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RESEARCH ARTICLE

Formulation and *In Vitro* Characterization of Tea Tree Oil Anti-Dandruff Shampoo

Humra Umar[1], Tariq Mahmood[1], Talib Hussain[2,3], Rabia Aslam[1], Yasser Shahzad[2] and Abid Mehmood Yousaf[2,*]

[1]*Faculty of Pharmacy, University of Central Punjab, Lahore, Pakistan;* [2]*Department of Pharmacy, COMSATS University Islamabad, Lahore Campus, Lahore, Pakistan;* [3]*Provincial Pharmacovigilance Centre, Provincial Drug Control Unit, Lahore, Pakistan*

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Abstract: ***Background*:** Dandruff is a common scalp condition affecting half of the population of the world.

***Objective*:** The current study aimed at developing anti-dandruff shampoos containing tea tree oil, which is believed to be effective against *Malassezia furfur*, a fungus involved in dandruff production.

***Methodology*:** Various shampoos containing tea tree oil in 0.5 to 3% concentration were prepared after careful selection of various shampoo ingredients. The formulated shampoos were subjected to various quality tests such as pH, viscosity, foam production, dirt dispersion, wetting time, surface tension, solid contents, and antimicrobial activity against a model fungal strain, namely *Candida albicans*. The formulated shampoos were also compared with the marketed shampoos for quality attributes.

***Results*:** The results revealed that tea tree oil shampoos had pH values in the range of 5 – 6, which is close to the slightly acidic skin's pH and considered as good for hair. All other quality attributes were comparable to the marketed products. The marketed shampoos had superior antifungal activity due to the presence of zinc pyrithione or a higher concentration of salicylic acid or selenium sulfide. Notwithstanding, the tea tree oil shampoos demonstrated an appreciable antifungal activity due to synergistic effects of tea tree oil, sodium lauryl sulphate, and salicylic acid. Furthermore, the tea tree oil shampoos were stable during two months-long stability testing.

***Conclusion*:** Thus, tea tree oil anti-dandruff shampoos have the potential to address the dandruff problem.

Keywords: Anti-dandruff, antifungal, formulation, shampoo, tea tree oil, marketed shampoos.

1. INTRODUCTION

Dandruff is a non-inflammatory chronic dermal disorder that affects almost half of the world's population [1]. Dandruff is characterized as an unpleasant post-puberty problem of hair and scalp, especially in males, which is often associated with itching due to flaky hair and scalp [2, 3]. A lipophilic yeast, namely *Malassezia furfur*, is associated with dandruff production in sebum-rich areas such as the scalp [4]. Dandruff is generally treated with shampoos containing synthetic or natural ingredients. Synthetic ingredients such as selenium sulfide or antifungals containing azole ring are often used in chemical-based shampoos; however complete eradication of dandruff is difficult with this regime [5]. Shampoos containing major components of synthetic origin are also responsible for adverse reactions such as skin irritation, allergy, hair breakage, skin and hair discoloration [6]. On the other hand, herbal shampoos have shown promising results in addressing the issue of dandruff. Herbal shampoos containing natural plant extracts are usually compatible with the skin and may not elicit side effects that are often associated with synthetic ingredients [7].

Many natural extracts from plants have beneficial effects on skin and hair due to the presence of vitamins, amino acids, sugars, glycosides, phytohormones, bioflavonoids, fruit acids, and essential oils and are commonly used in shampoos [8]. Various plants such as *Azadirachta indica* (Neem), *Ocimum sanctum* (Tulsi), *Aloe vera* (aloe), *Terminalia chebula* (harda, haritaki), *Terminalia bellirica* (bahera), *Sapindus mukorossi* (Reetha), *Phyllanthus emblica* (Amla), and *Acacia concinna* (Sheekakai) have been traditionally used for hair cleansing, conditioning, and removing dandruff in sub-continent including Pakistan and India [9]. Although these plants have been used for centuries for hair care, yet commercial herbal shampoos mainly

*Address correspondence to this author at the Department of Pharmacy, COMSATS University Islamabad, Lahore Campus, Lahore, Pakistan;
E-mail: abid.ucp@hotmail.com

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consist of synthetic ingredients boosted with natural products [10].

Essential oil from the plant *Melaleuca alternifolia*, (tea tree oil) has recently shown promising results against skin infections because of its strong antibacterial, anti-fungal, and anti-viral activities[11]. Tea tree oil contains a number of hydrocarbons and terpenes; however, terpene–4–ol is the major component.Terpene–4–ol is believed to have antimicrobial activity and could possibly be effective against *Malassezia furfur*[12]. Satchell and co-workers have demonstrated that 5% tea tree oil shampoo was effective against dandruff without any skin irritation [13]. However, they did not disclose the complete composition of the shampoo they used in their study.

Here, we have described a new shampoo formulation containing tea tree oil as a dandruff remedy. The prepared shampoos were extensively characterized for various quality attributes. More importantly, the prepared shampoos were compared with five commercially available shampoos in Pakistan, namely Vatika, Clear for Men, Head & Shoulder, Selsun blue, and Garnier. The anti-dandruff activity of tea tree oil shampoo and marketed shampoos was determined by anti-microbial activity against *Candida albicans* as a model fungal strain [14].

2. MATERIALS AND METHODS

2.1. Materials

Tea tree oil was procured from Xpel marketing Ltd (England). Sodium lauryl sulphate, salicylic acid, sodium EDTA, guar gum, Tween 80, sodium hydroxide, urea, and xanthan gum were purchased from Sigma-Aldrich (Germany). Lanolin was sourced from Suru Chemicals & Pharmaceuticals Private Ltd. (India). Triethanolamine was procured from Merk (Germany). The purified water that was prepared at an in-house facility was used throughout the studies.

2.2. Formulation of Anti-dandruff Shampoo

Initially, a total of eight formulation trials were conducted in order to find an optimal shampoo formulation. In all eight trials, we fixed the quantities of SLS, lanolin, sodium EDTA, Tween 80, urea, and salicylic acid, whilst we systematically studied the effect of xanthan gum, guar gum, and gelatin, as given in Table **1**. The shampoo formulations were prepared by mixing the ingredients in distilled water using a magnetic stirrer operating at 500 RPM. The final pH of the shampoo was adjusted between 5 – 6 either by 0.4N NaOH solution or 2% triethanolamine solution. The prepared shampoo trials were evaluated for physical appearance, and the most stable formulation was selected for making the anti-dandruff shampoo by adding tea tree oil at various concentrations, namely 0.5%, 1%, 1.5%, 2%, 2.5%, and 3% designated by S1, S2, S3, S4, S5, and S6, respectively.

2.3. Characterization of Shampoos

2.3.1. Organoleptic Properties

The prepared anti-dandruff shampoos were compared with the marketed anti-dandruff shampoos based on sensory evaluation. The shampoos were inspected for color, clarity, odor, andtexture [15].

2.3.2. Determination of pH

The pH values of prepared shampoos and marketed selected shampoos were determined using a digital pH meter at ambient temperature 25 ± 0.2°C.

Table 1. Various trials of shampoo formulations.

Ingredients (Grams)	F1	F2	F3	F4	F5	F6	F7	F8
SLS	15	15	15	15	15	15	15	15
Lanolin	1	1	1	1	1	1	1	1
Sodium EDTA	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Xanthan gum	3	-	2	-	3	-	2	-
Guar gum	-	1	-	-	-	1	-	-
Gelatin	-	-	-	2	-	-	-	2
Tween 80	1	1	1	1	1	1	1	1
Urea	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sodium hydroxide	q.s	q.s	q.s	q.s	-	-	-	-
Triethanolamine	-	-	-	-	q.s	q.s	q.s	q.s
Salicylic acid	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Distilled water	q.s. to make 100 mL							

2.3.3. Viscosity Measurements

The viscosity of shampoos was determined using a Brookfield viscometer (BDV-8S) equipped with an L3 spindle, and the spindle rotation speed was set at 1.5, 3, 6, 12, 30, and 60 RPM. Viscosity measurements were performed in triplicate at ambient temperature (25 ± 0.2°C).

2.3.4. Solid Contents Determination

The percentage of solid contents was measured by the loss-on-drying method. Briefly, 5 g of each shampoo formulation was poured in clean, dry, and pre-weighed Petri dishes, and the final weight was recorded. This was followed by placing Petri dishes in a convection oven set at 50°C for 1 h or until shampoos were completely dried. The driedPetri dishes were weighed again, and the solid content after drying was estimated using the following formula [9]:

$$Solid\ contents\ (\%) = \frac{W_\circ - W_1}{W_\circ}$$

Where,*Wo* is the initial weight of the sample while *W1* is the weight of solid contents.

2.3.5. Surface Tension Determination

Surface tension was measured using a stalagmometer by making a 10% dilution of each shampoo in distilled water at ambient temperature (25 ± 0.2°C). The surface tension of shampoos was determined in triplicate using the formula given below:

$$\gamma_1 = \frac{n_2 \times \rho_1}{n_1 \times \rho_2} \times \gamma_2$$

Where,γ_1 is the surface tension of shampoo, $n_1$is the number of drops of shampoo, n_2 is the number of drops of water, ρ_1 is the density of shampoo, ρ_2 is the density of water, and γ_2 is the surface tension of water.

2.3.6. Foam Volume

The foam volume of the prepared shampoos and the marketed shampoos was determined by the shake cylinder method, as described previously in a study[9]. Briefly, 50 mL of 10% shampoo solution was added to a 250mL graduated cylinder and shook 10 times. The total volume of foam after 1, 2, 3, and 4 min of shaking was recorded immediately using the graduates of the cylinder. The process was repeated three times.

2.3.7. Dirt Dispersion

Two drops of shampoo were added to 10 mL of distilled water in a test tube, followed by the addition of one drop of Indian ink. The test tube was stoppered and gently shaken10 times. The amount of Indian ink was visually estimated in the foam as none, light, moderate and heavy. Shampoos that caused the color to stay in the foam were considered low quality[16].

2.3.8. Wetting Time

The wetting time of shampoos was determined by dropping 50 mL of 1% aqueous shampoo solution on 1g of wool yarn in a 100 mL beaker [17]. The time when wool yarn started to float at the surface of the shampoo solution and when it started to sink was recorded carefully by a stopwatch. The mean values and standard error of at least three replicates were reported.

2.3.9. Detergency Power

Detergency power (DP) of each shampoo was estimated by soaking 5 grams of wool yarn in grease, weighing it, and then placing it in 100 mL of 1% aqueous solution of shampoo in a 250 mL capacity flask. The flask was then fixed in a shaking water bath that was set at 35°C and was shaken for 4 min at the rate of 50 agitations per min. Afterward, the solution was removed from the flask, and the wool yarn was allowed to dry. After drying, the wool yarn was weighed, and the detergency power was calculated by using the following equation [18]:

$$DP\ (\%) = 1 - \frac{T}{C} \times 100$$

Where, C is the initial weight of grease and T is the weight of grease after removing it from the shampoo solution.

2.3.10. Antimicrobial Activity of Shampoos

Anti-microbial activity of formulated shampoos and the marketed shampoos was determined using the agar well diffusion method against *Candida albicans* culture[19, 20]. Briefly, Sabouraud Dextrose Agar (SDA) media was prepared and poured into sterilized Petri dishes. After solidification of media in the Petri dishes, 4 mm diameter hole was made using a sterilized cork borer. This was followed by adding 3 mL of *Candida albicans* culture and was spread using a sterilized glass spreader. The holes were filled with 150 µL of shampoo, and Petri dishes were covered immediately with a lid and sealed with parafilm. The sealed Petri dishes were placed in an incubator operating at 37°C for 24 h. After 24 h, zones of inhibition were measured and reported as the mean and standard deviation of at least three replicates. Tea tree oil solutions of respective concentrations (as used in formulated shampoos) were used to compare zones of inhibition of each formulated tea tree oil shampoo. This was further compared with the antifungal activity of the marketed shampoos.

2.3.11. Stability Studies

Stability studies on shampoos were conducted by placing them in an incubator at 45°C and 75% relative humidity for 2 months and were regularly evaluated for a physical appearance at 1, 4, 7, 15, 30, and 60 days.

2.3.12. Statistical Analysis

All the experiments were conducted in triplicate, and their mean ± standard deviation was reported. One-way ANOVA was applied to check the significance of the difference, and the p values<0.05 were considered significant. The data was statistically analyzed using Origin software (version 8.5, OriginLab, USA).

3. RESULT AND DISCUSSION

Here we have described a new anti-dandruff shampoo formulation containing tea tree oil at various concentrations. Initially, we have performed various trials to find a suitable shampoo base to incorporate tea tree oil. Trial shampoos

were formulated using different natural polymers such as xanthan gum, guar gum, and gelatin and were evaluated for their physical appearance, pH, and day-long stability. Among eight trial formulations, F2 appeared to be more stable than any other formulation after keeping it at 45°C for 24 h, and it had a pH value of 5.8, which is very close to the skin pH. Additionally, the physical appearance of shampoo with a formulation containing guar gum (F2) was more appealing in terms of texture and clarity. Furthermore, shampoo formulation with triethanolamine as pH adjuster was found to have a pungent smell as compared to the formulation containing sodium hydroxide as a pH adjuster; thus sodium hydroxide was deemed suitable. Finally, based on initial observations, F2 formulation was chosen for incorporation of tea tree oil at 6 concentrations, namely 0.5%,1%, 1.5%, 2%, 2.5%, and 3%. The prepared tea tree oil shampoos were also compared with marketed shampoos, namely Vatika, Clear for Men, Head & Shoulder, Selsun blue, and Garnier Fructis.

3.1. Organoleptic Properties

All the formulated shampoos and marketed shampoos were evaluated for color, clarity, odor, and texture. The results of organoleptic evaluations are summarized in Table **2**. In the formulated shampoos, we did not add any color or fragrance; however, the presence of lanolin resulted in pale yellow colored shampoo formulations. On the other hand, all the marketed shampoos were perfumed and colored for their aesthetic appeal. The tea tree oil shampoos appeared cloudy with gel-type texture whilst the marketed shampoos appeared shiny with gel-type texture. While the formulated shampoos were acceptable, the marketed shampoos had a clear edge over the tea tree oil shampoos in terms of appearance and odor.

3.2. pH and Viscosity Measurements

Our prepared shampoos had pH in the range of 5.4 – 5.7, which is close to the pH of the skin, as given in Table **2**. However, the marketed shampoos showed a markedly variable pH value from 3.9 to 7.4. Hair consists of tiny scales known as cuticles, which are sensitive to pH, especially alkaline pH, which opens up these scales, ultimately leading to hair damage. Thus a slightly acidic pH is favourable [21]. Furthermore, the slightly acidic pH of shampoos increases the hair's quality, decreases eye irritation, and maintains the ecological balance of the scalp [22].

The viscosity of tea tree oil shampoos and marketed shampoos was determined at various spindle speeds. As can be seen from Fig. (**1A**), the viscosity of formulated shampoos decreased from 4170.5cP to 1114.8cP with increasing tea tree oil concentrations from 0.5% to 3%at a spindle speed of 1.5 rpm. It was not clear why the viscosity of shampoos decreased with increasing tea tree oil concentration; one possibility could be the modification in the gelling ability of natural hydrophilic polymer by adding the essential oil. This would, in turn, had reduced the overall viscosity of the shampoo formulation. Previous research has revealed an alteration in pH by increasing the tea tree oil contents, thereby reducing the viscosity [23], however, this was not true in our study, and the pH values remained fairly stable with increasing tea tree oil contents (Table **2**). Nevertheless, the important factor is pseudo-plastic behaviour which is a desirable attribute in shampoo formulations [24]. Results revealed that the viscosity of each of the tea tree oil shampoo was decreased with increasing spindle speed from

Table 2. Organoleptic properties and pH of anti-dandruff shampoos.

Shampoo	Color	Clarity	Odor	Texture	pH
Tea Tree Oil Anti-Dandruff Shampoos					-
S1	PY	C	PL	GT	5.7 ± 0.0
S2	PY	C	PL	GT	5.6 ± 0.0
S3	PY	C	PL	GT	5.7 ± 0.1
S4	PY	C	PL	GT	5.6 ± 0.1
S5	PY	C	PL	GT	5.4 ± 0.0
S6	PY	C	PL	GT	5.5 ± 0.1
Marketed Shampoos					-
Head & Shoulder	W	S	PL	GT	7.4 ± 0.2
Selsun Blue	BG	S	PL	GT	3.9 ± 0.0
Garnier Fructis fortifying shampoo	SB	S	PL	GT	4.9 ± 0.6
Clear	PB	S	PL	GT	5.4 ± 0.1
Vatika	Y	S	PL	GT	6.3 ± 0.1

Abbreviations: PY=Pale Yellow, C=Cloudy, GT=Gel Type, W=White, BG=Blue-Green, SB=Sky Blue, PB=Pale Blue, Y=Yellow, S=Shiny, PL=Pleasant.

1.5 rpm to 60 rpm, which confirmed the pseudo-plastic behaviour of prepared shampoos [25]. On the other hand, the marketed shampoos had significantly higher($p<0.05$) viscosity as compared with that of the formulated tea tree oil shampoos (Fig. **1B**), possibly because of compositional difference in formulated and marketed shampoos. Nevertheless, a similar trend of decreasing viscosity with increasing spindle speed was observed for the marketed shampoos. Generally, a decrease in viscosity with increasing shear rate is a favorable activity, which helps in the easy spreading of shampoo on hair [26].

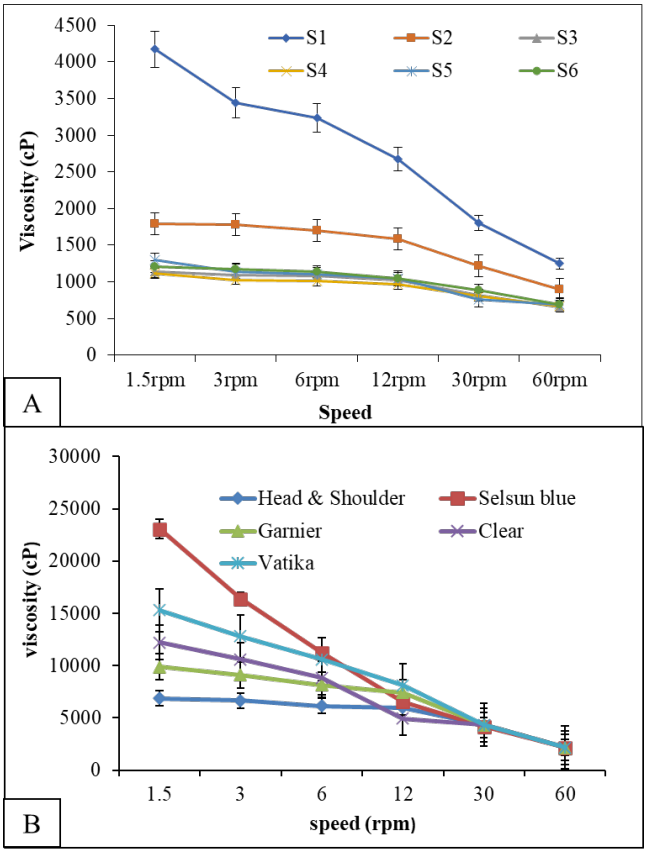

Fig. (1). Viscosities of (**A**) Tea Tree Anti-Dandruff shampoos and (**B**) marketed shampoos. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.3. Percentage Solid Contents

For good quality anti-dandruff shampoos, solid content should ideally be between 20 – 30%. This allows for easy rinsing and removal from the hair [7]. Fig. (**2A** & **B**) depicts solid contents in the prepared tea tree oil anti-dandruff shampoos and the marketed shampoos. The solid contents in formulated shampoos ranged from 20.03 – 22.25%, whilst the solid contents in marketed shampoos ranged from 21.96 – 32.20%. All the tea tree oil shampoos had a lower range of solid contents, thus deemed excellent. On the other hand, marketed shampoos, namely Garnier Fructis and Clear had slightly higher solid content, whilst Head & Shoulder, Selsun Blue, and Vatika shampoos had solid content within an ideal range.

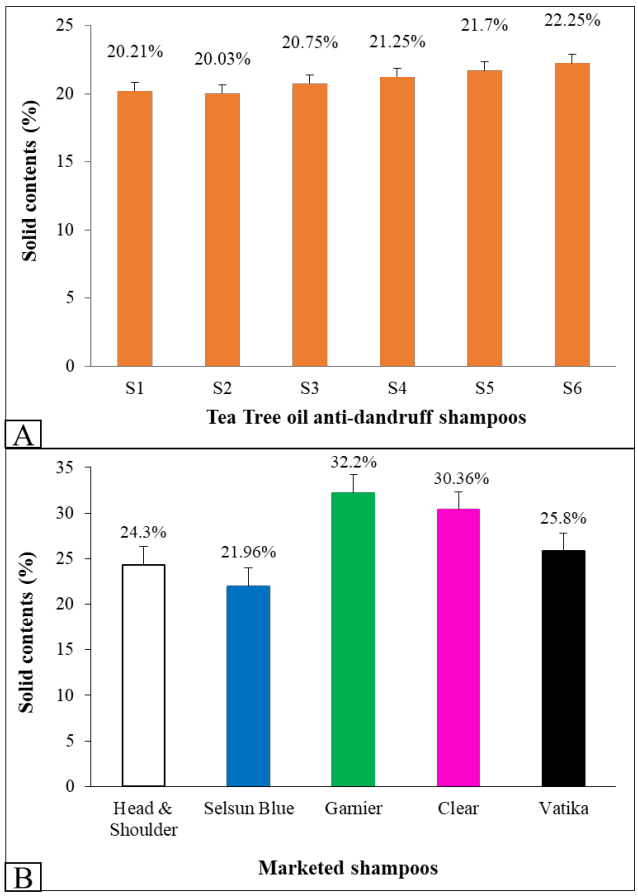

Fig. (2). Percentage of solid contents in (**A**) tea tree anti-dandruff shampoos and (**B**) marketed shampoos. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.4. Measurement of Surface Tension

Surface tension is linked with the cleaning ability of shampoos. Surfactants have the ability to reduce the surface tension and the lesser the surface tension, the higher the cleaning ability. Good shampoos generally reduce the surface tension of water, which is approximately 72.28 dyn/cm[25]. The measured surface tensions of tea tree oil and marketed shampoos are listed in Table **3**. As can be seen from Table **3**, all the shampoos had surface tension in the range of 24.02 – 36.27 dyn/cm, which is sufficiently below the surface tension of pure water. Thus, all shampoos were able to significantly reduce the surface tension of water and were deemed good for their cleaning ability. However, the surface tension of marketed shampoos was significantly less ($p<0.05$) than that of the tea tree oil shampoos. Furthermore, all tea tree oil shampoos reduced the surface tension significantly, yet no correlation existed between tea tree oil concentration and reduction in surface tension.

3.5. Foam Volume

From the customer perspective, foaming has paramount importance in the quality of shampoos, however, there is no correlation existed between foaming and cleaning [25]. Fig.

Table 3. A comparison of surface tension, dirt dispersion, and wetting time of tea tree oil and marketed shampoos.

Shampoo	Surface Tension (dyn/cm)	Dirt Dispersion	Wetting Time (Seconds)
S1	35.61 ± 1.12	None	5.46± 0.09
S2	36.27 ± 0.98	Light	4.65± 0.11
S3	34.73 ± 0.66	Light	4.54± 0.32
S4	36.09 ± 0.32	None	7.30± 0.15
S5	35.15 ± 0.11	Light	7.55± 0.12
S6	34.54 ± 1.09	Light	6.98± 0.50
Head & Shoulder	27.24 ± 0.87	Light	4.40± 0.08
Selsun Blue	32.96 ± 0.54	None	4.24± 0.57
Garnier Fructis fortifying shampoo	24.02 ± 0.21	Light	5.29± 0.03
Clear	27.03 ± 0.33	Light	6.03± 0.12
Vatika	29.28 ± 1.99	Light	5.04± 0.85

(**3A** & **B**) depicts the volume of foam produced by tea tree oil shampoos and marketed shampoos in distilled water at different time points. All the shampoos (formulated and marketed) resulted in foam in higher than 100 mL volume, and the foam was stable throughout the testing time. The foam volume produced by tea tree oil shampoos and marketed shampoos was comparable and no significant difference existed. The foaming ability of shampoos is correlated with the surface tension reduction by the surfactants. Without the presence of surfactants, there will be enough surface tension that could pull the bubbles closed, resulting in limited or no foam at all. However, the presence of surfactants at the air/water interface reduces the surface tension to a point where the pull caused by the tension becomes negligible and air bubbles stabilize for a longer duration of time[27]. Since our formulated shampoos and marketed shampoos had surfactants, a reduced surface tension resulted in bubble formation as revealed by the foaming of shampoos.

3.6. Dirt Dispersion

Dirt dispersion is an important quality attribute of shampoos and it is linked with the cleansing action of shampoos. Generally, shampoos that cause the ink to stay in the foam instead of the liquid portion are considered poor quality products because of difficulty in washing away the ink, consequently, the dirt would be difficult to remove from the hair [16]. Among the prepared tea tree oil shampoos, S2, S3, S5, and S6 had light ink in their foam section while no ink was detected in the S1 and S4 shampoo formulation (Table **3**). On the other hand, all marketed shampoos except Selsun Blue shampoo showed light ink distribution in the foam. Thus, tea tree oil shampoos were similar to marketed shampoos, while S1 and S4 shampoo formulations were found to be superior in terms of cleansing action on the basis of dirt dispersion.

3.7. Wetting Time

The wetting ability of shampoos describes their efficacy as cleansing products and it depends on the surfactant used. The marketed shampoos had wetting time in the range of 4.24 – 6.03s (Table **3**). In comparison, the formulated tea tree oil shampoos showed wetting time in the range of 4.54 – 7.55s (Table **3**). The results revealed that there was no significant difference ($p>0.05$) in the wetting time of formulated tea tree oil shampoos and the marketed shampoos.

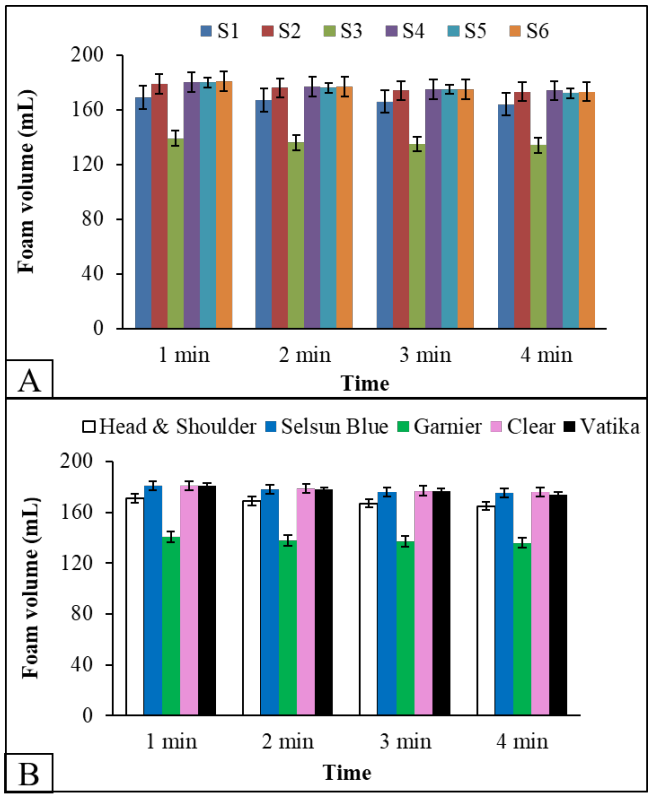

Fig. (3). Foam Volume of (**A**) tea tree oil anti-dandruff shampoos and (**B**) marketed shampoos. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.8. Cleaning Action

The prepared tea tree oil shampoos and the marketed shampoos were subjected to evaluation of detergency power or cleaning action. Although experimental assessment of detergency power is difficult to standardize, yet it is a general agreement that good quality shampoos should effectively remove the grease or oil from the hair [18]. Our results showed that the cleaning action of tea tree oil shampoos was in the range of 23.0 – 30.7% as compared to 28.6 – 30.5% for the marketed shampoos, as depicted in Fig. (**4A** & **B**). With little or no difference in cleaning action, we can say that our formulated shampoos were as good as the marketed shampoos.

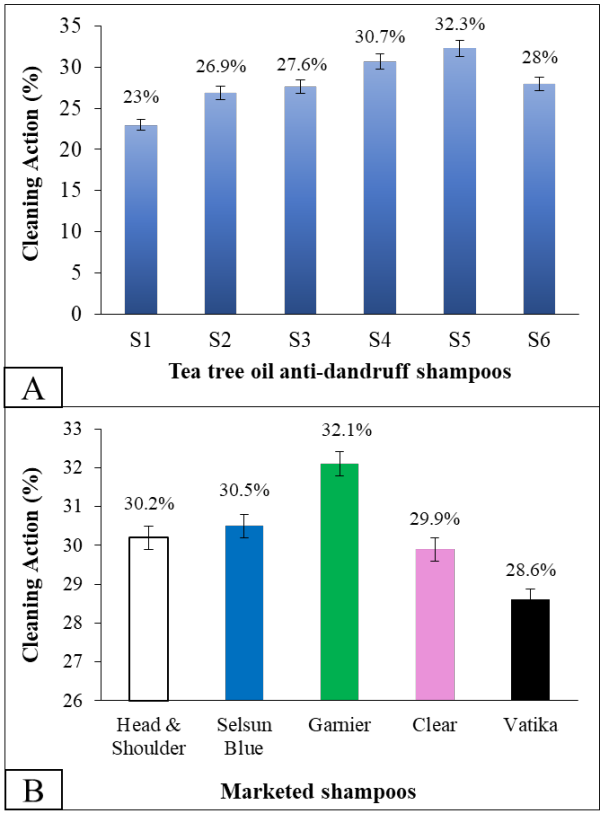

Fig. (4). Cleaning action of (**A**) tea tree oil anti-dandruff shampoos and (**B**) marketed shampoos. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

3.9. Antimicrobial Activity of Tea Tree Oil Anti-Dandruff Shampoos

Antifungal activity of tea tree oil shampoos and the marketed shampoos was studied against a model fungal strain, namely*Candida albicans*. Moreover, pure tea tree oil solutions were also studied to compare the shampoo formulation. In this case, pure tea tree oil solutions were considered as a control to compare the effect of other ingredients of formulations for their antifungal activity, and it was found that the solutions had the same concentration as used in the shampoo formulations. The antifungal activity in terms of zones of inhibitions of pure tea tree oil, tea tree oil shampoos, and the marketed shampoos is listed in Table **4**.

As can be seen from Table **4**, pure tea tree oil solutions had antifungal activity against *Candida albicans*, as demonstrated by zones of inhibition. The inhibition of fungal growth was concentration-dependent for pure tea tree oil solutions. The fungal growth inhibition was more pronounced in the formulated tea tree oil shampoos as demonstrated by the values. Although no concentration-dependent inhibition was observed, yet the inhibition zones were larger than those produced with pure tea tree oil solution. The enhanced antifungal activity of tea tree oil shampoos was attributed to the presence of SLS and salicylic acid, which have some antifungal activity and contributed synergistically against the fungal strain [28, 29]. On the other hand, the marketed shampoos had significantly higher ($p<0.05$) zones of inhibition, thus had the higher antifungal activity against *Candida albicans*. However, Selsun Blue had the lowest antifungal activity as compared to other marketed shampoos and its antifungal activity was insignificantly different ($p>0.05$) from our formulated shampoos (Table **4**). Overall, the higher antifungal activity of the marketed shampoos was attributed to a combined effect of zinc pyrithione, SLS, and salicylic acid. Conclusively, the tea tree oil shampoos had demonstrated an appreciable antifungal activity and could serve as an excellent alternative to the marketed shampoos.

3.10. Stability Studies

Finally, the tea tree oil shampoos and the marketed shampoos were compared in terms of their stability at various temperatures, such as 4°C, 25°C, and 40°C (75% RH). The shampoos were stored at each of the temperatures for 2

Table 4. Zone of inhibition of tea tree oil shampoos and their Standards.

Standard (Tea Tree Oil Solution)	Zone of Inhibition (mm)	Tea Tree Oil Shampoos	Zone of Inhibition (mm)	Marketed Shampoos	Zone of Inhibition (mm)
0.5%	14.2± 0.4	S1	23.8± 1.1	Head & Shoulder	33.35 ± 2.1
1%	16.2± 0.6	S2	22.3± 0.8	Selsun Blue	20.45 ± 0.9
1.5%	17.4± 0.2	S3	24.8± 0.3	Garnier Fructis	41.85 ± 2.8
2%	18.6± 0.8	S4	26.9± 1.4	Clear	35.5 ± 1.9
2.5%	22.9± 0.9	S5	20.1± 0.7	Vatika	35.8 ± 2.6
3%	21.7± 0.4	S6	25.7± 1.7	-	-

months, and the physical appearance and pH values were determined at various time points, as shown in Table **5**-**7**. As can be seen from the stability data at various conditions, no change in physical appearance was observed, however, an insignificant change ($p>0.05$) in pH values was observed at all storage conditions. Since the pH change was small, we can say that all the formulations were sufficiently stable over the 2 months stability testing period.

Table 5. Stability studies on tea tree oil shampoos and the marketed shampoos at 4°C.

Stability Studies at 4°C						
Shampoo Formulations	**At 1st Day**	**At 4th Day**	**At 7th Day**	**At 15th Day**	**At 1 Month**	**At 2 Months**
S1	5.70± 0.01	5.73± 0.07	5.75± 0.21	5.78± 0.42	5.81± 0.00	5.88± 0.13
S2	5.60± 0.18	5.61± 0.04	5.64± 0.08	5.66± 0.00	5.70± 0.02	5.76± 0.09
S3	5.70± 0.14	5.70± 0.21	5.73± 0.07	5.75± 0.03	5.82± 0.08	5.88± 0.00
S4	5.60± 0.00	5.63± 0.04	5.65± 0.03	5.69± 0.07	5.73± 0.00	5.76± 0.00
S5	5.40± 0.04	5.42± 0.18	5.45±0.09	5.48± 0.22	5.52± 0.11	5.59± 0.10
S6	5.50± 0.03	5.50± 0.00	5.53± 0.17	5.56± 0.2	5.60± 0.15	5.67± 0.06
Marketed Shampoos						
Head & Shoulder	7.41± 0.01	7.43± 0.01	7.46± 0.01	7.49± 0.01	7.54± 0.01	7.59± 0.01
Selsun Blue	3.90± 0.00	3.91± 0.11	3.95± 0.16	4.01± 0.03	4.08± 0.08	4.12± 0.10
Garnier fructis	4.92± 0.00	4.95± 0.02	4.97± 0.10	5.02± 0.20	5.09± 0.19	5.14± 0.12
Clear	5.43± 0.17	5.47± 0.05	5.49± 0.00	5.54± 0.02	5.60± 0.01	5.64± 0.09
Vatika	6.30± 0.04	6.33± 0.11	6.36± 0.03	6.41± 0.01	6.46± 0.08	6.52± 0.00

Table 6. Stability studies on tea tree oil shampoos and the marketed shampoos at 25°C.

Stability Studies at 25°C						
Shampoo Formulations	**At 1st Day**	**At 4th Day**	**At 7th Day**	**At 15th Day**	**At 1 Month**	**At 2 Months**
S1	5.70± 0.05	5.75± 0.00	5.77± 0.13	5.81± 0.05	5.84± 0.03	5.91± 0.09
S2	5.60± 0.00	5.63± 0.07	5.67± 0.01	5.68± 0.21	5.73± 0.11	5.79± 0.06
S3	5.70± 0.02	5.73± 0.09	5.76± 0.10	5.77± 0.11	5.85± 0.16	5.92± 0.05
S4	5.60± 0.06	5.65± 0.03	5.68± 0.14	5.72± 0.12	5.76± 0.04	5.79± 0.07
S5	5.40± 0.02	5.44± 0.07	5.49± 0.03	5.51± 0.05	5.54± 0.11	5.62± 0.00
S6	5.50± 0.09	5.52± 0.14	5.55± 0.11	5.59± 0.12	5.63± 0.07	5.69± 0.03
Marketed shampoos						
Head & Shoulder	7.41± 0.00	7.45± 0.04	7.48± 0.07	7.53± 0.03	7.57± 0.00	7.63± 0.15
Selsun Blue	3.90± 0.19	3.93± 0.11	3.97± 0.08	4.04± 0.05	4.11± 0.07	4.16± 0.10
Garnier fructis	4.92± 0.03	4.96± 0.00	4.99± 0.05	5.05± 0.02	5.09± 0.03	5.18± 0.08
Clear	5.43± 0.01	5.49± 0.05	5.52± 0.21	5.57± 0.11	5.63± 0.13	5.67± 0.09
Vatika	6.30± 0.22	6.36± 0.11	6.38± 0.06	6.43± 0.02	6.49± 0.03	6.56± 0.04

Table 7. Stability studies on tea tree oil shampoos and the marketed shampoos at 40°C.

Stability Studies at 40°C and 75% RH						
Shampoo Formulations	**At 1st Day**	**At 4th Day**	**At 7th Day**	**At 15th Day**	**After 1 Month**	**After 2 Months**
S1	5.70± 0.07	5.78± 0.02	5.80± 0.11	5.85± 0.21	5.86± 0.06	5.95± 0.00
S2	5.60± 0.02	5.66± 0.08	5.71± 0.04	5.73± 0.11	5.76± 0.04	5.84± 0.05
S3	5.70± 0.11	5.76± 0.05	5.79± 0.02	5.79± 0.08	5.88± 0.03	5.96± 0.13
S4	5.60± 0.06	5.68± 0.03	5.72± 0.11	5.75± 0.09	5.79± 0.00	5.83± 0.04
S5	5.40± 0.01	5.47± 0.04	5.52± 0.13	5.54± 0.07	5.57± 0.11	5.65± 0.05
S6	5.50± 0.01	5.55± 0.00	5.57± 0.01	5.63± 0.07	5.67± 0.02	5.73± 0.11
Marketed shampoos						
Head & Shoulder	7.41± 0.14	7.47± 0.21	7.51± 0.08	7.56± 0.19	7.62± 0.07	7.66± 0.02
Selsun Blue	3.90± 0.06	3.95± 0.11	4.02± 0.09	4.09± 0.04	4.18± 0.01	4.23± 0.03
Garnier fructis	4.92± 0.03	4.98± 0.05	5.04± 0.18	5.09± 0.10	5.15± 0.02	5.26± 0.01
Clear	5.43± 0.08	5.52± 0.03	5.56± 0.00	5.62± 0.09	5.67± 0.11	5.71± 0.06
Vatika	6.30± 0.07	6.39± 0.11	6.42± 0.15	6.48± 0.09	6.54± 0.03	6.62± 0.00

CONCLUSION

The current study demonstrated successful preparation of tea tree oil anti-dandruff shampoos at various concentrations of tea tree oil. The formulated shampoos had excellent properties in terms of pH, viscosity, wetting time, detergency and cleaning action, dirt dispersion, and antifungal activity. Furthermore, the formulated shampoos had comparable properties with the marketed shampoos. In conclusion, tea tree oil can be used to formulate anti-dandruff shampoos at various concentrations, and different concentrations may be advantageous to various skin and hair types. However, further studies are warranted to establish the efficacy of formulated shampoos in human volunteers with different skin and hair types.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable

AVAILABILITY OF DATA AND MATERIAL

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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