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Original Article

Formulation of a Herbal Shampoo using Total Saponins of *Acanthophyllum squarrosum*

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Abstract

Shampoos are products which remove surface grease and dirt from the hair shaft and scalp. The cleansing or detergent action of a shampoo is a primary function. However, the foaming characteristic of a shampoo has an important role in its acceptability. Often alkanolamides are used for the formation of a stable foam; but because of producing nitrosamines, they are potentially carcinogenic compounds. Hence, the main goal of this study was the elimination of these materials from shampoo formulations. *Acanthophyllum squarrosum* is one of the 23 species of the genus *Acanthophyllum* endemic in Iran. Due to the presence of saponin in its root, chubak, has been used traditionally as a detergent.

In this study, total saponins of *Acanthophyllum squarrosum* roots were extracted, using several solvents. A clear liquid shampoo base was formulated using Texapon as surfactant, sodium chloride as thickener, glycerin as viscosity modifier, methyl paraben as preservative and EDTA as sequestring agent. Then, a fixed amount of Texapon was substituted by 1, 2.5 and 5 percent of total herbal saponins. Foaming ability of the shampoos prepared was evaluated by the Ross-Miles method and the cleansing power by Thompson test.

The best result was found with the formula containing 20% Texapon and 5% total herbal saponins. Therefore, this formulation was selected as the best formula and evaluated for other characteristics. In addition to possessing the properties of a liquid shampoo, the unique characteristics of the formula containing 5% total herbal saponins was that it produced a stable foam without the use of foam stabilizers. Evaluation of the cleansing power by Thompson test showed that the final formula is a shampoo for normal hair, with very good cleansing ability. Rheological studies showed that the final formula had a pseudoplastic behavior. Organoleptic and physicochemical characteristics of the final formula were all found to be acceptable.

Keywords: *Acanthophyllum squarrosum*; Saponin; Shampoo; Formulation; physicochemical characteristics.

Introduction

Acanthophyllum C. A. Meyer is a genus belongs to the Caryophyllaceae family, with a total of 61 species in the world. Of these, 33 occur in Iran, in which 23 species are endemic (1). According to the literature (2-6), the highest number of species has been recorded in the eastern parts of Iran (Khorrasan province) and in the neighboring regions (Turkmenistan and Afghanistan).

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Acanthophyllum squarrosum Boiss. is a perennial shrub growing wild in different locations of Iran. The roots of this plant, because of triterpene saponins, are gathered traditionally in considerable quantity by local people. These roots, known as "soap roots", are used as soaps and detergents, especially for wooly cloths, as well as for provoking sneeze.

Saponins have detergent or surfactant properties, because they contain both water-soluble and fat-soluble components. They consist of a fat-soluble core, having either a steroid or triterpenoid structure, with one or more side chains of water-soluble carbohydrates (sugars). Yucca saponins (*Yucca schidigera)* have a steroidal core (steroidal saponins), while the Quillaja (*Quillaja saponaria*) and Acanthophyllum saponins have a triterpenoid core. As a consequence of their surface-active properties, saponins are excellent foaming agents, forming very stable foams. Because of their surfactant properties, they are used industrially in mining and ore separation, in the preparation of emulsions for photographic films, and extensively in cosmetic products, such as lipstick and shampoo. Quillaja bark has been used as a shampoo in Chile for hundreds of years, and native Americans used yucca to make soap. Saponin-rich Quillaja bark is one of the rare natural washing agents that helps to absorb excess sebum without causing reactive hyper-seborrhea. The antifungal and antibacterial properties of saponins are important in cosmetic applications, in addition to their emollient effects.

Shampooing is the most common form of hair treatment. Shampoos have primarily been products aimed at cleansing the hair and scalp. The diversity of qualities demanded from a good shampoo by today's consumer goes far beyond this general function. Selected ingredients of shampoo that have been popular with the consumer are currently under attack because of potential risks associated with their use (e.g. halogenated organic compound, formaldehyde, musk fragrance and crude coal tar).

Foaming characteristic of a shampoo has an important role in it's acceptability.A Shampoo should produce a stable and copious amount of foam. Often alkanolamides are used to prepare a stable foam, but because of producing nitrosamines they are potentially carcinogenic compounds (7).

In 1979, the FDA commissioner issued a call for the cosmetic industry to significantly reduce the level of nitrosamines in personal care products (8). Since that time the level of nitrosamines have been reduced, but not eliminated. Therefore, the main objective of this study was to eliminate these harmful materials from shampoo formulation and substitute them with a safe natural product, namely total saponins of *Acanthophyllum squarrosum.*

Experimental

Plant materials

Acanthphyllum squarrosum whole plant was collected in autumn 2003 from a region 40 km eastern south of Tabas, in the Khorrasan province (Iran). After scientific identification, a voucher sample was preserved for further reference at the Herbarium of the Department of Pharmacognosy, School of Pharmacy, Ahwaz Joundishapoor Medical Sciences University, Ahwaz, Iran.

All the chemicals used were of the analytical grade and purchased from reputable companies.

Isolation of saponins

After cleaning and washing with water, the plant roots were cut into small pieces, air-dried in shade and powdered. Using th soxhlet apparatus and petroleum ether as the solvent, powdered roots were defated at 45 °C for 4 h. Extraction of total saponins was conducted in several stages. First, the defated dried plant powder was extracted with methanol in a soxhlet apparatus set at 60 °C for 10 h, yielding a reddish crude extract. This methanolic extract, after concentration, was dissolved in a minimum amount of distilled water and decanted several times with n-butanol. In the final stage, the total saponins present in the butanolic extract was precipitated using diethylether and then filtered (9).

Shampoo formulation

To formulate a clear shampoo base, definite amounts of Texapon (Triethanolamine lauryl sulfate, Lovaken, Malaysia) and salt (sodium chloride) were added to an aqueous solution containing glycerin (5%) methyl paraben (0.25%) and EDTA (0.15%). If needed, pH

Table 1. Percentage of ingredients present within formulations A_2 and B_3.

Compound	Percentage (w/v)					
	A_1	A_2	A_3	A_4	A_5	A_6
Texapon	30	30	30	20	20	20
Sodium chloride	0.5	1	1.5	0.5	1	1.5

was adjusted to 6-8 by the addition of citric acid or triethanolamine solution. Table 1 shows different percentages of Texapon and sodium chloride in preliminary formulations (A series). Then, the formulations prepared were evaluated in terms of their clarity, foam producing ability and fluidity, and the best formulation was chosen.

In the next step, 10% of the total Texapon content of the best formulation was replaced by 1, 2.5 and 5 percent of *Acanthophyllum squarrosum* total saponins (ATS) to prepare series B formulations. The components and percentage of ingredients used within the final formulation (B_3) are listed in Table 2.

Also, for comparison purposes, formulations containing the same percentages of a brand saponin, Quillaja total saponins (QTS), named the "C series" were prepared.

To evaluate the formulations prepared, quality control tests including organoleptic assessment and physicochemical controls such as pH, density and viscosity were performed. Also, to assure the quality of products, specific tests for shampoo formulations including the determination of dry residue and moisture content, total surfactant activity, salt content, surface tension, thermal and mechanical stability and detergency tests were carried out. The results were compared with those of the appropriate base formulation (A formula) and formulations containing QTS. Some of the more important tests have been described below.

Rheological evaluations

After visual inspection, apparent viscosity of the samples was determined at room temperature using a rotational spindle Brookfield Viscometer

Table 2. Percentage of ingredients present within formulations A_2 and B_3.

Ingredient	Percentage (w/v)	
	A_2	B_3
Texapon	30	27
ATS	-	3
Sodium chloride	1	1
Glycerin	5	5
Methyl paraben	0.25	0.25
EDTA	0.15	0.15
Deionized water	qs to 100 ml	qs to 100 ml

(Model DV-I Plus, LV, USA) set at different spindle speeds from 0.3 to 1000 rpm.

Surface tension measurements

Surface tension of samples was determined using a Du-Nouy ring-type tensiometer (Torison Balance, Germany) at room temperature. The decrease in the values was plotted against the concentration of total saponins and compared with that of formulation A and QTS containing formulations.

Foaming ability and foam stability

To evaluate the foaming ability of formulations prepared, the Ross-Miles foam column method was used. Briefly, 0.25 and 0.5 percent aqueous solutions of each formulation were prepared and placed within a 100 mL burrette at room temperature. They were then individually poured from a height of 50 cm into a measuring cylinder and the height of produced foam was measured. To evaluate the foam stability, the same procedure was performed and the foam height after 10 and 20 minutes were also determined (10, 11).

Detergency ability

The thompson method was used to evaluate the detergency ability of the samples (11, 12). Briefly, a crumple of hair were washed with a 5% sodium lauryl sulfate (SLS) solution, then dried and divided into 3g weight groups. The samples were suspended in a hexane solution containig 10% artificial sebum (Table 3) and the mixture was shaken for 15 minutes at room

Table 3. Composition of the artificial sebum (12)

Ingredient	Olive oil	Coconut oil	Stearic acid	Oleic acid	Liquid paraffin	Cholesterol
Percentage (w/v)	20	15	15	15	15	20

Table 4. Apparent specifications of series A formulations.

Formulation	Specifications
A_1	Clear, good foaming, low viscosity
A_2	Clear, good foaming, suitable viscosity
A_3	Clear, good foaming, high viscosity
A_4	Clear, low foaming, low viscosity
A_5	Clear, low foaming, suitable viscosity
A_6	Clear, low foaming, high viscosity

temperature. Then samples were removed, the solvent was evaporated at room temperature and their sebum content determined. In the next step, each sample was divided into two equal parts, one washed with 0.1 ml of the 10% test shampoo and the other considered as the negative control. After drying, the resided sebum on samples was extracted with 20 ml hexane and re-weighed. Finally, the percentage of detergency power was calculated using the following equation:
in which, 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

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

Stability tests

To study the thermal stability of formulations, samples were placed in glass tubes, some placed in a 45°C oven and the others in a 5°C chiller. After one week, their appearance and physical stability were inspected (10, 14).

To assess their mechanical stability, each sample was centrifuged at 2400 rpm for 3 minutes and then its structural stability was inspected (10).

Statistical analysis

The presented data are the mean of three experiments and two measurements. Statistical tests employed were the analysis of variance along with Tukey post hoc test for repeated measurements or general linear model for repeated measurement, in order to evaluate the effect of concentration and the type of formulation on foaming ability, surface tension, viscosity and detergency activity of shampoos prepared. $P<0.05$ was considered as a significant difference.

Results and Discussion

The yield of the total saponins was based on the dried plant and found to be equal to 2.25% w/w. The results of visual inspection of series A formulations are listed in Table 4. As can be seen, among them, formulation A_2 had the best overall characteristics and was chosen as a suitable base formulation to incorporate total saponins. Subsequently, B and series C formulations were designed and prepared by the incorporation of 1, 2.5 and 5% ATS and QTS within the base shampoo formulation.

Foam producing ability

The results from Tukey statistical test showed that as the concentration of ATS or QTS increased in shampoo formulation, there was a significant decrease ($p<0.001$) in foam production. The maximum value was observed with the formulation containing 5% ATS (Table 5).

In comparison to the base formulation, there was a significant increase in foam height for 1, 2.5 and 5 percent ATS containing formulations ($p<0.001$). In addition, the results showed that foam production of ATS containing formulations was significantly more than the QTS containing shampoos. Regarding the achievement of an effective and stable foam producing property, the final formulation containing 5 and 20 percent ATS and Texapon, respectively, produced the highest and most stable foam, when compared with the other formulations (Table 5).

Rheological studies

The results obtained from the rheological studies were fitted into different flow behaviors, using the linear or non-linear regression. Table 6 shows the goodness of fitting indices for Newtonian, plastic and pseudoplastic flow

Table 5. The mean difference in foaming ability of series B and C formulations, compared with formulation A.

Formulation	B_1	B_2	B_3	C_1	C_2	C_3
Mean difference	0.9139	0.4386	-0.3000	1.1222	0.5611	-0.2111

Table 6. Goodness of fitting indices for Newtonian, plastic and pseudoplastic flow (**η′** and **n)** behaviors for formulations A_2 and B_3.

Formulation	η′ (cps.)				n			
A_2	196.42	199.23	216.84	181.79	1.69	1.69	1.70	1.68
B3	843.75	843.75	700.65	705.92	1.95	1.70	1.95	1.92

behaviors. As can be seen in the table, all the formulations followed a pseudoplastic rheogram. Basedon to the results, there was no significant difference in viscosity of different formulations.

Rheological studies conducted on the final formulation showed the best fitness to pseudoplastic behavior and a desirable viscosity which facilitates its daily application. Also, its rheological behavior was completely equal to formulations containing 2% sodium chloride and alkyl ether sulfate.

In addition to adequate foam producing ability, the final formulation produced a stable foam and there was no need to add foam stabilizers. Graph 1 shows the rheogram of the final formulation (B_3).

Surface tension studies

Although surface tension has no determining effect on the acceptance of shampoo formulations, it has been mentioned that a proper shampoo should be able to decrease the surface tension of pure water to about 40 dynes/cm (15, 16). To compare the effect of substitution of 10% Texapon by ATS on the surface activity of formulations prepared, different dilutions of formulations were prepared and their surface tensions were measured. Although the surface tension of all the samples decreased to 40 dynes/cm and there was no significant difference between them, statistical analysis (Tukey test) showed a significant decrease ($p<0.0001$) in the critical micelle concentration (CMC) for ATS containing formulations.

Considering the surface tension results, formulations containing ATS were able to decrease the surface tension of water to 40 dynes/cm. Moreover, the results showed that the combination of ATS and Texapon increased the surface activity of the final product.

Table 7. Comparison of the *mean* and *variation range* within the percentage of detergency power of formulations A_2 and B_3.

Formulation	A_2	B_3
Mean(%)	60.91	67.33
Variation range	0.44	0.82

Detergency ability

Table 7 shows the results of Thompson analysis on detergency data. Based on the results, formulation A_2 and the final formulation (B_3) could be classified as '*daily shampoo*' and '*shampoo for normal hair*', respectively (12). Statistical analysis, via Kruskal-Wallis followed by Steel-Dwass tests, showed a significant increase ($p<0.05$) in the detergency power of the final formulation B_3 (containing 5% ATS), compared to the base shampoo (A_2 formula).

The results of detergency studies (Thompson method) showed that the final formulation has a significantly greater detergency ability, when compared with the other formulations ($p<0.05$).

Thermal and mechanical stability

The obtained results from thermal and mechanical stability studies on the final formulation indicated that there was no change in foam production, detergency and viscosity during the test period. Stability and acceptability of organoleptic properties (odor and color) of ATS containing formulations during the storage period indicated that they are chemically and physically stable.

Conclusion

The final formulation (B_3) was a clear liquid shampoo with desirable organoleptic properties, and so there was no need to add any synthetic colorant or chemical essence to alter its color and odor, respectively. Due to acidic nature of saponins, the pH of final formulation was adjused with triethanolamine to prevent any irritation due to the consumption of the shampoo (16).

Furthermore, due to good homogeneity and appearance, there was no need to add any synthetic colorant or stabilizer to the final formulation.

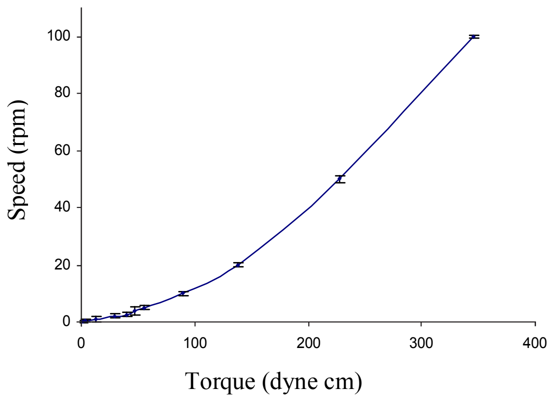

Figure 1. Rheogram of formulation B_3 (n=3, mean±SD).

Recent studies on synthetic surfactants have shown that they can not completely remove the existing sebum on normal hair (14). Hence, it could be suggested that ATS is a good candidate for substitution of all or at least a fraction of synthetic surfactants in shampoo formulations.

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