

FORMULATION AND EVALUATION OF HERBAL SHAMPOO FROM *ZIZIPHUS SPINA* LEAVES EXTRACT

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Received on: 05/09/11 Revised on: 19/10/11 Accepted on: 22/11/11

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ABSTRACT

Synthetic preservatives and detergents have sometimes been the cause of adverse effects among consumers. A more radical approach in reducing the synthetic ingredients is by incorporating natural extract whose functionality is comparable with their synthetic ingredients. Selection of *Ziziphus spina cristii* (*Z. spina cristii*) leaves extract was based on its particular properties such as antimicrobial and detergent activities. This study aimed to formulate a self preserving shampoo having low concentration of the detergent using *Ziziphus spina cristii* leaves with emphasis on safety and efficacy; will avoid the risk posed by chemical ingredients. Formulation of three investigated shampoos was prepared containing different amounts of *Ziziphus spina cristii* extract and sodium laureth sulfate as a surfactant. Evaluation of organoleptic, physicochemical and performance tests were performed and compared with herbal marketed product (Cedr shampoo[®], Sehat Company, Iran). The results indicated that F2 and F3 produced clear shampoos and their averaged pH values were in the range of 5.59-6.25 which were suitable to retain the acidic mantle of scalp. They provided stable foam, surface tension reduction, good cleaning and wetting effect and have pseudo plastic rheological behavior. Moreover, the aesthetic attributes, such as lather and clarity were comparable with the marketed shampoos. The foam volume was on a par and formulas showed higher detergency and foaming effects than the commercial herbal one ($p < 0.05$). Moreover they can be considered safe to children since less chemical contents were used and no side effects observed after application.

Keywords: Herbal Shampoo, Formulation, Evaluation, *Ziziphus Spina* Extract, Detergents, Sodium laureth Sulfate

INTRODUCTION

The shampoo sector is probably the largest unit sale amongst the hair care products since shampoos are one of cosmetic products used in daily life. Shampoos are primarily been products aimed at cleansing the hair and scalp. *Ziziphus spina-christii* is a tree indigenous to the north districts of United Arab Emirates. The leaves of this plant, which are locally known as "Sedr" and "Konar", have been traditionally used for washing the hair and body. Application of the powdered leaves is said to darken and lengthen women's hair¹. *Ziziphus Spina-christii* leaves are also used in the folk medicine as an antiseptic, antifungal and anti-inflammatory agent, and for healing skin diseases such as atopic dermatitis². The plant is already used in many parts of the world for the care of the skin³. The chemical composition and phytochemicals present in the plant would suggest and substantiate the ethnobotanical and cultural reliance on this plant. From the constituents, it contains four saponin glycosides⁴. Saponin is one of the natural washing agents that help to absorb excess sebum without causing adverse reactions. In addition to the emollient effect of saponins their antibacterial and antifungal properties are important in cosmetic applications. Selected ingredients of shampoo have been popular with the consumer are currently under attack because of potential risks.

Ziziphus spina-christii has shown to have antimicrobial activity against bacteria, fungi and other pathogens that are normally quite resistant⁵. Therefore the ideology of preparation of a self preserved with low concentration of a detergent will be advantageous and reduce the risk of chemicals.

Sodium lauryl sulfate based detergents are the most common but the concentration will vary considerably from brand to brand and even within a manufacturer's product range. Cheap shampoos may contain a high detergent concentration while expensive shampoos may contain very little of a cheap detergent⁵.

Our endeavor was to formulate a herbal shampoo from *Ziziphus spina* leaves. The prepared formulas were *in vitro* evaluated for detergency, foam volume and stability, surface tension, wetting properties, pH, viscosity, conditioning effects and stability in comparison to commercial brand (Cedr shampoo[®], Sehat Company, Iran). The test methods had been selected based upon their

simplicity, rapidity and reproducibility. Furthermore, *in vivo* eye irritation and skin sensitization tests were performed using the candidate formula.

MATERIALS AND METHODS**Plant collection**

Leaves of *Ziziphus spina* were obtained from Haj Seed gardens (Al-Muhaisanah 1, Dubai, UAE) in September 2011. Authentication of the samples was done by the Head of Department of Pharmacognosy at Dubai Pharmacy College.

Preparation of plant extract

The collected leaves were cleaned from unwanted foreign materials and accurately weighed. Then samples were homogenized and extracted using ethanol (70 % v/v). The extracts were filtered and concentrated to dryness under reduced pressure and controlled temperature (50-55°C) to obtain solvent-free semisolid extracts. The solvent-free semisolid extract obtained was washed, weighed and packed into plastic containers and stored at room temperature in the laboratory until used for the antimicrobial studies. The percentage of yield was 16.34%.

Preparation of herbal shampoos

To formulate a clear shampoo base, four samples, designated as F-0, F-1, F-2 and F-3 were prepared as shown in Table 1. F1, F2 and F3 were prepared by incorporation of 5, 10 and 20 % w/w of *Z. spina* extract in 10%, 5% and 0% w/w of sodium laureth sulfate respectively. The volume was completed with distilled water to 100mL. F-0 was control sample (without plant extract).

Evaluation of herbal shampoos

To evaluate the prepared formulations, quality control tests including organoleptic and physicochemical characterization such as pH, solid contents and viscosity were performed. As well to ensure the quality of the products, specific tests for shampoo formulations including: surface tension, foam volume and foam stability, detergency, eye irritation, skin sensitization tests and preliminary stability study were also carried out. The results were compared with frequently used marketed herbal shampoo (Cedr shampoo[®], Sehat Company, Iran) that was considered as reference

Physical appearance/visual inspection

All samples were observed for their physical appearance/visual inspection. The prepared formulations were evaluated in terms of their clarity, foam producing ability and fluidity⁶.

Determination of pH

The pH of shampoo solution (10% w/v) in distilled water was determined at room temperature⁷. The pH was measured by pH meter (Mettler Toledo, USA).

Percentage of solid contents

Four grams of the prepared shampoo were placed in a clean dry evaporating dish. The weight of the dish and shampoo was determined. The liquid portion of the shampoo was evaporated by placing on a hot plate. Then the weight of the shampoo solid contents after complete drying was determined.

Surface tension measurement

The surface tension measurement of the diluted shampoos (10% w/v in distilled water) was carried out at 20 °C using du Nuoy tensiometer. (Lauda, Germany)

Wetting time

The canvas was cut into 1-inch diameter discs having an average weight of 0.44 g. The disc was floated on the surface of shampoo solution 1% w/v and the stopwatch started. The time required for the disc to begin to sink was measured accurately and noted as the wetting time.

Rheological property

The viscosity of the prepared formulations was measured at room temperature using a programmable rheometer (Brookfield DV-III Ultra, Brookfield Engineering Laboratories Inc., USA) fitted with a spindle type S 17 while set at different spindle speeds. The best approach is to take multipoint measurements approximate to those of the process being modeled⁸. All measurements were performed in triplicate at room temperature and the viscosity profile of the shampoos was measured.

Dirt dispersion

Two drops of shampoo were added in a large test tube contain 10 ml of distilled water. One drop of India ink was added; the test tube was Stoppard and shaken for ten times. The amount of ink in the foam was estimated as None, Light, Moderate, or Heavy.

Foam, volume and stability

The foam volume test was determined by mixing the prepared formulas with distilled water, hard water (Ca⁺⁺ 150 ppm) and a standard soil. The foam was produced by mixing in a kitchen blender. An artificial sebum was used for detergency evaluation (0.25 mL of sebum 10% in hexane for 4g of the shampoo). Forty milliliters of shampoo solution 10% w/v in distilled water was blended for 5 sec in a kitchen blender. The height of the foam generated was measured immediately and after 3 min, the test was repeated in hard water, standard soil and in the presence of artificial sebum.

On the other hand, Ross-Miles foam column method was used to evaluate the foaming stability of the prepared formulations. Briefly, 0.25% and 0.5% of aqueous solutions of each formulation were prepared and placed in burette of 100 mL capacity at the 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. The foam height after 10 and 20 minutes were also determined^{9,10}.

Detergency evaluation

Thompson method¹¹ was used to evaluate the detergency of the prepared shampoos with minor modification since our results were evaluated gravimetrically. Hair tresses of Asian (Indian) origin were obtained from the market. The tresses were prewashed with 5% SLS solution, dried and cut into 10 inch, 3 g swatches. The sebum composition was chosen to include a variety of functional groups similar to that in actual sebum.

The actual formula for the artificial sebum used in our study was olive oil 2%, coconut oil 15%, stearic acid 15%, oleic acid 15%, paraffin wax 15% and cholesterol 20%. The hair swatch (3 g) was suspended in 20 ml of a 10% sebum solution in hexane for 15 min with intermittent shaking. The swatch was removed, the solvent evaporated at room temperature and the hair swatch weighed to determine the sebum load. Fifteen swatches were treated similarly and the soil levels were found to range from 9.96 to 11.05%, each swatch was then split into two equal samples of 1.5 g each: one for the shampoo treatment and the other to act as internal control to overcome the tress-to-tress variation in soil levels. The control swatch was left untreated. The test swatch was washed with 0.1 ml of a shampoo solution 10% using the finger method described by Thompson et al. It was then dried using a hair dryer and further dried in an oven at 60 degrees for 4 hours to ensure uniform moisture content.

The sebum remaining in the test swatch after shampooing and that in the unwashed control swatch was then extracted, using 20 ml hexane in a stoppered flask for 30 minutes on a rotary shaker. The hexane solution was then evaporated to dryness and the sebum extracted from the test and control swatches were weighed. Detergency was evaluated as a percentage of sebum removed after shampooing according to the following equation:

$$\text{Detergency} = 100 - (T \times 100/C)$$

Where T is the weight of sebum in test swatch and C is the weight of sebum in control swatch.

Eye irritation test

Animals (albino rats) were collected from animal house of Dubai Pharmacy College. Ethical approval to conduct this experiment was obtained from the Research Committee of the College. Shampoo solution 1% was dripped into the eyes of six albino rabbits with their eyes held open with clips at the lids. The progressive damage to the rabbits' eyes was recorded at specific intervals over an average period of five minutes. Reactions to the irritants can include swelling of the eyelid, inflammation of the iris, ulceration, hemorrhaging (bleeding) and blindness¹².

Skin sensitization test

Animals (guinea pigs) were divided into six groups (n=3). On the previous day of the experiment, the hairs on the backside area of guinea pigs were removed. The animals of Group I was served as negative control (without any treatment). Animals Group II, III and IV were applied with shampoo formulation F1, F2, F3, respectively. While animals group V was considered as a reference (applied with the commercial product). Shampoos were applied onto nude skin of animals groups. A 0.8% v/v of formalin aqueous solution was applied as a standard irritant on animals Group VI (positive control) up to 72 hours. The application sites were graded according to a visual scoring scale, always by the same investigator. The erythema scale was as follows: 0: none, 1: slight, 2: well defined, 3: moderate and 4: scar formation severe¹³.

Preliminary stability study

Stability and acceptability of organoleptic properties (odor and color) of formulations during storage indicate chemical and physical stability of the prepared shampoos formulations. This study was conducted on a candidate formula (F2). Samples were placed in dark glass tubes and stored in a chamber at 45°C and 75% relative humidity. Their appearance and physical stability was inspected for a period of 3 months at interval of one month^{13, 14}. To assess their mechanical stability, each sample was centrifuged at 2400 rpm for 3 minutes and then its structural stability was inspected¹⁵.

Statistical analysis

The results were given as mean± S.D. (n=5). One-way analysis of variance (ANOVA) comparison test was used to compare characteristics of different formulations with the commercial product. A p value of 0.05 was considered to be significant.

RESULTS AND DISCUSSION

Evaluation of herbal shampoos

Physical appearance

The results of visual inspection of shampoo formulations are showed in Table 2. It can be noticed that, all formulations had good characteristics with respect to appearance and foaming.

pH

The pH of shampoo formulations has been shown to be important for improving and enhancing the qualities of hair, minimizing irritation to the eyes and stabilizing the ecological balance of the scalp⁸.

The pH of shampoo solution 10% in distilled water was determined at 25 C and the results are presented in Table 3.

The current trend to promote shampoos of lower pH is one of the ways to minimize damage to the hair. Mild acidity prevents swelling and promotes tightening of the scales, thereby inducing sheen. As seen from Table 3, only shampoo F3 shows a higher pH nearly the same as the commercial one, whereas all the other shampoos are acid balanced and were ranged 5.5 to 5.8.

Solids contents

If the shampoo has too many solids it will be hard to work into the hair or too hard to wash out. The solids contents of the prepared formulas were in a range of 22-28%. Thus they can be considered easy to wash out shampoos (Table 3). No significant difference ($p < 0.05$) among the prepared formulas and the commercial brand (reference).

Surface tension

All shampoo formulations showed similar reduction in surface tension ($p < 0.05$), as shown in Table 3. Surface tension reduction is one of the mechanisms implicated in detergency. The reduction in surface tension of water from 72.8 dynes cm^{-1} to 32-37 dynes cm^{-1} by the shampoos is an indication of their good detergent action.

Wetting action

The rate of wetting or the wetting ability of surface-active agents is commonly used to determine their comparative efficacies. Although the Draves test¹⁶ has been adopted as the official test, the canvas disc wetting test¹⁷ offers a number of advantages over it. The canvas disc method is more accurate and time saving than the official test. Also, the unlike materials and apparatus required for the canvas test are easily available in any laboratory, unlike the Draves test, which requires cotton skeins, anchors and weights. Hence, we have used the canvas disc method for our work.

Different types of canvas were tried for the test and one that gave an effective balance between time saving and testing efficiency was chosen for the test.

The wetting ability of a substance is a function of its concentration. By comparing the results in Table 3 with those of detergency% in the next column it seems fair to conclude that shampoo F3 contains the maximum concentration of natural detergents, and hence shows maximum detergency and minimum wetting time. By contrast, shampoo F0 shows minimum detergency and maximum wetting time; and shampoos F1, F2 and reference showed intermediate detergency and wetting times.

Detergency evaluation

Although cleaning or soil/sebum removal is the primary aim of a shampoo, experimental detergency evaluation has been difficult to standardize, as there is no real agreement on a standard soil, a reproducible soiling process or the amount of soil a shampoo should ideally remove.

Earlier work on detergency evaluation was done using greasy wool yarn by Barnette and Powers¹⁸. Later, the approach turned to evaluating detergency using as little as 10 mg of hair clippings obtained from salons¹⁹. Thompson *et al.*¹¹ used hair tresses soiled with artificial sebum for evaluating detergency.

However our formulas were evaluated gravimetrically and results indicated a significant difference ($p < 0.05$) in the amount of sebum removed by different shampoos. Shampoo F0, being a frequent-use cleanser, was expected to have the minimum detergency. Shampoos F2 and F3 also show moderate detergency and may be regarded as mild cleansers. However, shampoos F1 and reference are 'active' cleansers and are more suitable for oily hair than normal hair, (Table 3).

Cleaning action

Cleaning action was tested on wool yarn in grease. Although cleaning or soil/sebum removal is the primary aim of a shampoo, experimental detergency evaluation has been difficult to standardize, as there is no real agreement on a standard soil, a reproducible soiling process or the amount of soil a shampoo should ideally remove. As seen from the results, there is a significant difference in the amount of sebum removed by the different shampoos. The results of detergency studies showed that the final formulation has significantly similar detergency ability, when compared with the marketed formulations and it was found in between 18-33%. Table 3.

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. All three shampoos (F1, F2 and F3) showed similar results as the marketed one, except the control (F0). These results indicate that no dirt would stay in the foam; so prepared and marketed formulations are satisfactory^{20, 21}.

Viscosity

Product viscosity plays an important role in defining and controlling many attributes such as shelf life stability and product aesthetics such as clarity ease of flow on removal from packing and spreading on application to hair and product consistency in the package. The flow characteristics of non-Newtonian materials are usually not measured with a single data point, because their viscosity is dependent on the shear rate. The best approach is to take multipoint measurements approximate to those of the process being modeled²². Fig.1. indicates that shampoo F0 and F1 are low viscosity products, whose viscosity remains almost unchanged over the range of r.p.m used. Shampoo F2 and F3 show pseudo plastic behavior the same as the commercial one (reference), which is a desirable attribute in a shampoo formulation. At a low r.p.m., these shampoos show high viscosity. On increasing the shear, the viscosity drops, which would allow ease of spreading on the hair.

Foam volume and stability

All the investigated shampoos showed similar foaming characteristics in distilled water as well as hard water. However, in the presence of soil, only shampoos F2 and F3 foamed well as the commercial one (Table 4). From a practical point of view, shampoos F0 and F1 would not foam well during the first hair wash, due to the presence of soil. However, during the second wash, i.e. once the sebum has been removed; all five shampoos would show comparable foaming properties. A point to be noted here is that there does not seem to be any direct correlation between detergency and foaming, which only confirms the fact that a shampoo that foams well need not clean well.

Eye irritation test

There was slight irritation on application of preparation. The animal recovered well within 3 days as the case with the reference. No eyes irritation, dryness or scaling caused by the tested formulations.

Skin sensitization test

The all formulation showed no skin sensitization including marketed shampoo except F1. The adverse reactions may occur to one of the primary constituents of the cosmetic formulation or contamination or procedural misconduct. Other additives are the second most

common cause of skin reactions besides fragrances. In most cases, these were only mild or transient such as stinging sensation. In few cases, reactions may be more severe with redness, edema.

Preliminary stability study

Stability and acceptability of organoleptic properties (odor and color) of the selected F2 during the storage period indicated complete chemical and physical stability of the tested formula. The stability of the herbal shampoo formulation is shown in Table 5.

CONCLUSION

The present study aimed to prepare a stable, self preserved a shampoo formulation contains low detergents to reduce the risk of chemicals. Results showed that no significant difference between both F2 and F3 in comparison with the reference regarding their physicochemical properties and their *in vivo* evaluation. However these formulas contain no preservative and less detergent contents as the reference that make them in favorite on the commercial product.

ACKNOWLEDGMENT

Authors are grateful for Dr. Naglaa Jamil, Head Pharmacognosy Department at Dubai Pharmacy College for her help in plant extraction. Also authors express gratitude to DPC undergraduate students, Fatma.A.Masoud, Mona.M. Alshihry and Sabah A. Memon, for their support in lab work.

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Table 1 Composition of the Prepared Ziziphus Shampoo Formulations

Ingredients (%w/w)	F0	F1	F2	F3
Ziziphus extract	-	5	10.00	20
Sodium laureth sulfate	15.00	10.00	5.00	0.00
Glycerin	1	1	1	1
EDTA	0.15	0.15	0.15	0.15
Distilled water (mL) q.s.	100.00	100.00	100.00	100.00

Table 2 Physical Appearance of the Prepared Ziziphus Shampoo Formulations

Formulations	Physical appearance	Foam producing ability
F0	Transparent	Mild foaming
F1	Dark green	Good foaming
F2	Dark green	Good foaming
F3	Light green	Good foaming
Reference	Light green	Good foaming

Table 3 In Vitro Evaluation of the Prepared Ziziphus Shampoo Formulations*

Formulas	pH	Solid contents (%)	Surface tension (dynes cm ⁻¹)	Wetting time (Sec)	Detergency (%)	Cleaning (%)
F0	5.51± 0.07	19.11± 0.02	34.9± 0.32	227± 0.22	61.10± 0.32	18.20± 0.02
F1	5.53± 0.03	22.51± 0.02	37.7± 0.02	171± 0.19	76.71± 0.12	24.50± 0.08
F2	5.81± 0.04	25.11± 0.02	32.8± 0.12	176± 0.74	67.67± 0.49	32.20± 0.05
F3	7.63± 0.02	28.11± 0.02	34.6± 0.62	159± 0.79	63.57± 0.26	33.61± 0.09
Reference	6.91± 0.01	29.11± 0.02	33.1± 0.42	185± 0.81	80.12± 0.90	34.20± 0.02

* Results are given as mean± S.D. (n=5).

Table 4 Foam Volume of Shampoo Formulations in Comparison to a Reference

Formulas	Foam volume (ml)					
	DW		Hard Water		DW + soil	
	0 min	3 min	0 min	3 min	0 min	3 min
F0	168	109	158	105	63	59
F1	153	116	153	101	57	53
F2	158	131	144	123	136	90
F3	175	138	162	114	92	90
Reference	166	135	153	114	142	98

Table 5: Stability Study of Ziziphus Shampoo (F2)

Parameters	1month	2month	3month
Physical appearance/visual inspection	Clear, dark green	Clear dark green	Clear dark green
pH	5.51± 0.02	5.53± 1.02	5.61± 0.82
Solids contents (%)	22.51± 0.02	24.11± 0.92	26.51± 1.02
Surface tension (dyne. cm ⁻¹)	33.22± 0.12	32.52± 0.32	35.20± 0.72
Viscosity (cps) at 20 rpm	67.00± 1.89	65.28± 0.99	66.19± 5.89
Detergency ability (%)	65.12± 0.12	67.10± 0.10	54.11± 0.52
Foaming volume (ml)	160	160	158

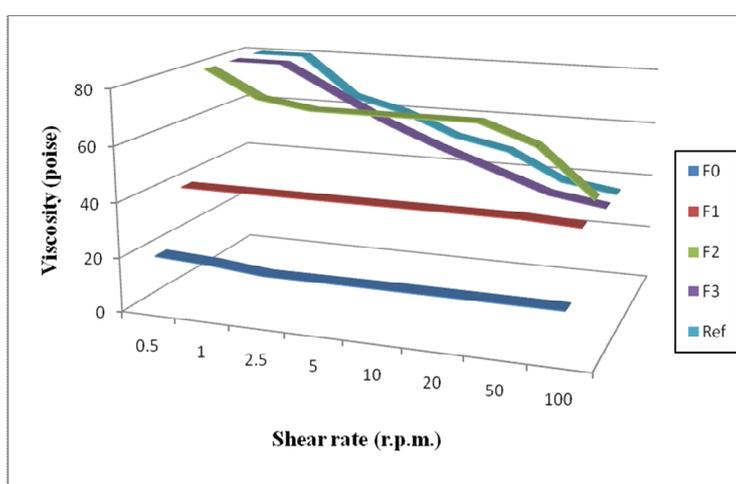


Fig. 1 Viscosity profile of the prepared shampoo formulations in comparison to the reference.

Source of support: Nil, Conflict of interest: None Declared