



# Development of Lab-Based Wheat Protein Shampoo for Healthier Hair

Payal Puri\*, Muskan Roshan, Harsh Singh and Ajay Dabar  
Department of Bioscience, Soft vision College and Research Institute (Affiliated to Devi Ahilya University), Scheme No. 54, Indore- 452010, Madhya Pradesh, India

Received: 14 Mar 2020 / Accepted: 8 Apr 2020 / Published online: 1 Jul 2020

\*Corresponding Author Email: [payal.shgl@gmail.com](mailto:payal.shgl@gmail.com)

## Abstract

The aim of the present study was to develop a novel protein shampoo containing natural ingredients with an emphasis on safety and efficacy. The study presents a shampoo formulated with wheat protein as its active constituent, which strengthens hair roots, brings lusture, softness and prevents premature greying and loss of hairs. The shampoo was formulated by adding the herbal extracts of Wheat sprouts Wheatgrass, Aloe vera China rose, Bhringraj and Reetha in different proportions. Evaluation of physicochemical and performance tests for developed shampoo were carried out in terms of visual inspection, wetting time, pH, percentage of solid contents, surface tension, detergency, dirt dispersion, conditioning performance, foam volume and its stability. The formulated protein shampoo was evaluated for conditioning performance by its application in 20 volunteers. The formulated herbal shampoo is appealing, yellow in color with demonstrable good foam stability, detergency, cleansing property and surface tension. The pH of protein shampoo was 6.5, near to skin pH. Percentage of solid content of shampoo was found to be 21% after drying. Dirt dispersion of formed herbal shampoo was light. 1% of shampoo gave 23 cm foam height and viscosity of 42.5 millipoise. The highest score of the conditioning performance of the developed protein shampoo was found to be 3.0 out of 4. The results indicated that formulated protein shampoo can be potentially used for cleaning and managing hairs in daily life. Future works should focus in the development of refined protocol towards formulating protein shampoo to improve its overall quality and safety.

## Keywords

Conditioning, Formulation, Herbal, Stability, Wheat sprouts

\*\*\*\*\*

## INTRODUCTION:

Shampoos play an important role in the hair care routine of an individual <sup>[1]</sup>. Hair is one of the external measures of internal body conditions. It is an important part of human body derived from ectoderm and is protective appendages on the body <sup>[2, 3]</sup>. The purpose of using shampoo is to remove surface grease, dirt from the hair and to provide nourishment and give healthier look to the hairs <sup>[4]</sup>. Shampoos are a viscous solution of detergents containing suitable additives, preservatives and active ingredients <sup>[5, 6]</sup>. Now-a-days many synthetic,

herbal, medicated and non-medicated shampoos are available in the market but popularity of herbal shampoo among consumers is on rise because of their belief that these products being of natural origin are safe, economical and free from side effects. Herbal shampoo is a cosmetic preparation which uses herbs from plants for washing of hair and scalp just like a regular shampoo <sup>[7, 8]</sup>.

In synthetic shampoos, surfactants (chemicals) are added mainly for their cleansing and foaming property, but the continuous use of these surfactants leads to deleterious effects such as eye irritation,

scalp irritation, hair loss, and dryness of hairs [9]. Some international researchers have claimed that the chemicals of shampoo are also responsible for cancer [10]. Alternative to synthetic shampoo one can use shampoos containing natural herbal ingredients. Many herbal plants such as *Hibiscus rosa-sinensis*, *Lawsonia inermis*, *Citrus aurantifolia*, *Phyllanthus emblica*, *Ocimum sanctum*, *Zingiber officinalis*, *Azadirachta indica*, *Sapindus mukorossi*, *Aloe barbadensis* have been used in many shampoo formulations. These plant products may be used in their powdered form, crude form, purified extracts, or derivative form [11]. However, formulating cosmetic products containing only natural substances is very difficult. The selection of active ingredients for hair care is based on the ability of the ingredient to prevent skin damage as well as to improve the quality of skin by cleansing, nourishing and protecting the skin [12].

A protein shampoo contains added proteins that strengthen the hair and cleanse the scalp. Protein shampoos offer nourishment and moisturize dull and lifeless hair strands [13-15]. Growing air pollution, use of hair color and increasing occurrence of harmful chemicals in the surroundings have led to growth in the number of hair issues among people across the globe. Use of protein shampoo provides a necessary solution for such hair issues. The most common types of protein shampoos are those that contain keratin, collagen, silk and are of animal origin [13, 14]. All of them although are effective but expensive in the long term.

In the present study, we made an attempt to develop a basic protocol for formulation of protein-based shampoo for effective hair care considering economical aspect. The protein shampoo was formulated containing suitable ingredients such as Wheat sprout (*Triticum aestivum*), Wheatgrass, China rose (*Hibiscus rosa-sinensis*), Reetha (*Sapindus mukorossi*), Bhringraj (*Eclipta prostrata*), Aloe vera (*Aloe barbadensis*), Amba haldi (*Curcuma amada*) etc. in different proportions and evaluated for its physicochemical properties.

#### **Functions of herbal ingredients in protein shampoo formulation:**

##### **1. Wheat sprouts**

Sprouted wheat protein, a key ingredient in our formulation, contains low molecular weight proteins and essential amino acids that aid in its absorption into the inner hair fibers for long lasting strength [16]. It tends to repair capillaries to get strong & thick hair [17]. It protects and increases shine and smoothness of hairs due to high essential amino acid and protein content. Wheat sprout extract offers a high

concentration of vitamins A, C, B1, B2, B3, D, E, K that are required for a healthy and beautiful hairdo [18].

##### **2. Wheatgrass**

It treats dandruff & scalp problems. It is high in antioxidants thereby preventing hair damage from free radicals including environmental toxins and UV exposure. It prevents and reduces graying of hair [19].

##### **3. China rose**

It helps to stimulate hair growth, makes hair smooth and shiny and deep conditions hair [20].

##### **4. Reetha**

It is used as cleansing agent, keeps scalp gentle & removes any microorganisms responsible for infection due to its high saponin content. It is also helpful against dandruff [20].

##### **5. Bhringraj**

It retains the original color of hair [20].

##### **6. Aloe vera**

It helps in thickening of hair. It also helps to nourish hair [21].

##### **7. Amba haldi**

It is used for imparting yellow color to the shampoo besides its anti-inflammatory property.

##### **8. Guar gum**

It is used to increase the viscosity of the shampoo [22].

#### **Function of chemicals:**

1. Glycerin helps in moisturizing of hair.
2. Sodium Chloride is used as a thickener.
3. Sodium lauryl sulphate (SLS) is used as a surfactant.
4. Sodium benzoate is used as a preservative.
5. Jasmine oil is used for fragrance.

#### **MATERIALS AND METHODS:**

##### **ETHICS:**

Approvals to conduct experimental protocols to study conditioning performance on human volunteers were approved by the Softvision College Institutional Ethical Committee where work was done. Consent to participate in this study was obtained from each subject after the procedures had been explained.

##### **COLLECTION OF PLANTS:**

The parts of plants like Wheat (seeds), Reetha (fruit), Bhringraj (leaves), Amba haldi (rhizome) and Guar gum powder were collected from the local market. Red colored China rose (flowers) and Aloe vera (leaves) were obtained from the nursery locally. The sprouting of wheat and hydroponic culture of wheat grass was done in house as shown in figure 1. These were washed under running water to remove contaminants. They were dried in sunlight, converted into coarse powders, sieved using 60 meshes and were stored in well-closed containers.



**Figure 1: (a) Sprouting of wheat in house (b) Hydroponic culture of wheat grass (c) Drying of plant materials**

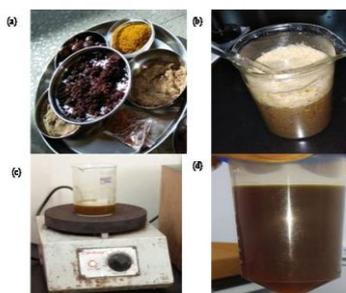
#### PREPARATION OF HERBAL EXTRACTS:

Extracts were prepared by decoction method [23]. 4.1g of powdered Wheat sprouts, 4.1 g of Wheatgrass powder, 3.5 gram of China rose powder, 4.1 g of Bhringraj powder, 6 g of Aloe vera gel, 10 g of Ritha fruit and 1 g Amba haldi (table 1) were

placed in a stainless steel vessel with 50 ml of distilled water and covered. The mixture was kept for boiling until the water reduced to one quarter and then filtered. The clear extract obtained was used as herbal extract. The different stages of preparation of herbal extract are presented in figure 2.

**Table 1: Ingredients of herbal extract**

Plant	Parts	Quantity for 100g
Wheat sprout	Seeds	8.2%
Wheat grass	Grass	8.2%
China rose	Flowers	7%
Bhringraj	Leaves	8.2%
Aloe vera gel	Leaves	12%
Reetha	Fruits	20%
Amba haldi	Rhizome	2%



**Figure 2: (a) Dried plant materials (b) Mixing of herbal ingredients (c) Boiling of herbal ingredients (d) Filtered herbal extract**

**Table 2: Composition of herbal shampoo**

Ingredients	Quantity
Herbal extract	20 ml
0.1 M NaCl	20 ml
SLS (7.5%)	20 ml
Guargum	10 ml
Glycerine	2 ml
Vitamin E capsule	1
Sodium benzoate (0.2%)	2 ml
Essential oil (Jasmine)	q.s.
Amba haldi	q.s.
Water	26 ml

**Table 3: Conditioning performance of formulated shampoo**

Score	Formulated shampoo	Control
1	2	15
2	6	5
3	11	0
4	5	0

Score 1 - poor, score 2 - fair, Score 3 - good, and Score 4 – excellent

**Table 4: Results for physiochemical evaluation of formulated protein shampoo**

S.No.	Parameters	Observations
1	Physical appearance	Yellow and translucent
2	Ph	6.5
3	Percentage of solid contents	21%
4	Wetting time	9.46 sec
5	Viscosity 42.5 millipoise	
6	Dirt dispersion	Light
7	Cleansing action	Good
8	Surface tension	33.01 dynes/cm
9	Foam ability and stability	23 ml after 1 min and 18 ml after 20 min
10	Skin irritation	None
11	Microbiological count	50CFU/ml
12	Antimicrobial activity Zone of inhibition against Gram positive bacteria	

Volume of sample	Zone diameter
25 ul	0.6 cm
50 ul	1.0 cm
100ul	2.0 cm
150ul	2.5 cm

#### FORMULATION OF PROTEIN SHAMPOO:

Formulation of the herbal shampoo was done as per the formula given in table 2. To increase the thickness of formulation, SLS (7.5%) solution was prepared using 0.1 M NaCl. Twenty ml of the herbal extract was added to 20 ml SLS solution with 20 ml NaCl solution and mixed by shaking gently. The final volume was made to 100 ml by adding 10 ml guar gum extract, 2 ml of glycerine and 26 ml of water. To improve aroma in the formulation, sufficient quantity of essential oil (jasmine oil) was added. The shampoo also included one capsule of Vitamin E for conditioning and 2 ml 0.1 % of sodium benzoate as preservative.

#### EVALUATION OF PROTEIN SHAMPOO:

To evaluate the prepared formulations, quality control tests including visual assessment and physicochemical controls such as pH, density, viscosity surface tension, foam volume, foam stability and wetting time were performed using standard protocol.

##### 1. Physical appearance/visual inspection

The formulation prepared was evaluated for the clarity, color, odor and foam producing ability and fluidity<sup>[24]</sup>.

##### 2. Determination of pH

The pH of 10% v/v shampoo solution in distilled water was measured by using calibrated pH meter at room temperature<sup>[25]</sup>.

##### 3. Determination of solid content percentage

A clean dry evaporating dish was weighed, and 4 grams of shampoo was added to the evaporating dish. The evaporating dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight of the solid contents present in the shampoo was calculated after drying.

##### 4. Wetting time

Wetting time was calculated by noting the time required by the canvas paper to sink completely<sup>[26]</sup>. A canvas paper weighing 0.44 g was cut into a disc of diameter measuring 1-inch. Over the shampoo (1% v/v) surface, the canvas paper disc was kept, and the time taken for the paper to sink was measured using the stopwatch.

##### 5. Rheological evaluation

The viscosity of herbal shampoo was determined by using Ostwald's viscometer<sup>[27]</sup> as shown in figure 3. The viscosity of the herbal shampoo was measured by counting drops of herbal shampoo from the mark to bottom.

$$n_y = n_w \frac{d_y t_y}{d_w t_w}$$

$n_w$ : viscosity of water

$n_y$ : viscosity of tested liquid

$d_w$ : density of water

$d_y$ : density of tested liquid

$t_w$ : timing of run off of water

$t_y$ : timing of run off of tested liquid



**Figure 3: Stalagmometer for surface tension determination**

### 6. Dirt Dispersion

Two drops of herbal shampoo were added in a wide mouthed test tube containing 10ml of distilled water. 1 drop of India ink was added to this solution and the test tube was covered and shaken for ten times. The amount of ink in the foam was estimated as None, Light, Moderate, or Heavy [7].

### 7. Cleansing action

The cleansing property of the herbal shampoo was evaluated by self-application of the shampoo on hairs. The shampoo was used to wash the hair of human subject that had applied oil 4-5 hours before washing. The performance of the shampoo was assessed on its ability to remove oily dirt from scalp.

### 8. Surface tension measurement

Surface tension measurement was carried out with herbal shampoo through stalagmometer depicted in figure 4. The principle is to measure the weight of the drops of herbal shampoo falling from a capillary glass tube, and thereby calculate the surface tension of the fluid. We can determine the weight of the falling drops by counting them. From it we can determine the surface tension as shown below [28].

$$ST = \frac{nl}{nw} \times \frac{dl}{dw} \times tw$$

$nl$ : no. of drops of liquid

$nw$ : no. of drops of water

$dl$ : density of liquid

$dw$ : density of water

$tw$ : 71.2 dyne/cm



**Figure 4: Ostwald's viscometer for viscosity determination**

### 9. Foaming ability and foam stability

Cylinder shake method was used for determining foaming ability. 50ml of the 1% herbal shampoo solution was put into a 250ml graduated cylinder which was covered with hands and shaken for 10 minutes. The total volume of the foam content after 1-minute shaking was recorded. The foam volume was calculated. Immediately after shaking the

volume of foam at 1minute intervals for 20 minutes were recorded [29]. Foam stability was evaluated by recording the foam volume after 1 min and 5 min of shake test.

### 10. Stability study

The stability of the formulation was studied for a period of 1 month by keeping at temperature of 25-30°C.

### 11. Skin irritation test

Prepared herbal shampoo was applied on skin for 5 minutes after that was washed and tested for irritation or inflammation to the skin.

### 12. Conditioning performance

Twenty normal male and female subjects (age range 19–60 years) who were concerned about their dry and rough hairs were enrolled in the study. The examination was conducted from November to December in 2019. These subjects applied the formulated shampoo twice a week for a period of 15 days. The control group was the one with no hair washing and the test group used the formulated shampoo for hair wash. They were asked to rate for conditioning performance from score 1 to 4 (1=poor; 2=satisfactory; 3=good; 4=excellent). Conditioning properties include all desirable benefits imparted to the hair such as increased body to the hair, improved lusture, softness and silkiness<sup>[30]</sup>.

### 13. Microbial examination

100 microliter of shampoo was mixed with melted Mueller Hinton agar and poured to sterile Petri dishes under ascetic conditions. The plates were rotated to mix thoroughly and then allowed to set.

The plates were incubated at 37°C for 24 hours and observed for microbial growth<sup>[11]</sup>.

### 14. Anti-microbial activity

This test was carried out to determine the susceptibility or resistance of organisms to formulation ingredients according to the method described by Cheesbrough<sup>[31]</sup>. The Gram positive (*Bacillus*) and Gram negative (*E. coli*) test organisms were subcultured on nutrient broth and incubated at 37°C till desired turbidity. The developed culture was streaked on the surface of Mueller Hinton agar on which four wells were punched with sterile cork borer. 25, 50, 100 and 150 ul shampoo were filled in these wells in increasing order. The plates were incubated at 37°C for 24 hrs and zone of inhibition around the wells were measured using a ruler.

## RESULTS AND DISCUSSIONS:

### 1. Physical appearance / Visual inspection

The protein based formulated shampoo as shown in figure 5 is translucent and yellow in color. It has a good odor given by the fragrance in the ingredients and also a good foam producing ability.

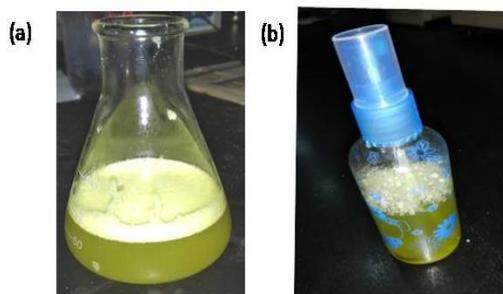


Figure 5: Protein based herbal shampoo (a) Before packaging with herbal extracts (b)After packaging with herbal and chemical constituents.



Figure 6: Dirt dispersion test



Figure 7: Result for cleansing action (a) Before shampoo (b) After shampoo



Figure 8: Foam height

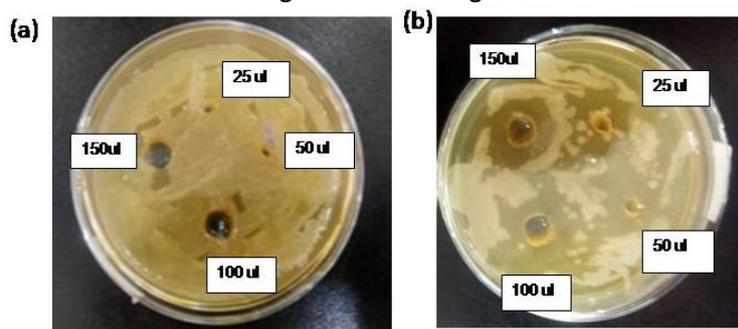


Figure 9: Antimicrobial activity against a) Gram negative and b) Gram positive bacteria.

## 2. pH

The pH of formulated shampoo was 6.5, falling within the ideal pH range for shampoo which is between 5 and 7.8. The formulated shampoo is acid balanced which is near to the skin pH. The pH of shampoo is important for improving and enhancing the qualities of hair, minimizing irritation to the eyes and stabilizing the ecological balance of the scalp. Mild acidity prevents swelling and promotes tightening of the scales, there by inducing shine.

## 3. Viscosity

The viscosity of shampoo plays an important role in determining its shelf life stability, the ease of flow on removal from packing and spreading on application to hair and product consistency in the package. The viscosity of formulated shampoo was found to be 42.5 millipoise which is good enough for its applicability.

## 4. Percentage of solid contents

Good shampoos usually have 20-30% solid content as it is easy to be applied and rinse out from the hair. If the shampoo has too many solids it will be hard to work into the hair or too hard to wash out. The result of percent of solids contents of tested shampoo was found to be 21% and thus that it can be washed out easily.

## 5. Dirt Dispersion

Shampoo that causes the ink to concentrate in the foam is considered poor quality; the dirt

should stay in water. Dirt that stays in the foam will be difficult to rinse away. It will redeposit on the hair. The estimated amount of ink in foam was light as can be seen in figure 6 and so results indicate that prepared formulation is satisfactory.

## 6. Cleansing action

The cleansing action was tested on human hairs that have applied oil and not been washed for seven days. The results of detergency studies showed that the formulation has significant cleansing ability as it was able to remove both dirt and oil from hairs. This is presented in figure 7.

## 7. Surface Tension

Surface tension reduction is one of the mechanisms implicated in detergency. The reduction in surface tension of water (72.8 dynes/cm) by the herbal shampoos is an indication of its good detergent action. The surface tension value of formulated shampoo was found to be 33.01 dynes/cm which is good.

## 8. Foaming ability and foam stability

Although foam generation has little to do with the cleansing ability of shampoos, it is of importance to the consumer. The final formulation produced stable foams as shown in figure 8. There was little bit change in foam volume. The foam volume remains same throughout the period of about 5 min showing that the generated foam by the shampoo has

good stability and the prepared shampoo exhibits good foaming ability due to the presence of soapnut and wheat sprout protein.

#### 9. Wetting time

The wetting ability of a surfactant is dependent on its concentration and is commonly used to test its efficacy. The canvas disc method is a quick, efficient and reliable test to evaluate the wetting ability of a shampoo. The wetting time of herbal shampoo was found to be 9.46 sec which is good.

#### 10. Skin irritation test

The skin irritation tests revealed that the herbal shampoo shows no harmful effect on the skin. This due to the absence of harmful synthetic ingredients. Most of the synthetic chemicals produce inflammation and causes irritation to the skin. But in this formulation, all ingredients are obtained naturally. So it does not produce any harmful effect on the skin.

#### 11. Conditioning performance

Conditioning performance of the formulated shampoo based on the scores of human volunteers is presented in table 3. Majority of the volunteers rated that the hairs washed with formulated shampoo provided the best conditioning performance and as expected the control hairs (without washing) got the minimum score (1). The score of the conditioning performance of the hairs washed with formulated shampoo was found to 3.0 out of 4 (table 3). The results revealed that the formulated shampoo showed good conditioning effect.

#### 12. Stability Study

Stability of formulation during the storage period indicated that it is chemically and physically stable at standard room temperature of 25-30°C. The results showed that it possessed good stability within the 4 weeks of stability study with negligible changes in its physicochemical properties.

#### 13. Microbiological examination

The microbiological count of the formulated shampoo was quite low (50 CFU/ml) so it is safe to use. According to Jordanian standards, shampoo formulation should not contain more than  $10^2$  CFU/ml<sup>[32]</sup>.

#### 14. Anti-microbial activity

The result for anti-microbial activity revealed that the formulated shampoo was effective against Gram positive bacteria at all volume. The shampoo showed no activity against Gram negative bacteria as shown in figure 9. The results are determined by appearance of zone

of inhibition on agar plate around punched wells. The diameter of zone of inhibition ranged from 0.6-2.5 cm for 25-150 ul volume of shampoo.

The overall results for evaluation of formulated protein based herbal shampoo are shown in table 4.

#### CONCLUSION:

Hair care is a part of routine regime for an individual. Growing awareness regarding personal care among youngsters and adults has increased the sale of personal care products such as skin care, hair care, and oral care products. It is essential to have a green revolution for healthier and long-lasting life in modern times. The objective of the study was to develop a stable and functionally effective protein-based shampoo by excluding most of the synthetic chemicals, which are normally incorporated in such formulations. The investigation of herbal protein shampoo formulation was carried out to fulfill this goal along with its standardization.

The factors like UV radiations, pollution, cosmetic treatments, grooming practices, and cleansing have direct and indirect impact on to the hair. UV-B degrades the keratin protein of hair and UV-A is responsible for free radical generation leading to hair loss and graying. The developed shampoo provides good source of essential amino acids and low molecular weight proteins in the form of wheat sprouts therefore it is unique in this context for promoting healthy hair growth and preventing early hair loss by inducing keratin protein synthesis. Wheat grass can delay premature graying of hairs due to its antioxidant property. The natural color of the hair can be retained due to incorporation of Bhringraj in the formulation. Although the formulated shampoo contains synthetic chemical as SLS (7.5%) but its percentage is too small to cause any adverse effects (removal of oil from hairs) as compared to synthetic shampoo (10-40%) available in the market. The evaluation study on our shampoo showed good cleansing action, better foaming capacity, and quick wetting time. The formulated shampoo was not only safer than the chemical conditioning agents, but also greatly reduced the protein loss during combing. The pH of the formulated shampoo was 6.5 which can retain the acidic mantle of scalp and softness of hair. The study showed that our shampoo has good foam productivity, volume and stability which indicate its efficiency for hair cleaning. The high foam productivity is due to saponin and protein content of soapnut and wheat sprouts respectively. The volunteers reported that hair rinsing was pretty convenient after using our protein shampoo. Our

shampoo has a proper viscosity as it can easily come out from the container. We have used guar gum (10%) to make the shampoo viscous. Apart from this, protein content of wheat sprouts may also account for its viscosity.

The hair lusture and its softness are the two attributes for consumers showing the suitability after using a shampoo. This has been confirmed in our conditioning experiments. The high amount of hydrolyzed proteins in wheat sprouts and deep conditioning property of China rose might account for these two traits. We have also used Aloe vera gel to provide the conditioning and moisturizing effects. Based on the results from volunteer's view, it was demonstrated that 95% of them were satisfied with hair combing comfort, smooth and shiny hair with no skin irritation. The formulated shampoo has fair antidandruff activity efficiency as revealed by its inhibitory action against Gram positive bacteria This might be due to cumulative effect of wheat grass and saponin content of soap nut.

It is concluded that the formulated shampoo is safe, economical and effective to use as an alternative to its synthetic counterpart. These favorable outcomes clearly support the use of the listed ingredients with development of more refined protocol for protein shampoo formulation, but further scientific validation is needed for its overall quality determination.

#### ACKNOWLEDGEMENTS:

We would like to thank all volunteers for their participation in the required experimental studies. The authors are grateful to Director, Soft vision College and Research Institute, Indore (M.P.), India for providing us necessary laboratory infrastructure and facilities in conducting this research work.

#### REFERENCES:

1. Gavazzoni Dias, M.R., Hair cosmetics: An overview. *Int J Trichol*, 7(1): 2-15, (2015).
2. Akula, N.P., Preparation and evaluation of shampoo powder containing herbal ingredients. *Asian J Pharm Clin Res*, 8(1): 266-270, (2015).
3. Patil, S.S., Mane, Y.J. and Mohite, S.K., Formulation and evaluation of herbal shampoo powder. *Int J Adv Res*, 3(3): 939-946, (2015).
4. Wani, S., Khot, N. and Buchake, V.V., Preparation and evaluation of antidandruff polyherbal powder shampoo. *Pharmacophore (Int Res J)* 5(1): 77-84, (2014).
5. Fatima, G.X., Rahul, R. S., Sowmya, K.V., Reshma I. and Shanmuganathan S., Development and evaluation of a polyherbal shampoo. *Adv. J. Pharm. Life Sci Res*, 3(1): 13-16, (2015).
6. Maniker, A.R. and Jolly, C.I., Formulation of natural shampoo. *Int J Cosm Sci* 23(1): 59-62, (2001).
7. Mendhekar, S.Y., Tajane, A.S., Shitole, P.B., Jadhav, S. and Gaikwad, D.D., Formulation and evaluation of polyherbal shampoo and compare with marketed shampoos. *World J Pharm Pharm Sci*, 6(12): 1388-1397, (2017).
8. Pandey, S., Meshya, N. and Viral, D., Herbs play an important role in the field of cosmetics. *Int J Pharm Tech Res* 2(1): 632-639, (2010).
9. Shinde, P.R., Tatiya, A.U. and Surana, S.J., Formulation development and evaluation of herbal antidandruff shampoo. *Int J Res Cosmet Sci*. 3(2): 25-33, (2013).
10. Thitiltdecha, N., Teerawutgulrag, A., Kilburn, J.D. and Rakariyatham, N., Identification of major phenolic compounds from *Nephelium lappaceum* L. and their antioxidant activities. *Molecules*, 15(3): 1453-1465, (2010).
11. Pooja, A., Arun, N. and Maninder, K., Shampoos based on synthetic ingredients vis-à-vis shampoos based on herbal ingredients: A review. *Int J Pharm Sci Rev Res*, 7(1): 41-46, (2011).
12. Swaminathan, D., Studies on the addition of activated charcoal in herbal shampoo. *Chem Technol*, 13(1): 1-9 (2018).
13. Noudeh, G.D., Sharififar, F., Khazeli, P., Mohajeri, E. and Jahanbakhsh, J., Formulation of herbal conditioner shampoo by using extract of fenugreek seeds and evaluation of its physicochemical parameters. *Afr J Pharm Pharmacol*, 5(22): 2420-2427, (2011).
14. Roddick-Lanzilotta, A., Kelly, R., Scott, S. and Chahal, S., New keratin isolates: actives for natural hair protection. *J Cosmet Sci*, 58 (4): 405-411, (2007).
15. Challoner, N.I., Chahal, S.P. and Jones, R.T., Moisture regulation of hair using cosmetic proteins. *Croda NC* 04910. 1-12, (1999).
16. Sramkova, Z., Gregova, E., Sturdik, E., Chemical composition and nutritional quality of wheat grain. *Acta Chim Slo*, 2(1): 115-138, (2009).
17. Burnett, C.L., Bergfeld, W.F., Belsito, D.V., Hill R.A., Klaassen C.D., Liebler D.C., Marks J.G., Shank R.C., Slaga T.J., Synder P.W., Anderson F.A. and Heldreth B., Safety assessment of hydrolyzed wheat protein and hydrolyzed wheat gluten used in cosmetics. *Int J Toxicol*, 37(1): 555- 66S, (2018).
18. Dhabekar, S.S. and Hajare, J., Encapsulation of sprouted wheat and its incorporation in cosmetics. *Int Edu Res J*, 1(5): 43-45, (2015).
19. Aate, J., Urade, P., Potey, L. and Kosagle, S., A review: Wheat grass and its health benefits. *Int J Pharm Pharm Res*, 9(4): 288-298, (2017).
20. Jadhav, A.V., Morale, D., Daunderkar, Bhujbal, N. and Kshirsagar, S., Herbal cosmetics-An overview. *World J Pharm Sci*, 6(9):144-152, (2018).
21. Qadir, M.I., Medicinal and cosmetological importance of Aloe vera. *Int J Natural Therapy*, 2: 21-26, (2009).
22. Karsheva, M. and Georgieva, S. and Handjieva, S., The choice of the thickener-a way to improve the cosmetics sensory properties. *J Univ Chem Technol and Metallurgy*, 42(2): 187-198, (2007).
23. Kameswararao, K., Lakshmi prasanna, B., Aparnadevi, M., Nagadevi, G. and Rajeswari, S., Formulation and evaluation of polyherbal shampoo. *Int J Pharmacogn Phytochem*, 13(1): 251-268, (2018).

24. Aghel, N., Moghimipour, B. and Dana, R.A., Formulation of a herbal shampoo using total saponins of *Acanthophyllum squarrosum*. *Iran J Pharm Res*, 6(3): 167-172, (2007).
25. Dash, G.K. and Razak, A., Formulation and evaluation of a herbal shampoo. *Indo Am J Pharm Sci*, 4(9): 2860-2865 (2017).
26. Manikar, A.R. and Jolly C.I., Evaluation of commercial herbal shampoos. *Int J Cosmet Sci*, 22(5): 385-391, (2000)
27. Yamini, N.S., Sudha, Jyotsna, Pratyusha, K., Pratyusha, J. and Kartheeka., Formulation and evaluation of polyherbal hair oil. *J Pharmacogn Phytochem*, 7(3): 3254-3256, (2018).
28. Kumar, A. and Mali, R.R., Evaluation of prepared shampoo formulations and to compare formulated shampoo with marketed shampoos. *Int J Pharm Sci Rev Res*, 3(1): 120-126, (2010).
29. Klein, K. Evaluation of shampoo foam. *Cosmetics and Toiletries Mag*, 119(10): 32-35, (2004).
30. Igwebike, O., Iroha, I.R. and Oke, B., Formulation and antimicrobial activity of Triclosan-based medicated shampoo. *J Chem Pharm Res*, 9(7): 100-104, (2017).
31. Cheesebrough, M., *District Laboratory practice in tropical Countries part II*, Cambridge University Press, UK 2006, pp. 67-75,
32. Jordan Institution for Standards and Meterology. Shampoo specification. JS 483, (2002).