74

The Open Biology Journal

Content list available at: https://openbiologyjournal.com/

RESEARCH ARTICLE

Formulation of Herbal Shampoo against Head Louse (*Pediculus humanus capitis De Geer*)

Deeksha[1], Rishabha Malviya[1,*], Pramod K. Sharma[1], Dharmendra Singh[2] and Akanksha Sharma[1]

[1]*Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Greater Noida, Gautam Buddh Nagar, Uttar Pradesh, India*
[2]*Institute of Pharmacy, VBS Purvanchal University, Jaunpur, Uttar Pradesh, India*

Abstract:

Background:

Natural herbal shampoos are appealing to the consumers as they contain natural herbs without any harmful effects. The shampoo is used to remove dirt or other debris from hairs. Natural herbs are used in a herbal shampoo, which becomes more beneficial, safe, or efficacious than synthetic ones.

Aim:

The present study aims to formulate and evaluate a herbal shampoo containing various herbal extracts for anti-lice activity.

Materials and Methods:

Natural herbs such as neem, hibiscus, henna leaves, amla fruit and reetha epicarp are the components of the natural herbal shampoos used in this study. Soxhlet apparatus was used for water-based extraction of these natural herbs, whereas the maceration process was used for alcohol-based extraction. Nine formulations were prepared and evaluated by using different parameters like physical appearance, viscosity, surface tension, foam volume, pH, solid content, dirt dispersion and wetting time.

Results:

All nine shampoo formulations were successfully prepared at the pH range of 5.86-6.06. The physical appearance was observed to be between light brown to greenish-brown for F1 to F9 batches. The viscosity of the formulations was found to be between 1.05±0.011 to 0.96±0.011. Mortality of head lice (Lethal time (LT50)) for all the formulations was found in the range of 7.5 min- 52.5 min. Formulation F_5 was found to be the best in the context of all the parameters. Formulation F_5 showed good foaming property. The wetting ability was found to be good due to reduced surface tension (33.05±0.86); solid content was found to be 1.74% so that it would be easy to wash out from the hair.

Conclusion:

All the shampoo formulations showed good quality and could be used safely and effectively. These formulations also showed anti-lice activity so they can be used for the problem of head lice.

Keywords: Antidandruff agent, Anti-lice activity, Herbal extracts, Herbal shampoo, Lethal time, Natural herbs.

1. INTRODUCTION

Hair is one of the external indicators of internal body conditions. Shampooing is the most common form of hair treatment [1 - 3]. For the removal of oil, dirt, environmental pollutants, skin particles, dandruff and other contaminants that gradually grow up in hair, shampoo is used as a cleaning agent and is known as a hair care product being liquidy, cream-yorgel-like preparations. The main focus is on the removal of contaminants without any stripping of sebum to make hair unmanageable [4]. The consistency of the preparation is based on the inclusion of natural or synthetic fatty alcohols or the thickening agents (*e.g.* resin, gum, and PEG) and traditional soaps saturated with glycerides. Indian women use natural herbs such as reetha and shikkakai that are natural cleansing agents without harmful effects.

Nowadays, people are attracted to natural sources as they

* Address correspondence to this author at the Department of Pharmacy, School of Medical and Allied Sciences, Galgotias University, Plot No.2, Sector 17-A, Yamuna Expressway, Greater Noida, Gautam Buddh Nagar, Uttar Pradesh, India; Tel: +91-9450352185; E-mail: rishabhamalviya19@gmail.com

have no harmful effects as that of synthetic ones. So, natural herbal shampoos are popular among consumers.

The head lice *Pediculus humanus capitis* De Geer, an ectoparasite, confined to the scalp of hair of humans causes pruritis, skin irritation, and sleep loss as well as occasional secondary bacterial infection [1, 5]. The plant essential oils are used as the best source for insect control due to their bioactive chemicals [1, 6].

The present study deals with the formulations of shampoo and their further characterization, along with the anti-lice activity.

2. MATERIALS AND METHODS

2.1. Specimen Collection

The plant specimens (Neem leaves, Hibiscus leaves, Henna leaves, Amla fruit, Reetha epicarp, and Urad dal) were collected from the gardens, the local market and surroundings of Greater Noida, India and were authenticated. The raw materials collected are shown with their biological source/ family in Table **1** [7, 8]. The chemicals like hydroxypropylmethylcellulose, sodium lauryl sulphate, and glycerine were of analytical grade and provided as "required no purification before use".

Vatika premium naturals (henna & olive) shampoo, Dabur India Ltd was used as a marked formulation (control sample) for comparative study.

2.2. Preparation of Aqueous Extract

The leaf portions were washed thoroughly with water and dried in shadow, and powdered. Powdered material was passed through 60# sieve and stored in a desiccator for further use. Powdered leaves of neem (10 g), hibiscus (17 g), henna (10 g) and amla fruit (62.82 g) were kept in a round-bottom flask fitted with a reflux condenser and extracted with distilled water (250 ml) for 8-10 hours using soxhlet apparatus. The obtained extract was concentrated up to the semisolid form under reduced pressure and stored in a refrigerator till use.

The reetha epicarp (25 g) was taken in a beaker containing distilled water (100 ml), kept for 3 days and filtered, concentrated up to the semisolid form and stored till use.

2.3. Preparation of Alcoholic Extract

The dried, powdered leaves of neem (5 g), hibiscus (5 g), and amla fruit (23 g), urad dal (25 g) were taken in a beaker containing ethyl alcohol (50 ml) and kept for 24 hours and the

extract was obtained using maceration process. It was further filtered, concentrated to semisolid form and stored till use.

Reetha epicarp (25 g) was extracted in the same way by butanol (100 ml) using the maceration process, kept for 3 days and filtered, concentrated up to the semisolid form and stored till use.

2.4. Preparation of Shampoo

Hydroxypropylmethylcellulose (15 cps) was found to be suitable for the preparation of herbal shampoo and used as a base as it was clear, transparent and had no bubbles. Now, 0.5% hydroxypropylmethylcellulose solution was prepared as it had the same consistency as that of 10% marketed shampoo solution and referred to as the base for herbal shampoo. Then all the other herbal extracts were added in the increasing order to the base and mixed well by stirring so that the ingredients were uniformly distributed with good consistency and volume was made up to 100 ml. The composition of the formulation is described in Table **2**.

Table 1. Herbs used in the prepared shampoos.

S. No.	Ingredients	Part Used	Biological Source	Family	Uses
1.	Neem	Leaf	*Azadirachta indica*	Meliaceae	Prevents dryness and flaking of hairs, anti-lice activity.
2.	Hibiscus/ Gurhal	Leaf	*Hibiscus rosa sinensis*	Malvaceae	Prevents hair loss and promotes hair growth .
3.	Henna	Leaf	*Lawsonia inermis*	Lythraceae	Growth of hair, conditioner
4.	Amla	Fruit	*Emblica officinalis*	Euphorbiaceae	Darkening of hairs and Hair growth promoter
5.	Reetha	Fruit epicarp	*Sapindus mukorossi*	Sapindaceae	Detergent and antidandruff agent
6.	Urad	Seeds	*Vigna mungo*	Fabaceae	Saponifying agent and nourishes hair

2.5. Evaluation of Formulation

Different formulations of shampoo were evaluated using different parameters such as follows:

Table 2. Composition of shampoos.

S. No.	Ingredients	Batches								
		F₁	F₂	F₃	F₄	F₅	F₆	F₇	F₈	F₉
1.	Hydroxypropyl methyl Cellulose (15 cps) (% w/v)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
2.	Saponin (Reetha water based) (% w/v)	0.4	0.5	0.6	0.75	-	-	-	-	-
	Reetha (butanol based) (% w/v)	0.4	0.5	0.6	0.75	-	-	-	-	-
3.	Sodium lauryl sulphate (% w/v)	-	-	-	-	0.2	0.2	0.2	0.2	0.2
4.	Glycerine (% w/v)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

(Table 2) contd.....

S. No.	Ingredients	Batches								
5.	Neem (water based) (% w/v)	0.5	1.0	1.2	1.5	2.0	-	-	-	-
	Neem (ethanol based) (% w/v)	-	0.5	-	-	1.2	1.2	1.5	-	1.0
6.	Hibiscus (water based) (% w/v)	0.5	1.0	1.0	1.2	1.2	1.0	1.5	1.0	-
	Hibiscus (ethanol based) (% w/v)	-	0.5	0.5	-	-	1.5	1.2	0.5	1.0
7.	Amla (water based) (% w/v)	0.5	-	1.0	1.5	1.0	1.0	1.5	1.5	0.5
	Amla (ethanol based) (% w/v)	-	0.5	1.2	1.0	-	1.2	1.2	1.5	1.0
8.	Henna (water based) (% w/v)	0.5	0.5	1.0	1.0	1.2	1.2	1.5	1.5	0.5
9.	Urad dal (ethanol based) (% w/v)	0.5	-	-	1.2	1.2	-	1.5	1.5	1.0
10.	Distilled Water q.s.	100 ml								

2.5.1. Physical Appearance/Visual Inspection

The formulations prepared were evaluated in terms of their clarity, foam producing ability and fluidity [4].

2.5.2. The pH Measurement

The pH was measured using a pH-meter (Model no. µP pH-meter (Decibel)) on the undiluted shampoo. Three determinations were done for each shampoo [4]. For marketed formulation, 10% v/v solution of shampoo was prepared using distilled water and pH was determined.

2.5.3. Viscosity Measurement

The viscosity of 10% v/v shampoo solution (also for marketed shampoo) was measured by using an Ostwald Viscometer as per equation 1, and measurements were performed three times for each shampoo [4]:

$$\eta_{sol} = \eta_{H_2O} \times \frac{t_{sol}\rho_{sol}}{t_{H_2O}\rho_{H_2O}} \qquad (1)$$

η = Viscosity of solution, t = Time, ρ = Density

2.5.4. Determination of Solid Contents

Percent solid content was measured by taking a weighed clean, dry evaporating dish in which 4 g of shampoo was added. Then the dish and shampoo were weighed again. The exact weight of the shampoo was calculated, and the evaporating dish with shampoo was placed on the hot plate until the liquid portion was evaporated. The weight of shampoo only (solids) after drying was calculated [4].

2.5.5. Dirt Dispersion

To a large test tube containing 10 ml of distilled water, two drops of shampoo were added. One drop of ink was added in the test tube, fitted with a stopcock and shaken ten times. The amount of ink in the foam was estimated as none, light, moderate or heavy [4].

2.5.6. Wetting Time

The canvas was cut into a 3.5 cm diameter disc having an average weight of 0.34 g. The disc was floated on the surface of the shampoo solution (1% w/v) and a stopwatch was started. The time required for the disc to begin to sink was measured accurately and noted as wetting time [4].

2.5.7. Foam Volume

About 20 ml shampoo solution was taken in a measuring cylinder fitted with stopcock and was shaken for 10 sec. Foam volume was then calculated immediately, after 3 min and after 6 min [9].

2.5.8. Surface Tension Measurement

The surface tension of the undiluted shampoo was determined by the drop weight method, using a stalagmometer as per equation 2 [10]:

$$\sigma_{sol} = \sigma_{H_2O} \times \frac{m_{sol}}{m_{H_2O}} \qquad (2)$$

Where, δ_{sol}= Surface tension of solution, δ_{H_2O}= Surface tension of water, m_{sol}= Weight of drops of solution, m_{H_2O}= Weight of drops of water

The surface tension of marketed formulation was determined by preparing a 10%v/v solution of shampoo using distilled water.

2.5.9. Anti-lice Activity

Anti-lice activity was measured by placing live lice in Petri dish containing 2 ml shampoo. The time of mortality of lice mortality is defined as lack of movement of gut and limbs and failure to respond when the legs were stroked with forceps) was checked and noted [1, 11].

2.5.10. Cleansing Ability

Wool yarn (5 g) was dipped in grease. After that, it was placed in a flask containing 200 ml of water with 1 g of shampoo. The temperature of the water was maintained at 35 °C. The flask was shaken for 4 min at the rate of 50 times a minute. The solution was removed and the sample was taken out, dried and weighed. The amount of grease removed was calculated as per equation 3:

$$DP = 100\left(1 - \frac{T}{C}\right) \qquad (3)$$

DP is the percentage of detergency power, C is the weight of sebum in the control sample and T is the weight of sebum in the test sample [12].

Table 3. Evaluation parameters for shampoos.

S. No.	Evaluation Parameters	Batches								
		F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
1.	Physical appearance	Light brown	Dark greenish brown	Light greenish brown	Dark brown	Dark brown	Green	Greenish brown	Brown	Greenish brown
2.	pH	5.86 ±0.057	6.06 ±0.057	6.20 ±0.100	6.13 ±0.057	6.03 ±0.057	6.20 ±0.000	6.10 ±0.100	5.93 ±0.152	6.06 ±0.152
3.	Viscosity (cP)	1.05 ±0.011	1.04 ±0.005	0.98 ±0.011	0.98 ±0.00	1.00 ±0.011	1.02 ±0.005	0.99 ±0.03	0.96 ±0.00	0.96 ±0.011
4.	Dirt Dispersion	Light foam	Light foam	Light foam	Light foam	Light foam	Dark foam	Moderate foam	Moderate foam	Light foam
5.	Solid Content (%)	1.74 ±0.042	1.98 ±0.031	2.49 ±0.039	2.72 ±0.049	1.74 ±0.012	2.48 ±0.053	3.25 ±0.045	2.02 ±0.036	1.48 ±0.017
6.	Surface tension (dyne/cm)	39.86 ±7.86	36.83 ±5.47	36.60 ±1.42	40.15 ±7.65	33.05 ±0.86	35.56 ±0.66	34.02 ±0.53	33.41 ±1.19	34.89 ±1.22
7.	Wetting time (sec)	10 ±0.052	8 ±0.049	5 ±0.051	6 ±0.056	8 ±0.051	9 ±0.057	10 ±0.000	7 ±0.012	11 ±0.017

2.6. Stability Studies

The stability and acceptability of organoleptic properties (odor and color) of formulations during the storage period indicate that they were chemically and physically stable. The thermal stability of formulations was studied by placing shampoo in glass tubes and they were placed in a humidity chamber at 45°C and 75% relative humidity. Their appearance and physical stability were inspected for 2 months [4].

3. RESULTS AND DISCUSSION

Nowadays, natural products are continuously appealing consumers as they are inexpensive, readily available, less toxic, potentially degradable, capable of a multitude of chemical modifications and compatible when compared with synthetic products.

The formulations were tested for pH, viscosity, foam quality, dirt dispersion, % solid content, surface tension, wetting time and anti-lice activity. The results are shown in Table **3**.

HPMC base was advantageous over carbopol and xanthan gum; these polymers were of poor grade, so their purity was required for use. For purification, acetone or ethyl alcohol was used, but due to the carcinogenic effect of acetone, ethyl alcohol should be used, but ethyl alcohol is expensive. So, HPMC was used as a base due to its cost-effectiveness.

Water/alcohol-based extract of neem leaves was used for the control of lice because it contains bitter limonoids, which also act as an antidandruff agent. Water/alcohol-based extract of hibiscus leaves was used to stimulate thicker hair growth, prevent premature graying of hair, hair loss, and scalp disorders, and is also used as a conditioner due to its mucilaginous nature. Amla aqueous/alcoholic extract was used for pigmentation of hair, hair growth and nourishment of hair due to the presence of minerals. The use of a combination of the both aqueous and alcoholic extract was found to be beneficial. Water-based extract of henna leaves was used as hair colour due to the chemical interaction of lawsone to the keratin and it is also used as a conditioner. Urad alcoholic extract was used

for hair shining (white shine).

A combination of water and butanol extract of reetha was more effective as it had good foaming property along with detergency rather than aqueous or alcoholic extract alone.

The prepared formulations were evaluated for their physical appearance visually for consistency. The evaluation studies revealed that all the formulations had pH range within 5.9-6.2, which was adjusted to pH 7 by adding 0.1N NaOH (sodium hydroxide) dropwise. It influenced the hair quality and tolerance at the skin and eye level. A substance that was too alkaline causing the hair cuticle to open, while a substance that was too acidic causing the cuticle to contract was prone to infection so, the pH was adjusted to neutral. The viscosity of all the formulations of herbal shampoo was such that they could be easily pourable when shaken due to their pseudoplastic nature.

Foam quality of F_5 was found to be of high quality as it produced thick foam amongst all the formulations. The result is shown in Fig. (**1**).

The dirt should stay in the water, not in the foam, but if the dirt stays in the foam, it would be difficult to rinse and it would redeposit in the hair. The results indicated no dirt in the foam; so F_1, F_2, F_3, F_4, F_5 and F_9 formulations were satisfactory. If the shampoo has high solid content, it will be difficult to properly smudge it into the hair or too hard to wash out. The result of the percent of solid content is tabulated in Table **3** and it was found between 1.48-3.25%. As a result, all the formulations were easy to wash out. Surface tension was measured to find out the cleansing ability of shampoos. If the surface tension is low, the cleansing ability of the shampoo is enhanced in two ways, either facilitating the spread of the aqueous solution and by increasing its wetting ability or by the removal of dirt from hair by keeping it in the suspension. So, batch F_5 showed good wetting ability due to its reduced surface tension. The wetting time of all the formulations was found to be within 5-11 sec. When the wetting time was reduced, wettability was increased. So, formulation F_3 had good wetting power, whereas formulation F_9 had low wetting power.
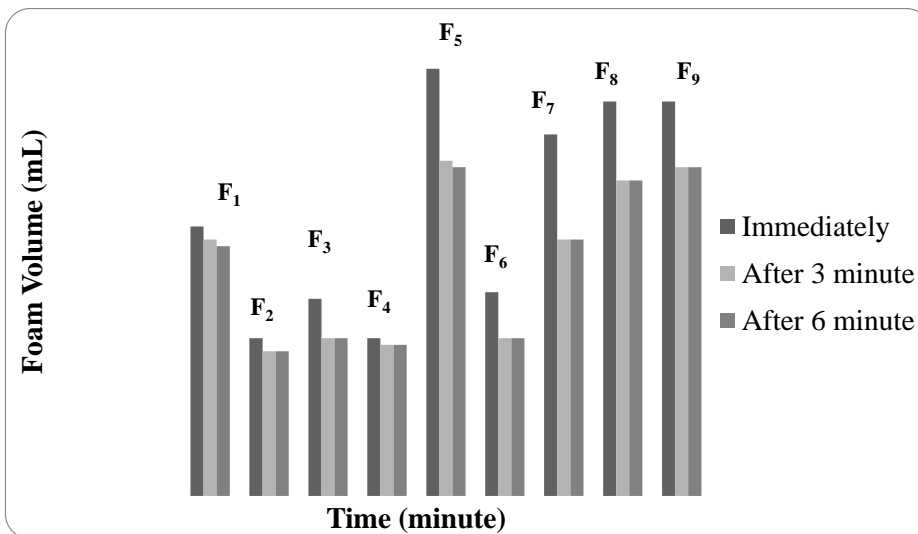
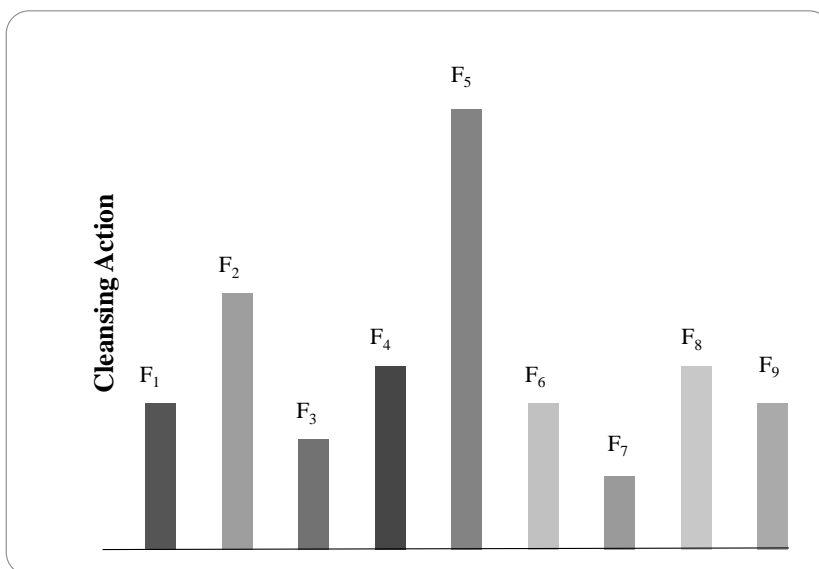**Fig. (1).** Foaming capacity of formulations.

Fig. (2). Cleansing action of shampoo formulations.

The anti-lice activity was assessed by using live lice and the mortality time was measured. Formulation F_2, F_3 and F_6 were prone to cause anti-lice activity leading to their mortality . The results are shown in Table **4**.

Table 4. The anti-lice activity of herbal shampoo formulations.

Organism	Batch- Fainting/Mortality Time (in minutes)								
	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Lice	Fainted in 15 min	Died in 60 min	Died in 120 min	Fainted in 30 min	Fainted in 30 min	Died in 105 min	Fainted in 15 min	Fainted in 30 min	Fainted in 30 min

Cleansing ability was also tested on all the formulations and results are shown in Fig. (**2**). The cleansing ability was good if it removed dirt and grease from hair easily. So, F_5 had good cleansing action.

Stability studies of shampoo formulations F_1, F_3 and F_5 were carried out for 2 months. During the studies, it was found that the solid content of the formulations was increased. In the context of all other parameters, formulations were found to be stable (Table **5**). The prepared shampoo data was compared with the marketed formulation (Vatika Premium Naturals (Henna & Olive) Shampoo). The physical appearance of the marketed shampoo was found to be light green colour with pH 6.1 ± 0.057, while the F5 batch of the prepared shampoo showed brown colour with pH 6.03 ± 0.057. The Vatika premium shampoo showed a viscosity of 0.98 ± 0.011 cP. Both the marketed and the prepared shampoo showed light foam property. The Vatika premium shampoo has solid content 1.94 ± 0.011 (%). The surface tension of the marketed product was found to be 34.05 ± 0.86 dyne/cm. The wetting time of the marketed shampoo was found to be 7 ± 0.051 sec.

Table 5. Stability studies of herbal shampoo formulations.

Evaluation Parameters	Stability Studies of Herbal Shampoo Formulations (for 60 Days)											
	F_1				F_3				F_5			
	0	15	30	60	0	15	30	60	0	15	30	60
Physical Appearance	Light brown	Light brown	Light brown	Light brown	Light greenish brown	Light greenish brown	Light greenish brown	Light greenish brown	Dark brown	Dark brown	Dark brown	Dark brown
pH	5.93 ±0.057	5.83 ±0.057	5.90 ±0.000	5.80 ±0.000	6.16 ±0.057	6.00 ±0.000	6.20 ±0.100	6.13 ±0.057	6.10 ±0.100	6.00 ±0.000	6.03 ±0.057	6.10 ±0.000
Viscosity (cP)	1.06 ±0.010	1.06 ±0.011	1.08 ±0.01	1.07 ±0.000	0.98 ±0.005	0.99 ±0.005	1.00 ±0.000	1.03 ±0.057	1.01 ±0.011	1.02 ±0.000	1.00 ±0.011	1.02 ±0.005
Dirt Dispersion	Light foam	Light foam	Light foam	Light foam	Light foam	Light foam	Light foam	Light foam	Light foam	Light foam	Light foam	Light foam
Solid Content (%)	1.75	1.78	1.80	1.84	2.54	2.59	2.61	2.65	1.76	1.79	1.83	1.86
Surface tension (dyne/cm)	42.18 ±0.965	42.48 ±1.49	41.65 ±0.406	39.67 ±0.465	35.88 ±1.65	35.61 ±0.587	36.79 ±0.625	37.14 ±0.201	32.96 ±0.140	34.12 ±0.230	33.80 ±0.786	32.95 ±0.178
Wetting time (sec)	11	10	11	11	5	4	5	4	9	8	8	9

CONCLUSION

The study showed that herbal shampoo was successfully formulated using different natural herbal extracts and evaluated using different parameters. The result of the evaluation revealed that all the formulations were of good quality, which were used to clean hair effectively. As they were naturally extracted so, consumer acceptance was increased without any side effect as compared to the synthetic ones. The anti-lice activity was also shown by these formulations and could be used for the same. Formulation F_5 was found to be effective in almost all aspects. In the context of the anti-lice activity, formulations F_2, F_3 and F_6 were effective. The prepared formulations were observed to be comparable to marketed (control) formulation.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

No animals or humans were used in the study.

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIALS

Reference data can be provided by the corresponding author [R.M.] on reasonable request.

FUNDING

None.

CONFLICT OF INTEREST

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

REFERENCES

[1] Sawant PS, Sankpal PB, Jagtap AM, Gavade AS, Vambhurkar GB. Formulation and evaluation of herbal shampoo. Res J Top Cosmet Sci 2020; 11(1): 1-4.
[http://dx.doi.org/10.5958/2321-5844.2020.00001.1]

[2] Patel I, Talathi A. Use of traditional Indian herbs for the formulation of shampoo and their comparative analysis. Int J Pharm Pharm Sci 2016; 8(3): 28-32.

[3] Moghimipour E, Jasemnezhad M, Mohammad Soleymani S, Salimi A. Preparation and evaluation of a free surfactant herbal shampoo with *Acanthophyllum Squarrosum* Saponins J Cosmet Dermatol 2020.
[http://dx.doi.org/10.1111/jocd.13483] [PMID: 32413183]

[4] Deeksha , Malviya R, Sharma PK. Advancement in shampoo (a dermal care product): Preparation methods, patents and commercial utility. Recent Pat Inflamm Allergy Drug Discov 2014; 8(1): 48-58.
[http://dx.doi.org/10.2174/1872213X08666140115110727] [PMID: 24433548]

[5] El-Sharawy RT, Elkhateeb A, Marzouk MM, Abd El-Latif RR, Abdelrazig SE, El-Ansari MA. Antiviral and antiparasitic activities of clovamide: The major constituent of *Dichrostachys cinerea* (L.) wight et arn J Appl Pharm Sci 2017; 7(9): 219-23.

[6] Gutiérrez MM, Werdin-González JO, Stefanazzi N, Bras C, Ferrero AA. The potential application of plant essential oils to control Pediculus humanus capitis (Anoplura: Pediculidae). Parasitol Res 2016; 115(2): 633-41.
[http://dx.doi.org/10.1007/s00436-015-4781-8] [PMID: 26462802]

[7] Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 36th ed. New Delhi: Nirali Prakashan 2006.

[8] http://habmath.hubpages.com/hub/Natural-Homemade-shampoos

[9] Moldovan M, Parauan S. Cosmetic evaluation of some commercial shampoos. Clujul Med 2012; 85(3): 378-83.

[10] https://www.fpharm.uniba.sk/fileadmin/user_upload/english/Fyzika/The_surface_tension_of_liquids_measured_with_the_stalagmometer.pdf

[11] Thompson D, Lemaster C, Allen R. Evaluation of relative shampoo

detergency. J Soc Cosmet Chem 1985; 36: 271-86.

[12] Deeksha, Malviya R, Sharma PK. Evaluation of marketed shampoo (synthetic and natural) for their hair cleansing, dirt dispersion, wetting time, solid content and foaming capacity properties. Glob J Pharmacol 2014; 8(4): 490-3.