customers' demand for cosmetic, skin, and hair care products derived from natural ingredients has increased so far. Traditional plants, herbs, and shrubs have been used extensively since prehistoric times for health care and cosmetic purposes. Medicinal plants are enriched with various bioactive constituents, namely phenolic compounds, beta carotene, tannins, flavonoids, saponins, fixed oils, essential oils, amino acids, terpenoids, alkaloids, hormones, enzymes, minerals, and probiotics [6, 7]. These bioactive phyto-constituents have moisturizing, nourishing, cleansing, and conditioning effects on hair and improve the texture and overall appearance of the hair. Additionally, it stimulates the growth of new hair, retains natural moisture, prevents premature graying of hairs, and removes dandruff flakes owing to its antibacterial and antioxidant properties [8]. Several hair care products described with natural ingredients, for instance, hair tonics, hair oil, shampoo, hair mask, conditioner, and serums have been used as an alternative to synthetic ones. For instance, organic green tea, aloe vera, shikakai, reetha, henna, amla, hibiscus, marigold, neem, fenugreek, licorice, and onion promote hair growth and nourish the hair follicles [9, 10]. Though the harmful effects of synthetic chemical-based shampoos are detrimental, natural ingredients could aid in improving properties, added benefits, and cost-effective manufacturing processes [11]. Therefore, the aim of the present study was to design and develop a natural shampoo using traditional plant extracts, namely sprouted fenugreek seeds, peas, soapnut, amla, marigold, and orange peel extracts. The physic-chemical properties of the formulations were ascertained and compared with the marketed shampoo, as well as performance, quality, and effectiveness were measured.

1. Materials and Methods

1.1. Plant Materials

The plant materials used for shampoo formulation include amla, soapnut, marigold, dry orange peel, pea, and fenugreek, which were acquired from the local market of Karachi-Pakistan. It was then authenticated via Department of Pharmacognosy, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Pakistan. All reagents and chemicals used in the studies were of analytical grade and procured from the local supplier of Karachi, Pakistan.

1.2. Preparation of Sprout Fenugreek Seed

Approximately 250 g seeds of *Trigonella foenum-graecum* were soaked in aloe gel (100 g) for 5-7 days. It was covered with muslin cloth and kept moist with distilled water for germination. After 7 days the seeds were sprouted. The germinated seeds were then dried under the shed and ground into coarse powder.

1.3. Preparation of Plant Extract

The plant material, for instance, *Sapindus mukorossi*, *Emblica officinalis* (fruit), *Citrus sinensis* (dry peels powder), *Calendula officinalis* (petals), sprouted seeds *of T. foenum-graecum* and *Pisum sativum* (250 g each) were ground separately and subjected to cold maceration using 70% ethanol-water for about 5-7 days. Subsequently, all liquid extracts were filtered separately and concentrated in a vacuum rotary evaporator at 40-50°C. The obtained semi-solid extracts were then stored in a glass bottle and placed in a refrigerator at 4°C until further study.

1.4. Phytochemical Screening

Phytochemical screening of sprouted fenugreek and pea extract was performed using the standard method [12].

1.5. Preparation of Shampoo Formulations

Shampoo formulations (F1 and F2) were prepared using an emulsion formula of oil: water: gum (O: W: G; 4:2:1) as the reported method with slight modification [13]. The xanthan and acacia gum made oil in water (o/w) emulsion. Formulation F1 contains crude extracts of amla, soapnut, marigold, orange peel and sprouted seeds extract of fenugreek as an active ingredient. While F2 contains pea extract as an active ingredient, other ingredients remain the same as the F1 formulation. All extracts were weighed accurately and mixed thoroughly in container. Castor oil was then added gradually and the mixture was triturated till an ideal homogenous dispersion was attained. The acacia and xanthan gum was dissolved separately in warm glycerin (50%) and occasionally for 5-10 minutes until completely dissolved. The xanthan gum and acacia gum solutions were mixed and stirred periodically. The mixture of plant extracts and castor oil was then added to the gum solution (1%) and gently mixed with constant stirring for 10-15 minutes. Additionally, a few drops of rose oil were added to the shampoo formulation to impart the aroma. The pH of designed formulation was adjusted with citric acid. The final volume was made 100 ml with distilled water and glycerin. The solution was then set aside for 24 hours at room temperature to thicken the shampoo solution. After that, it was stored in a glass container and placed in a refrigerator until further studies.

1.6. The Physicochemical Evaluation of Developed Shampoo

The physical appearance (color, odor, texture) and physicochemical parameters, for instance, pH, viscosity, and surface tension of both formulations (F1 & F2) were evaluated as the reported method [14].

1.6.1. Stability Study

The shampoo formulations were placed in a stability chamber at 40 ± 2°C temperature and 75% ± 2 relative humidity. The stability studies were recorded for 3 months at an interval of one month [15].

1.6.2. Determination of Solid Content Percentage

Approximately 4g of developed shampoo (F1 & F2) was transferred to the pre-weighed evaporating dish. The weight of the shampoo with the evaporating dish was noted. It was then positioned in the muffle furnace at 600°C for about 5 to 10 minutes until the liquid portion of the shampoo was completely dried. Subsequently, the solid content was determined using the following equation:

% age of solid content = Final weight – weight of the empty dish/4 x 100 (1)

1.6.3. Foam Stability

The cylinder shake method was executed to assess the foaming capacity of the shampoo formulations [16]. Around 50 ml of shampoo solution (1%) was shaken vigorously for 8-10 minutes. The volume of the foam content produced was noted immediately in the first seconds and after 2 min, 4 min, 8 min and 10 min of regular intervals of time.

1.6.4. Wetting Time

A canvas paper was cut into a 1-inch diameter in the form of a disk (average weight; 0.44 g). The paper was then saturated in 1% (v/v) shampoo solution. The time required for the paper to soak completely was then noted. It was considered as the wetting ability of the shampoo [17].

1.6.5. Measuring the Detergency Ability

The Thompson method was used to evaluate the detergency capacity of the shampoo formulation. The hair swatch was rinsed with 5% solution of sodium lauryl sulfate. After drying, it was separated into 3 small hair bunches (weight; 3 g; length; 10 inches each). The hair samples (3 g) were suspended in 20 ml of hexane solution having artificial sebum (10%). It was occasionally for 10-15 min. Subsequently, the solvent was removed, the hair samples were weighed and the content of sebum was noted. After that, each hair sample was divided into 2 equal portions; one portion was washed with a 10% shampoo solution. It was then dried with hair dryer. After drying, the residing sebum on the hair sample was again treated with hexane (20 ml) and weight was recorded. The other part of the hair sample was considered a negative control. The detergent capacity was determined using Equation 2.

% age of DP = 100(*1-T/C*) (2)

where DP - % age of detergency power, C - the weight of sebum in the control sample, T- the weight of sebum in the test sample.

1.6.6. Cleaning Action

Around 4-5 g of wool yarn was greased and then transferred into a conical flask having 1% shampoo solution. It was then agitated vigorously for 2-4 minutes. After drying the weight of wool sample was noted. Equation 2 was used to assess the amount of grease removed.

1.6.7. Dirt Dispersion

In a test tube filled with distilled water (10 ml), a few drops of shampoo formulation were introduced. The test tube was shaken vigorously for 3 to 5 min after 1 drop of Indian ink was added. Heavy, moderate, light and none were noted as the ink concentrations in the foam.

1.6.8. The Evaluation of Conditioning Performance

The conditioning effect of the shampoo was assessed as described in the method [18]. Women hair clusters were procured from the local beauty salon. It was cut into 4 swatches (length 10 cm; weight 5 g). One sample of hair swatch without washing with shampoo was considered as control. The remaining 3 tresses were washed identically with the marketed shampoo formulations and served as a test swatch. Each tress was then shaken with the shampoo solution (10 g shampoo; 15 g water) in a flask for about 2-3 minutes. After that, it was washed with 50 ml water. Each hair tress was then dried at room temperature. The procedure was repeated for 4-5 cycles. The conditioning performance of the formulated shampoo was scored from 1 to 4 (1-poor; 2-satisfactory; 3-good; 4-excellent). The volunteers were blindfolded and asked to touch the tresses and rated from scores 1 to 4.

1.7. Surface Characterization of Hair

A scanning electron microscope (JSM-63680, Leo Electron Microscopy Ltd., Cambridge, England) was used to evaluate the morphology of the hair surface [19]. Under SEM, the hair strands were affixed with double-side stitching tape coated with gold film (thickness 200nm) under low pressure (0.001 mm of Hg). Sodium lauryl sulfate (SLS; 1%) solution was used to wash the hair sample, and it was then dried with a blow dryer. The hair sample was subsequently divided into 4 categories.

Control (without treatment) was considered as Group I, whereas Group II hair strands were washed with a marketed shampoo solution (10%). The hair samples in Group III and IV were treated with F1 and F2 shampoo solutions (10%) respectively. The photomicrographs were taken at appropriate magnification to characterize the surface of hair strands.

1.8. Biological Activity

1.8.1. DPPH Free Radical Scavenging Activity

The antioxidant capacity of the shampoo formulation was ascertained using the DPPH free radical scavenging method [20]. Around 100 µl of shampoo formulation with concentrations ranging from 50-400 µg/ml were mixed with 3 ml of DPPH solution (0.1 mM). After agitation, the mixture was then kept in dark room for 25-30 minutes at room temperature. Subsequently, the absorbance was noted at 517 nm using UV-visible spectrophotometer. Ascorbic acid (50-400 µg/ml) was used as a reference standard. All experiments were conducted in triplicate. The following equation 3 was used to determine the percentage of inhibition:

$$Inhibition\ (\%) = (A0 - A) / A0 \times 100 \quad (3)$$

where A0-absorbance of the control (DPHH solution), A-absorbance of the shampoo solution/standard.

1.8.2. Antifungal Activity

The antifungal property of the formulated shampoo was determined using the disc diffusion method with minor modifications [21]. *Candida albicans* were cultured on sabouraud dextrose agar medium (around 20 ml) and incubated overnight. Sterilized filter paper disks (5 mm in diameter) were impregnated with shampoo solution at 20-60 µl/disc. The filter paper loaded with shampoo solution was placed in an inoculated Petri dish and incubated at room temperature for 48 hours. Subsequently, the diameter of the zone of inhibition after incubation was recorded in mm. The sterilized water (20 µl/disc) was used as a negative control, while fluconazole (20 µl/disc) served as a positive control. The experiment was performed in triplicate.

1.9. In Vivo Studies

1.9.1. Ethical Approval

The research protocol was approved by Advanced Board of Studied and Research (ABSR) Resolution No. 10(P) 06, University of Karachi, Karachi - Pakistan. Additionally, ethical approval was obtained from the Institutional Bioethical Review Committee (IBCKU-219/2021B), University of Karachi, Pakistan.

1.9.2. Experimental Animals

A group of healthy Albino Wistar rats (180-250 g) and rabbits (2-3 kg) of both sexes were procured from the animal house of the Department of Pharmacology, Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Pakistan. The animals were housed under standard laboratory conditions with 12/12 hours light/dark cycles, relative humidity (50-60 ± 0.2%), and 22 ± 2°C temperature, respectively. Prior to the study, the animals were fed a standard diet with water *ad libitum* and acclimatized to laboratory conditions for 7 days. The experimental animals were split into 4 groups (n = 7; in each). Group I was considered as a control (without treatment). Groups II, III and IV were treated with shampoo formulation (F1 & F2) and marketed shampoo accordingly. In addition, the protocol for the care of laboratory animals was reviewed by the Animal Experimental Ethics Committee of Faculty of Pharmacy & Pharmaceutical Sciences, University of Karachi, Pakistan.

1.9.3. Skin Irritation Tests

The open patch method was executed to determine the skin irritation test. A small quantity of shampoo solution (1%) was applied on the posterior side of the rats after shaving an area of 1-2 cm^2 to each group (n = 7). Any signs of irritation like prickling, abrasion, redness or rashes, soreness, or edema on the skin were noted for 2 hours at a regular interval of 30 min after application.

1.9.4. Eye Irritation Tests

Optical inflammation analysis of the formulated shampoo was investigated in the eyes of rabbits [22]. Approximately 2% of the shampoo solution was dripped into the rabbit's eyes (n = 7). The rabbits' eyes were kept open with clips at the lid; the irritation was noted at particular intervals above 2 min for 5 seconds. Reactions to the irritants, for instance, ulceration, inflammation of the iris and eyelids, hemorrhaging, and blindness were noted.

2. Results

2.1. Phytochemical Screening

The results of phytochemical analysis showed the presence of several active ingredients, for instance, saponins, alkaloids, flavonoids, tannins, phenolic compounds, phytosterols, triterpenoids, sugars, and Amino acids. The results are illustrated in Table 1.

Table 1 Phytochemical screening of plant extracts

Test	Observation	Sprouted fenugreek seed extract	Pea extract
Test for Alkaloids			
Wagner's Test	Reddish brown	++	--
Dragondroff's Test	Reddish orange precipitate	++	--
Test for carbohydrate			
Molish Test	Violet ring is formed	+++	+

Continuation of Table 1			
Fehling Test	Brick red precipitate	+	+
Test for cardiac glycosides			
KillerKillani Test	Reddish brown	+	--
Test for Protein and amino acids			
Biuret Test	Green precipitate	--	+
Ninhydrin Test	Purple solution	++	+
Xanthoproteic Test	Brown solution	--	--
Test for Flavonoids			
Sulphuric Acid (Conc.) Test	Reddish orange	+	++
Test for Phenol & Tannins			
Ferric Chloride Test	Dark bluish black	+++	+++
Lead Acetate Test	Off-white precipitate	+++	+++
Test for Saponins			
Foam Test	Foam persistent (1-2 min)	+++	+++
Test for phytosterols			
Libermann Burchard's Test	Brown	++	+++
Test for Triterpenoids			
Salkwoski Test	Red in lower layer	++	+
Test for Quinones			
Hydrochloric acid (Conc.) test	Brown ring at the bottom	--	+
Test for anthraquinones			
Ammonium hydroxide tests	Orange	--	+++
Test for Coumarins			
Sodium hydroxide tests	Orange solution	--	++
Test for Fixed Oils &Fats			
Saponification test	Red color	--	--
Stain test	Oil stain on paper	+	--

+++ Large quantity; ++ good amount; + small amount; - absent

Fig. 1 Sprouted fenugreek seeds and pea seeds shampoo formulation (Developed by the authors)

2.2. Formulation of Shampoo

The composition of the shampoo formulation (F1 & F2) is depicted in Table 2. The presence of phytochemicals in the crude extracts provides additional benefits to the shampoo formulation.

2.3. Physicochemical Properties of the Developed Shampoo

The colour of the F1 shampoo formulation was yellowish green, and the formulation F2 was caramel brown (Figure 1). Both formulations are semi-liquid in nature and have a uniform texture and pleasant rosemary odor. The marketed shampoo was odourless, brown in colour, and had a smooth and uniform texture. The pH value of the shampoo formulation ranged from 5.59 to 5.76 (F1), 5.32 to 5.96 (F2). Even so, the pH of the marketed shampoo was 6.

The result indicated that the developed shampoo had an optimal pH, nearer to the pH of the marketed shampoo. The viscosity of the designed shampoo (F1 & F2) ranged from 2.3 to 2.08 poise and 2.8 to 2.71 poise, respectively, falling within an acceptable range. The viscosity of the marketed shampoo was 2.65 poise. The designed formulations (F1 & F2) reduced the surface tension to 49.08 and 50.7 dynes/cm respectively. Although the marketed shampoo reduced the surface tension to 41.57 dynes/cm, indicating the strongest cleaning ability. Several studies have stated that a good quality shampoo should decrease the surface tension of water from 72.8 to 40 dynes/cm. The reduction shows the good detergency ability of the shampoo [23].

Table 2 Composition of shampoo formulation

Ingredients	Part used	Uses	F1	F2
Phyllanthus emblica (Amla)	Dried ripe fruit	Hair growth promoter, Conditioner	5 g	5 g
Sapindus mukorrossi (Soap nut)	Dried pericarp fruit	Nonionic surfactant, detergent	5 g	5 g
Calendula officinale (Marigold)	Petals	Antidandruff	5 g	5 g
Citrus sinensis (Orange)	Peel	Antidandruff/pH modifier/preservative	5 g	5 g
Trigonella foenum-graecum (Fenugreek)	Sprouted seed	Nourishing/preserve thickness	4 g	-
Pisum sativum (Pea)	Fruit	Stimulates hair growth at the root/antioxidant	-	4 g
Ricinus communis (Castor)	Seed oil	The hair growth promoter/conditioner	26 ml	26 ml
Xanthomonas campestris (Xanthan)	Gum	Thickener/stabilizer/viscosity modifier	1%	1%
Acacia Senegal (Acacia)	Gum	Thickener/stabilizer/viscosity modifier	1%	1%
Salvia rosmarinus (Rosemary)	Essential oil	Fragrance	1-2 drops	1-2 drops
Glycerin	Vehicles	Humectant	Q.S.	Q.S.
Water (Q.S.)	Vehicles	Diluent	Q.S.	Q.S.
Total			100 g	100 g

2.3.1. Stability Studies

The designed formulation remained stable at 42 ± 2°C temperature and relative humidity 75 ± 2% for 90 days. The appearance, color, and the odor of the formulation did not change and degradation was also not recorded at this temperature and humidity. The findings of the stability study demonstrated that the shampoo formulation was stable during the entire storage period. The results are presented in Table 3.

Table 3 Stability studies of shampoo formulations

	Stability results at initial level				
Formulations	**Color**	**Odor**	**pH**	**Surface tension**	**Viscosity**
F1	Green	Rosemary	5.59	49.08 dynes/cm	2.3 poise
F2	Light brown	Rosemary	5.32	50.7 dynes/cm	2.8 poise
F3	Brown	Herbal	6.00	41.57 dynes/cm	2.65 poise
	Stability results after 1 month				
F1	Green	Rosemary	5.63	49.08 dynes/cm	2.27 Poise
F2	Light Brown	Rosemary	5.45	50.38 dynes/cm	2.77 Poise
F3	Brown	Herbal	6.00	41.57 dynes/cm	2.65 Poise
	Stability results after 2 months				
F1	Green	Rosemary	5.72	49.37 dynes/cm	2.1 Poise
F2	Light Brown	Rosemary	5.64	50.76 dynes/cm	2.75 Poise
F3	Brown	Herbal	6.00	41.57 dynes/cm	2.65 Poise
	Stability results after 3 months				
F1	Green	Rosemary	5.76	49.52 dynes/cm	2.08 Poise
F2	Light Brown	Rosemary	5.96	50.92 dynes/cm	2.71 Poise
F3	Brown	Herbal	6.00	41.57 dynes/cm	2.65 Poise

Notes: F1 - fenugreek formulation; F2 - pea formulation; F3 - marketed shampoo

2.3.2. The Percentage of Solid Content

Approximately 20-30% solid content is ideal for a good quality shampoo that could be easy to apply and wash out from the hair [23]. The solid content of the formulated shampoo (F1 & F2) was recorded as 20.57 and 21.87% (Table 4), indicating that the solid content

of the shampoo falls within the acceptable range, whereas the marketed shampoo had 22.8% of the solid content.

2.3.3. Foaming Stability

The foam produced by the shampoo (F1 & F2) was denser, uniform, compact, and stable compared to the marketed shampoo and maintained the volume even after 5-10 minutes. The foaming property may be owing to the combination of plant extracts, namely amla, soapnut, marigold, and orange peel, that were incorporated in the shampoo formulation.

2.3.4. Wetting Time

The shampoo formulation (F1 & F2) yielded a higher wetting time, i.e., 60 and 75 seconds compared to the marketed shampoo (36 seconds). Though the designed formulation did not contain any added detergents like sodium laureth sulfate or ammonium laureth sulphate, it was extensively used in several synthetic shampoos to shorten the wetting time. Various natural ingredients like amla, soapnut and fenugreek that were assimilated in the formulation have detergent-like properties.

2.3.5. Detergency Ability

According to this study, the developed shampoos (F1 and F2) had good detergency ability. The amount of sebum removed from the hairs was found to be 25% and 21.8% respectively. The results are listed in Table 4.

2.3.6. Cleaning Action

The findings of the present studies demonstrated that the cleansing power of the developed shampoo and the marketed formulation almost have a similar cleansing ability.

2.3.7. Dirt Dispersion

According to this study, the estimated quantity of ink in foam was light and moderate, indicating that the formulations were satisfactory. Whereas in the marketed formulation, the water concentrated on dirt and yielded clean foam.

2.3.8. The Evaluation of Conditioning Performance

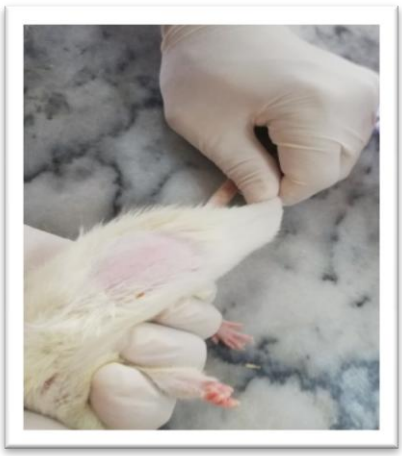
The conditioning effect of the shampoo formulation was based on the average scores reported by the volunteers. It was noted that most study participants rated a score of 3 for the tresses washed with developed shampoo (F1 & F2), whereas the marketed shampoo (F3) scored 4. Hair tress (without washing) was noted with a minimum (1) score. The results demonstrated that the developed shampoo has good conditioning performance. The mass of hair was soft, smooth and silky.

Table 4 Physicochemical parameters of developed and marketed shampoos

Characteristics	F1 (Fenugreek formulation)	F2 (Pea formulation)	F3 (Marketed Shampoo)
% Age of Solid Content	20.57%	21.87%	22.8%
Foaming Stability	4 cm	3 cm	5 cm
Wetting Time	60 sec	75 sec	36 sec
Detergency Ability/Artificial Sebum	25%	21.8%	29%
Cleaning Action	16%	14%	18%
Dirt Dispersion	Light	Moderate	Light
Conditioning Performance	Good	Good	Excellent
Skin Irritation	None	None	None
Eye Irritation	None	None	None

2.4. Skin Irritation Test

The shampoo formulation (F1 & F2) did not produce any kind of hypersensitive reactions on the skin of the animal and was found to be safe (Figure 4).



a

b

c

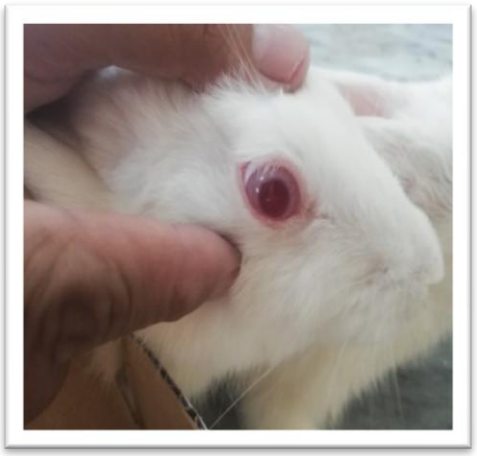
d

Fig. 4 Skin irritation test of control (a), F1 shampoo (b), F2 shampoo (c), F3 marketed shampoo (d) (Developed by the authors)

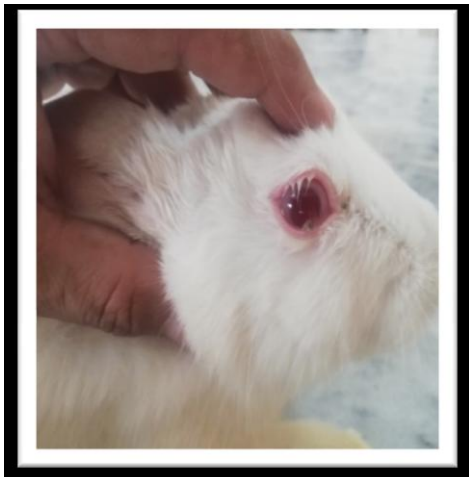
2.5. Eye Irritation Tests

Several synthetic surfactants may cause Inflammation of the eyelid, corneal irritation, and skin ulceration. Nonetheless, the developed shampoo contained natural ingredients that did not produce any irritation or harmful effect on the eyes (Figure 5).

a



b



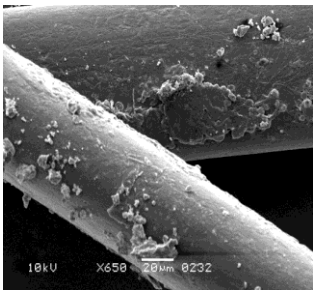
c

d

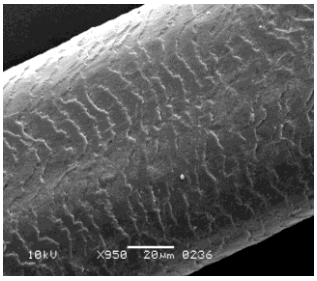
Fig. 5 Eye irritation test of control (a), F1 shampoo (b), F2 shampoo (c), F3 marketed shampoo (d) (Developed by the authors)

2.6. Surface Characterization of Hair Using a Scanning Electron Microscope (SEM)

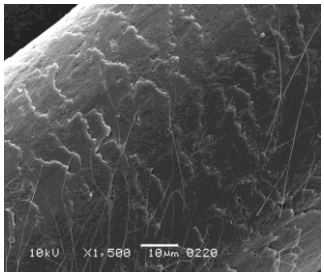
The results indicated that the formulated shampoo (F1 & F2) showed a good conditioning effect. Although the hair cleaned with the marketed shampoo exhibited a greater conditioning effect due to the presence of synthetic agents. The untreated hair showed normal structural design. Digital images of hair samples obtained by SEM are illustrated in Figure 6 (A-D).



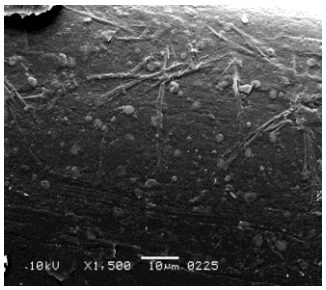
1



2



3



4

Fig. 6 Scanning electron microscope images of hair samples, clean or untreated hair (1), hair washed with marketed shampoo (2), Hair washed with developed (F1 & F2) shampoo (3 & 4) (Developed by the authors)

2.7. Antioxidant Activity

In contrast to the marketed shampoo (IC50 value: 44.18 µ/ml), the shampoo formulation (F1 & F2) demonstrated substantial antioxidant activity with IC_{50} values of 53.46 and 69.4 µ/ml, respectively. Even though, the IC_{50} for the reference standard drug (ascorbic acid) was only 36.17 µ/ml. The results are listed in Table 5. According to the preliminary phytochemical screening, several bioactive compounds including flavonoids, tannins, phenolic compounds, and saponins are present in the designed formulation and may contribute to its antioxidant activity.

Table 5 *In vitro* DPPH antioxidant activity

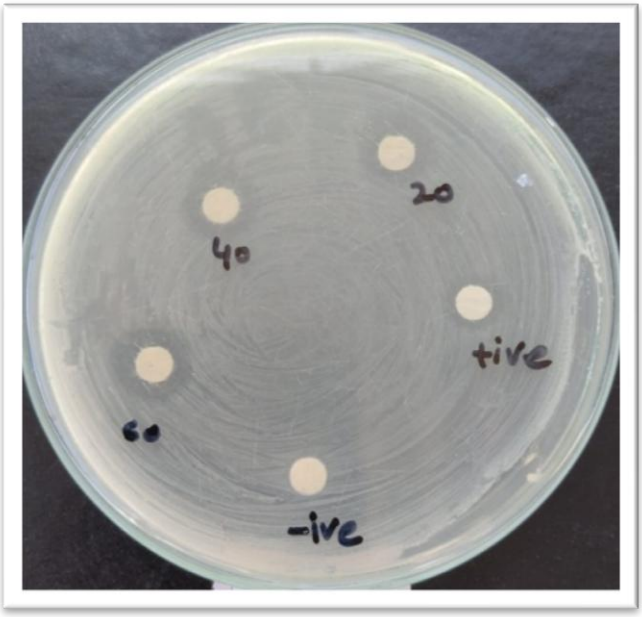
Concentration (µg/ml)	Ascorbic acid	Marketed shampoo	The shampoo formulation (F1)	The shampoo formulation (F2)
50	49.63%	52.59%	50.37%	46.66%
100	56.30%	53.33%	51.11%	48.15%
200	72.59%	63.70%	68.89%	68.89%
400	88.44%	83.70%	83.70%	70.37%
IC_{50} (µg/ml)	36.17	44.18	53.46	69.40

2.8. Antifungal Activity

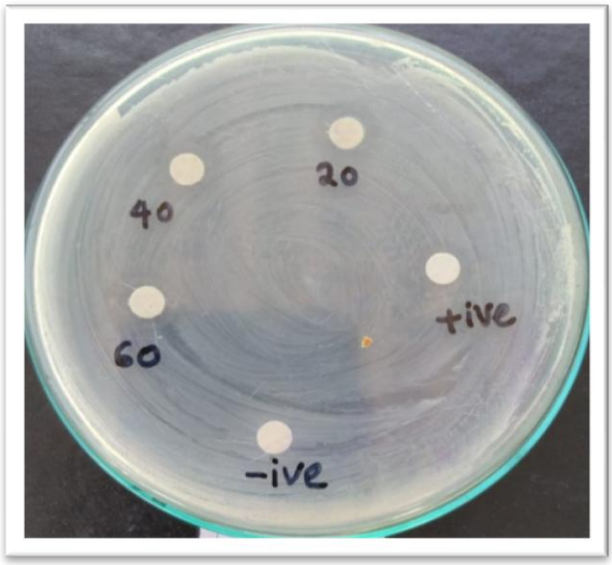
The zone of inhibition formed by fluconazole was 7.66 mm at 20 µl/disc, and samples were found to be 10.33 and 11mm zone of inhibition, respectively. Nonetheless, the developed shampoo (F1 & F2) showed a maximum inhibition of 12.33 and 12.66 mm at 60 µl/disc (Table 6). The antifungal activity of the designed formulations (F1 & F2) exhibited positive results against the fungal strain *C. albicans* (Figure 7) due to the presence of an optimized combination of traditional plant extracts that have established anti-dandruff properties [24, 25].

Table 6 Antifungal activity of shampoo formulation (F1 & F2)

Drugs	The zone of inhibition (mm)		
	20 µl/disc	40 µl/disc	60 µl/disc
Control (-ve)	0	0	0
F1 (Fenugreek)	10.33	12	12.66
F2 (Pea)	11	12	12.33
Fluconazole	7.66	-	-



F1



F2

Fig. 7 Zone of inhibition of formulated shampoo (F1 & F2) (Developed by the authors)

3. Discussion

According to the present study, the formulations were found to be stable, had good cleansing action, wetting capacity, and excellent conditioning performance as commercial shampoos. The results are depicted in Tables 3 and 4. The shampoo formulation exhibited antifungal and antidandruff activity. The formulation did not show any irritation on the skin or eyes. All the ingredients incorporated in the shampoo formulation were safe, effective and displayed good performance. Shampoo developed with hydroalcoholic extracts of natural ingredients, namely Indian gooseberry, soap nut, orange peel, marigold, sprouted fenugreek, pea, and rosemary, had been used traditionally for hair growth, cleansing, conditioning, and removal of dandruff flakes. Calendula flowers are enriched with several bioactive compounds that have antioxidant and anti-lice properties. Rosemary oil increases the blood circulation in the scalp, helps clean hair follicles, and enhances hair growth. Aloe gel is a good source of many enzymes, vitamins, and bioactive ingredients and has been used in various cosmetic, pharmaceutical, skin, and personal care products. Xanthan gum is a viscosity modifier, accordingly used in various hair care products, shampoos, conditioners, and styling products. The preservatives or harsh surfactants may cause irritation and dryness of the scalp that could be reduced with the use of natural surfactant soapnut, which also provides antidandruff activity with fewer side effects [26, 27]. Pea extract is a source of essential amino acids and has antioxidant, anti-ageing, reconstructive, conditioning and strengthening properties, and is used in many hair care formulations [28]. Though sprouted fenugreek seeds have been long hailed for several health benefits and used for various types of skin and hair care treatments. Sprout seeds are enriched with enzymes, protein, minerals, and vitamins A and C [29]. The presence of vitamin C in sprouts helps in hair growth and reduces hair fall and premature graying of hair as well as prevents dandruff. Additionally, germinated fenugreek seeds have higher antioxidant content that nourishes the hair from the roots, combats follicular problems, and promotes hair growth due to anti-inflammatory, antifungal and immunity-booster properties [30]. Owing to amazing health benefits, an extract of pea and germinated fenugreek seeds in aloe gel were incorporated into the shampoo formulation as a major constituent that could be beneficial to the health of the consumer.

4. Conclusions

We developed two types of herbal shampoo using germinated fenugreek seeds in aloe gel and pea extract as the main components of shampoo along with amla, reetha, marigold, orange peel extract, xanthan, and acacia gum that contribute to cleansing and beautification of hair. Although synthetic shampoo helps to remove dandruff and provides instant shine and luster, it also causes the hair to become dry and rough after prolonged usage, producing various scalp problems. Consumers had experienced several side effects owing to synthetic preservatives, conditioners, coloring, and antimicrobial agents. Therefore, a natural shampoo devoid of any chemical preservatives that can be used as an alternative to synthetic ones was developed to lessen the risk caused by chemicals. The uniqueness of the shampoo formulation was its bioactive ingredient including sprouting fenugreek seeds in aloe gel and pea extract, which have remarkable health benefits. The findings of the present study revealed that the natural ingredients incorporated in the shampoo formulation were safe, showed good performance with aesthetic appeal and were effective to use. The results were comparable to those of the marketed shampoo. Further research studies are needed to design a more refined protocol for the formulation of herbal shampoo in human volunteers with various hair types.

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