

A Completely Polyherbal Conditioning and Antioxidant Shampoo: A Phytochemical Study and Pharmaceutical Evaluation

Riham O. Bakr¹, Reham I. Amer^{2,3}, Marwa A. A. Fayed⁴, Tamer I. M. Ragab⁵

¹Departments of Pharmacognosy and ²Pharmaceutics, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Giza, ³Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, Al-Azhar University, Cairo, ⁴Department of Pharmacognosy, Faculty of Pharmacy, University of Sadat City, Sadat City, ⁵Department of Chemistry of Natural and Microbial Products, Pharmaceutical Industries Research Division, National Research Centre, Giza, Egypt

ABSTRACT

Aim: A recent approach in shampoo research has been to find a natural alternative for synthetic detergents that have deteriorative effects on hair follicles. This study aimed at the formulation of a completely herbal shampoo containing a natural foaming agent, in addition to having conditioning, antioxidant, and antimicrobial effects. **Material and Methods:** The leaves of *Salix babylonica* L., *Ziziphus spina-christi* L. (Willd), and *Glycyrrhiza glabra* rhizomes were extracted with 70% methanol then quantified for their phenolic and flavonoid contents using colorimetric assays that were qualitatively identified by Ultra-performance liquid chromatography (UPLC) with tandem mass spectrometry (MS-MS). The mineral content was also estimated. The radical scavenging activity was estimated using 1,1-diphenyl-2-Picrylhydrazyl (DPPH) and the half maximal inhibitory concentration (IC50) was determined. Additionally, the antimicrobial activity was tested using agar diffusion assay and compared to vancomycin and ketoconazole. Four formulations, consisting of the different plant extracts and a combination of the extracts, were prepared and evaluated for several physicochemical properties. The best formula was evaluated for its conditioning effects using scanning electron microscope and blind touch tests by asking volunteers for grading the formulations. **Results and Discussion:** UPLC-MS-MS analysis of *S. babylonica* and *Z. spina-christi* allowed tentative identification of 12 phytoconstituents in each. *Z. spina-christi* showed the highest phenolic content and a high copper, zinc, and manganese content beside the best antioxidant activity, whereas *G. glabra* had a high potency against *Bacillus cereus* and *Candida albicans*. The polyherbal shampoo formulation (F4) was selected as an optimized formulation because of a high foam stability after 4 min, low wetting time (2 s), surface tension reduction, and comparable results for percent solid content. F4 showed good conditioning effect and consumer contentment. **Conclusion:** The formulated polyherbal shampoo is chemical free, extra-nourishing shampoo with excellent conditioning, cleansing, and antimicrobial effects.

KEYWORDS: Antimicrobial, antioxidant, conditioning, minerals, phenolics, polyherbal shampoo

INTRODUCTION

Everyone does their best to look and smell good.^[1] Therefore, the beauty industry is one of the most stable and consistently growing industries and is resistant to economic downturns. In 2010, the global beauty market generated a total revenue of US\$382.3 billion.^[2] Hair care represents the largest segment in the global beauty market. Shampoo is probably the highest

selling product among hair care products. Although it is easy to formulate a shampoo that can remove all of the sebum from the hair and scalp, the lack of

Address for correspondence: Dr. Riham O. Bakr, Department of Pharmacognosy, Faculty of Pharmacy, October University for Modern Sciences and Arts, Giza, 11787, Egypt.
E-mail: romar@msa.eun.eg

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sebum will leave the hair frizzy, dry, and unattractive. The challenge lies in removing just enough sebum to allow the hair to appear clean and leave behind enough conditioning agents to keep the clean hair attractive. The best shampoos for hair loss combine cleansers with a conditioning agent in addition to a foaming agent to form an appealing froth, as consumers link the detergent effect with the formed foam, although the two are unrelated.^[3,4] Sodium lauryl sulfate (SLS) is one of the hazardous detergents used widely in cosmetics and in shampoos as a foaming agent. SLS is a known skin and eye irritant, even causing cataracts in adults, and has been proven to inhibit the proper formation of eyes in small children.^[5]

Modern medicine is based on the indigenous flora that is traditionally used to treat different ailments. The potential use of traditional herbal medicines for the development of novel cosmeceuticals has been greatly increased because of growing consumer awareness regarding healthy products with natural ingredients.^[1] A large number of plants have been explored by the cosmetic industry to provide specifically targeted products that are appealing to consumers and have good pharmacological activity. In general, bioactive extracts or phytochemicals from various plants can be used as ingredients in cosmetics to care for the body, as components that affect the biological function of the skin, and to provide nutrients for healthy skin.^[6] However, the preparation of botanical-based cosmeceuticals is complex because it is rarely supported by evidence-based science.^[7,8] A recent approach for the development of hair shampoos has been to search for an effective substitute for SLS from a natural source with negligible side effects compared with synthetic products.

Ziziphus spina-christi L. Willd (Family: Rhamnaceae) has traditionally been used to treat diarrhea and malaria, to provide relief from spasms, as painkillers, and for wound healing. The beverage made from the fruit of this plant is considered to be sedative for the treatment of measles, chest pain, and respiratory problems and to cleanse the stomach, for detoxification and as a tonic. For topical use, the leaves are boiled in water and used as a shampoo or mixed with lemon and applied to the face and hair to soften or soothe; in addition, the leaves can be mixed with vinegar for the treatment of snake bites.^[9,10] *Z. spina-christi* has documented antimicrobial, antifungal,^[11-13] anti-inflammatory,^[14] antioxidant,^[15,16] and antidiabetic properties.^[17,18] The chemical composition, that is, the presence of flavonoids,^[12] alkaloids,^[19] and saponins,^[20] is consistent with these activities.

Salix babylonica L. (Salicaceae), often called weeping willow or Babylon weeping willow, is widely used in herbal medicine for its analgesic and antipyretic effects and is the plant of origin for synthetic aspirin.^[21] This biological activity has been demonstrated and correlated with phenolic constituents, such as the benzyl ester of gentisic acid 2'-*O*-acetyl β -D-glucoside, trichocarpin, salicin, kaempferol 7-*O*-glucoside, apigenin 7-*O*-galactoside, luteolin 4'-*O*-glucoside, and an ester of terephthalic acid.^[22-24] In terms of anti-inflammatory activity, this plant was reported to be effective at treating fungal inflammation; therefore, willow extract is used in anti-dandruff shampoos.^[25]

Glycyrrhiza glabra L. (licorice, Family: Fabaceae) is well known for its use as a prophylactic to treat gastric and duodenal ulcers in dyspepsia, as an anti-inflammatory agent, laxative, emmenagogue, contraceptive, galactagogue, antiasthmatic agent, and antiviral agent, among other uses.^[26,27] Biological activity has been shown as antioxidant, cytotoxic, anticancer, antibacterial, hepatoprotective, and anti-inflammatory. Licorice is very rich in different classes of phytoconstituents, such as phenolics (liquiritin, isoliquiritin, liquiritigenin, isoliquiritigenin, glabridin, and glabrol), terpenoids and saponins (β -amyryn, glycyrrhizin, glycyrrhetol, galabrolide, licoric acid, and liquiritic acid), volatile oils (benzaldehyde, fenchone, linalool, anethole, estragole, eugenol, and hexanoic acid), vitamins (B1, B2, B3, B6, C, E, biotin, folic acid, and pantothenic acid), coumarins (glycyrin, umbelliferone, ligcoumarin, and herniarin), and mineral content (aluminum, calcium, iron, magnesium, cobalt, zinc, phosphorus, sodium, silicone, potassium, and stannous).^[28] Licorice was added to a herbal shampoo for its antimicrobial effect in addition to its demulcent effect and washability.^[29]

All the plants of choice are present in separate commercially available herbal shampoos with different purposes. This study was aimed at formulating a polyherbal shampoo containing *S. babylonica*, *Z. spina-christi*, and *G. glabra* as foaming agents and detergents to replace SLS, investigating the phytoconstituents and mineral content, and evaluating the physicochemical characteristics of the shampoo and its conditioning effect on the hair compared with that of a commercial synthetic brand. The tests were selected for their simplicity, rapidity, and reproducibility.

MATERIALS AND METHODS

Plant material

Leaves of *S. babylonica* L. and *Z. spina-christi* L. (Willd) were collected from the Orman garden and were identified by Dr. Theres Youssef Labib (Orman Botanical Garden,

Giza, Egypt). *G. glabra* rhizomes were collected from the local market and were identified by the Department of Medicine and Aromatic Plant Research, Horticulture Research Institute (Giza, Egypt).

Preparation of crude extracts

Air-dried and powdered (500 g) *Z. spina-christi*, *S. babylonica*, and *G. glabra* were subjected to extensive extraction with (70% v/v) methanol until exhaustion of the solvent. The methanolic extract was concentrated using a rotary evaporator under reduced pressure to yield semisolid residue (50, 60, and 75 g, respectively).

Phytochemical screening

Phytochemical screening was performed using standard procedures.^[30]

Quantitative estimation of total phenolic and flavonoid content

The total phenolic content of all the plant extracts was determined by the Folin-Ciocalteu method.^[31] Gallic acid was used as a standard, and the total phenolic content was expressed in terms of gallic acid equivalents (GAE). The total flavonoid content was determined based on the formation of an aluminum chloride complex. Quercetin was used as a standard, and the flavonoid content was determined in terms of quercetin equivalents (QE). The absorbance of the reaction mixture was measured at 510 nm.^[32] All procedures were performed in triplicate.

Estimation of mineral content

The mineral content was determined where the sample was digested by wet digestion with concentrated sulfuric acid in the presence of a digestion catalyst (a mixture of copper sulfate and anhydrous sodium sulfate, 1:10). The resulting solution was then measured using an atomic absorption spectrophotometer.^[33]

Ultra-performance liquid chromatography with tandem mass spectrometry analysis

The UPLC-MS-MS spectra were obtained by using an Xevo TQD triple quadrupole system (Waters Corporation, Milford, Massachusetts) equipped with an ESI source (electrospray voltage, 3.0 kV; sheath gas, nitrogen; and capillary temperature, 440°C) in negative ionization mode. The ion trap mass spectrometry (MS) system was coupled with a Waters aquity UPLC instrument (USA) equipped with a reversed-phase C-18 column (ACQUITY UPLC BEH C18 column (Waters Corp., Milford, MA, USA), 1.7 µm particle size, 2.1 × 50 mm column). Mobile phase elution was conducted with a flow rate of 0.2 mL/min by using a gradient mobile phase comprising two eluents: eluent A was H₂O acidified with 0.1% formic acid, and eluent B was MeOH acidified with 0.1% formic acid. The MSn

spectra were detected in negative mode between *m/z* 100 and 1000 with a starting collision-induced dissociation energy of 30 eV. The peaks and spectra were processed using MassLynx 4.1 software (Waters, USA) and tentatively identified by comparing retention times (*R_t*) and mass spectrum with previously reported data.

Free radical scavenging activity

The free radical scavenging activity of the extracts was determined by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method.^[34] All plant extracts were screened at 100 µg/mL, whereas the most active extract (with greater than 90% activity) was evaluated for its IC₅₀ (half maximal inhibitory concentration). The absorbance was measured at 517 nm in a microplate reader.

$$\text{DPPH radical scavenging activity (\%)} = 100 - [(A_0 - A_1)/A_0] \times 100]$$

where, *A*₀ is the absorbance of the control reaction and *A*₁ is the absorbance in the presence of the sample.

Antimicrobial activity

The antimicrobial activity of the plant extracts was evaluated using the agar well diffusion method and compared with the optimized shampoo formulation against the following pathogenic microorganisms:^[35] *Staphylococcus aureus* (ATCC 43300), *Bacillus cereus* (gram-positive bacteria), *Proteus* spp., *Escherichia coli* (ATCC 25922) (gram-negative bacteria), and *Candida albicans* (NRRL Y-477) (yeast). The experiment was performed using a culture grown at 37°C for 24 h. Twenty milliliters of nutrient agar for bacteria or potato dextrose agar for yeast were poured into sterile petri dishes and allowed to solidify. Wells were made in the agar plates using sterile cork pores that were 4 mm in diameter. The cultures were adjusted to approximately 10⁶ colony forming unit (CFU)/mL with sterile saline solution. One hundred and fifty microliters of the suspensions were spread over the agar plates using a sterile glass spreader. Each tested sample was dissolved in 1 mL of dimethyl sulfoxide and sterilized by filtration through a 0.22-µm membrane filter (using a Millipore membrane filter apparatus). Then, 100 µL of each sample was added separately to the appropriate wells in the petri dishes. Vancomycin was used as a standard drug for bacteria, whereas ketoconazole was used as a reference for fungi. The experiment was carried out in triplicate, and the average zone of inhibition was calculated.

Minimum inhibitory concentration measurement

The bacteriostatic activity of the active extracts (with inhibition zone [IZ] >16 mm) was then evaluated and compared with the optimized shampoo formulation using the twofold serial dilution technique.^[36] Twofold serial dilutions of the tested extract solutions were

prepared using the appropriate nutrient broth. The final concentrations of the solutions were 500, 250, 125, and 65 µg/mL. The tubes were then inoculated with the test organisms, which were grown in the appropriate broth at 37°C for 24 h (1×10^8 CFU/mL for bacteria and 1×10^6 CFU/mL for yeast); every 5 mL of medium received 0.1 mL of the above inoculum, and the culture was incubated at 37°C for 24 h. The minimum inhibitory concentration (MIC) (lowest concentration showing no microbial growth) was determined.

Formulation of the polyherbal shampoo

Four formulations of herbal shampoos (F₁–F₄) were prepared [Table 1] using different plant extracts (*Z. spina-christi*, *S. babylonica*, *G. glabra*, and a combination of the three plants) in the absence of SLS solution. The ingredients were ground with a mortar and pestle and mixed until the desired uniform dispersion was achieved. The mixture was then mixed well with gelatin (15%) solution on a magnetic stirrer until no precipitate remained at the bottom. Small amount of citric acid solution was added to adjust pH of the shampoo between 5 and 7.^[37,38] Few drops of lemon juice were added to impart aroma, and the volume was adjusted to 100 mL with gelatin solution. F₀ was prepared as a control sample (without plant extract and with SLS).

Physicochemical evaluation of the herbal formulations and commercial shampoos

To ensure product quality, specific tests were performed to compare the prepared shampoo formulations with commonly used commercially available herbal shampoos, including organoleptic testing, physicochemical characterization, and analysis of the conditioning effects. The results were used as references.

Physical appearance/visual inspection

The herbal preparations were assessed for transparency, color, odor, and foam-generating ability.^[38]

pH determination

The pH of different herbal shampoo formulations was measured using a digital pH meter (Mi 151, Martini Instruments, North Carolina).^[37]

Table 1: Compositions of different herbal shampoo formulations

Ingredient (% w/w)	F0	F1	F2	F3	F4
Sodium lauryl sulfate	15	-	-	-	-
Licorice extract	-	50	-	-	50
<i>Ziziphus spina-christi</i>	-	-	10	-	10
<i>Salix babylonica</i>	-	-	-	20	20
Lemon juice	1	1	1	1	1
Gelatin solution, complete to	100	100	100	100	100

Evaluation of percent solid content

Approximately 4 g of shampoo was placed in a dry, clean, and previously weighed evaporating dish. The liquid content of the shampoo was evaporated by placing the porcelain evaporating dish on a hot plate. The weight, and thus, the percent solid content of shampoo remaining after drying, was determined.^[39]

Surface tension measurement

The surface tension of a 10% w/v shampoo solution in distilled water was measured using a previously cleaned stalagmometer at room temperature.^[40] The obtained data were calculated using the following equation:

$$R_2 = (W_3 - W_1) n_1 / (W_2 - W_1) n_2 \times R_1$$

where, R_1 = the surface tension of distilled water at room temperature, W_1 = the weight of empty beaker, W_2 = the weight of the beaker filled with distilled water, W_3 = the weight of the beaker filled with shampoo solution, n_1 = the number of drops of distilled water, and n_2 = the number of drops of shampoo solution.

Evaluation of foaming ability and foam stability

The foaming ability was measured using the cylinder shake method.^[41] The total volume of foam formed after shaking 50 mL of a 1% formulated shampoo solution 10 times was recorded. Foam stability was evaluated by recording the foam volume immediately after 1 and 4 min of shaking.

Dirt dispersion performance

Approximately two drops of each shampoo formulation and one drop of India ink were added to 10 mL of distilled water, then shaken 10 times, and the amount of ink dispersed in the foam was described as none, light, moderate, or heavy.^[42]

Wetting time

The time required for a 1-inch diameter discs of canvas paper (average weight of 0.66 g) placed on 1% v/v shampoo solution to begin to settle was recorded as the wetting time.^[43]

Estimation of conditioning performance

To evaluate the conditioning effect of the optimized polyherbal shampoo (F4) and compare its effect with the commercial shampoo, a hair tress of an Egyptian woman was obtained from a local beauty salon.^[44] The hair tress was cut into three swatches that were approximately 5 cm in length. One part remained unwashed, which served as control (unwashed), whereas the other two swatches were washed with the optimized polyherbal shampoo formulation (F4) and commercial shampoo. Twenty-five randomly selected female

volunteers evaluated the conditioning performance of the tested shampoos based on the smoothness and softness using a blind touch test. All blindfolded women were asked to rank the conditioning performance of the three hair tresses after touching them. For rating, scores from * to **** were used, where, * = poor, ** = satisfactory, *** = good, and **** = excellent.

Surface characterization of hair using scanning electron microscope

Scanning electron microscope (SEM) is an important tool that is used to validate the effects of different beauty care products on hair. SEM was used to evaluate the conditioning effects of the optimized polyherbal shampoo (F4) compared with those of the commercial product on hair containing sebum. The hair strands were mounted under a SEM, and photomicrographs were acquired at two different magnifications: 1000 \times and 2000 \times .^[45] The following four samples were characterized by SEM: sample (A), clean hair; sample (B), hair with sebum; sample (C), hair with sebum washed with the optimized polyherbal shampoo (F₄); and sample (D), hair with sebum washed with commercial product.

RESULTS AND DISCUSSION

Phytochemical and plant extracts' activities

All plant extracts showed the presence of carbohydrates, flavonoids, and tannins, *Z. spina-christi* and *G. glabra* showed an abundance of saponins, whereas alkaloids were present in only *Z. spina-christi*, which is consistent with previous reports.^[20,28,46]

Quantitative estimation of total phenolic and flavonoid contents

The total phenolic content was estimated by the Folin-Ciocalteu method in terms of GAE (standard curve equation: $y = 0.0011x + 0.0009$, $r^2 = 0.9867$). The highest phenolic content was observed for *Z. spina-christi*, followed by *G. glabra* [Table 2]. The phenolic content was as previously reported.^[47] The total flavonoid content was quantified based on the aluminum chloride assay in terms of QE (standard curve equation: $0.0015x$, $r^2 = 0.9854$), where *G. glabra*

had the highest flavonoid content, followed by *S. babylonica* and *Z. spina-christi*.

Ultra-performance liquid chromatography with tandem mass spectrometry analysis

A detailed study performed on licorice allowed a full identification of the phytoconstituents.^[48] However, data regarding the phytoconstituents of *S. babylonica* and *Z. spina-christi* remain scarce. Therefore, Ultra-performance liquid chromatography (UPLC) with tandem mass spectrometry (MS-MS) was used for qualitative analysis of the components of both plants. Tentative identification was performed by comparison of the mass data of the detected compounds with values reported in the literature. The identities, retention times, and fragmentation patterns for all the identified compounds are presented in Tables 3 and 4. On investigating the *S. babylonica* extract [Table 3], 12 phytoconstituents were identified, where hexose loss was monitored by a loss of 162 amu, rhamnose by a loss of 146 amu, and glucuronate by a loss of 175 amu. Peak (Pk) 2 exhibited the marker of the *Salix* genus, namely, salicin, with a molecular ion peak at m/z 285 and daughter ions at m/z 123 and 121. Several kaempferol derivatives were identified, including dihydrokaempferol hexoside, kaempferol hexoside, kaempferol glucuronide, and kaempferol hexoside rhamnoside (Pk 4, 7, 8, and 11, respectively), in addition to eriodyctol, apigenin hexoside, rutin, and diosmetin. UPLC-MS analysis of the methanolic leaf extract of *Z. spina-christi* allowed the tentative identification of 12 flavonoids [Table 4], including highly glycosylated quercetin (Pk 1, 3, 4, 6, and 9), and diosmetin. This finding was consistent with a previously reported study.^[15]

Although extensively studied for their biological effects, further phytochemical investigation is required to explain the medicinal uses of *S. babylonica* and *Z. spina-christi*.

Estimation of mineral content

Minerals are very important for hair health and are therefore of significant importance in hair care product

Table 2: Total phenolic and flavonoid content of the methanolic extracts of *Salix babylonica*, *Ziziphus spina-christi*, and *Glycyrrhiza glabra* and their free radical scavenging activity

Plant	Phenolics (mg/g GAE)	Flavonoids (mg/g QE)	IC ₅₀ (μg/mL)
<i>Salix babylonica</i>	177.38 ± 0.56	27.05	89.1
<i>Ziziphus spina-christi</i>	327.46 ± 130	16.28	39.1
<i>Glycyrrhiza glabra</i>	205.02 ± 0.01	41	73.4
Butylated hydroxyanisole (BHA)			53 ± 3.1
Vitamin C			12 ± 3.5

Table 3: Compounds tentatively identified by ultra-performance liquid chromatography with tandem mass spectrometry in the leaf methanolic extract of *Salix babylonica*

No.	Rt	[M – H] ⁻ m/z	MS ² ions	Identification
1	2.95	315	152.9 (-162) 249.6 108	Protocatechuic hexoside
2	3.50	285	123 121	Salicin
3	6.82	305	225 151 147 96	Epigallocatechin
4	8.83	449	286.2 285 284 255 151 133	Dihydrokaempferol hexoside
5	8.85	463	287 219 133	Eriodictyol hexoside
6	9.06	609	300.3 301 271 255	Rutin
7	9.62	447	284.3 285.3 199 167 151 133	Kaempferol hexoside
8	9.62	461	285 175 143 133	Kaempferol glucuronide
9	10.54	431	269 239	Apigenin hexoside
10	11.81	285	243 223 200 185 149 133	Kaempferol
11	12.36	593	285 284 174 161 155	Kaempferol hexoside rhamnoside
12	13.58	299	284 256 155	Diosmetin

Boldface digits reflects the base peak (100% abundance)

Fragment ions are listed in order of relative abundances

Table 4: Compounds tentatively identified by ultra-performance liquid chromatography with tandem mass spectrometry in the leaf methanolic extract of *Ziziphus spina-christi*

No.	Rt	[M – H] ⁻ m/z	MS ² ions	Identification
1	7.96	901	755 (-146) 300 299 179	Quercetin 3- <i>O</i> -(2,6-di- <i>O</i> -rhamnosyl-galactoside) 7- <i>O</i> -rhamnoside
2	8.78	595	287 (-308) 270.8 259 217 193 181 179 167 163 137	Eriodictyol diglucoside
3	9.38	771	301 299 254	Quercetin dihexoside rhamnoside
4	9.38	755	609 (-146) 301(-308) 300 273	Quercetin dirhamnosyl hexoside
5	8.84 in the second 9.8	597	357 209 193 167 137 123	Phloretin 3',5' dihexoside
6	9.98 first in second at 10.95	579	300 271 255 243 179 151	Quercetin rhamnosyl pentoside
7	10.28	607	299 271	Diosmetin hexoside rhamnoside
8	10.29	739	299 350 242	Diosmetin hexoside rhamnoside pentoside
9	10.29	725	300 271 179	Quercetin pentoside dirhamnoside
10	11.31 first	301	243 216 179 171 161 151 135 121 107 63	Quercetin
11	11.60	523	299 271 255 188 173 163 145 119	Naringenin derivative
12	12.36	593	285 284 174 161 145	Kaempferol glucoside rhamnoside

Boldface digits reflects the base peak (100% abundance)

Fragment ions are listed in order of relative abundances

formulations. The mineral content in *S. babylonica*, *Z. spina-christi*, and *G. glabra* was estimated [Table 5]. *S. babylonica* and *Z. spina-christi* had high levels of iron, whereas *Z. spina-christi* showed high levels of manganese, zinc, and copper compared to a low mineral content for *G. glabra*. Manganese is necessary for the production of natural antioxidant enzymes that can defend against free radicals that promote thinning hair. Zinc is an important mineral for healthy hair, skin, and nails. Topical antiaging compounds of interest in recent research include minerals such as selenium, copper, manganese, and zinc.^[49]

Free radical scavenging activity

Hair is deteriorated by many external factors (sunlight, pollution, cosmetic treatments, grooming practices,

and cleansing), which results in fiber degradation and production of free radicals in addition to hair aging,^[50] which necessitates the use of topical antioxidants to prevent further degradation. The DPPH spectrophotometric method is one of the most widely applied methods for estimation of radical scavenging activity and is appreciated for its reliability. The potency of the plant methanolic extracts is presented in Table 2, where *Z. spina-christi* exhibited the best radical scavenging effect, with the lowest IC₅₀, followed by licorice, which correlated well with the results of phenolic content measurement.

Antimicrobial activity

The extracts were screened for potential antimicrobial activity by measuring the IZ (in millimeter) against

Table 5: Mineral content of *Salix babylonica*, *Ziziphus spina-christi*, and *Glycyrrhiza glabra*

Plant extract	Mineral content (mg/kg)				Mineral content (%)			
	Cu	Zn	Mn	Fe	K	Ca	P	Mg
<i>Salix babylonica</i>	31.67	47.78	166.4	1618.33	1.78	0.4	0.08	0.59
<i>Ziziphus spina-christi</i>	65.63	65.31	1082.8	1161.88	2.8	0.13	0.06	0.29
<i>Glycyrrhiza glabra</i>	8.700	14.70	3.8	2.00	0.21	0.040	0.008	0.263

Table 6: Antimicrobial activity of *Salix babylonica*, *Ziziphus spina-christi*, *Glycyrrhiza glabra*, and the polyherbal shampoo expressed as the mean diameters of the inhibition zones (mm)

Pathogens	<i>G. glabra</i>	<i>S. babylonica</i>	<i>Z. spina-christi</i>	Polyherbal shampoo	Vancomycin	Ketoconazole
Gram positive						
<i>Staphylococcus aureus</i> (ATCC 43300)	-	-	11	17	22	-
<i>Bacillus cereus</i>	20	11	-	26	22	-
Gram negative						
<i>Proteus</i> spp.	-	16	17	22	22	-
<i>Escherichia coli</i> (ATCC 25922)	-	-	-	11	22	-
Yeast						
<i>Candida albicans</i> (NRRL Y-477)	20	14	-	25	-	22

gram-positive and gram-negative bacteria in addition to yeast. The data presented in Table 6 showed the mean diameters of the IZs (in millimeter) of the different extracts against all the tested pathogens. The licorice extract exhibited the highest activity, which was comparable with that of the standard, against *B. cereus* and *C. albicans*, whereas *S. babylonica* showed moderate activity against *B. cereus*, *Proteus* spp., and *C. albicans*. In addition, *Z. spina-christi* exhibited weak activity against *S. aureus* and moderate activity against *Proteus* spp.

Minimum inhibitory concentration measurement

The MIC values of *G. glabra* and *Z. spina-christi* against representative microorganisms [Table 7] ranged between 125 and 250 µg/mL, where *G. glabra* exhibited better activity than *Z. spina-christi*. *G. glabra* has been reported to have significant antimicrobial activity.^[51]

Evaluation of herbal shampoos

Physical appearance/visual inspection

On visual inspection [Table 8], no significant difference was observed in terms of odor and transparency between the commercial and formulated shampoos, except in color and foaming characteristics.

pH determination

It is recommended that shampoos be formulated with either neutral or slightly alkaline pH values (between 7 and 5) to minimize hair damage and eye irritation and stabilize the ecological balance of the scalp.^[52] The pH values are presented in Table 8. Acid-balanced pH values were observed with the commercial shampoo and F0 (6.80 and 6.15, respectively), whereas the final pH values

of the different herbal shampoo formulations ranged from 5 to 5.90, which confirmed that these formulations met the requirements for an ideal shampoo and had pH values similar to that of the skin.

Evaluation of percent solid content

Any shampoo should have acceptable amount of solid content (20%–30%) to prevent a watery consistency, washing away quickly if within a lower percentage, or showing a difficulty to wash out if higher.^[53] The results shown in Table 8 revealed that the herbal shampoo formulation (F4) had an acceptable range of solid content (allowing ease of washing).

Surface tension measurement

Surface tension measurements reflect the amount of surfactant present in a formulated shampoo; surfactants are required for reduction of the surface tension of water. As the surface tension decreases, the cleansing ability of the shampoo increases. An ideal, high-quality shampoo should decrease the surface tension of pure water from 72.28 to approximately 40 dyne/cm.^[54] All herbal shampoo formulations and commercial products showed similar reductions in surface tension, ranging from 31.65 to 37.15 dyne/cm [Table 8]. However, among all the formulations, the polyherbal shampoo formulation (F4) had the lowest surface tension (31.15 dyne/cm), indicating that this formulation had the strongest cleansing ability.

Foaming ability and foam stability

Foaming ability represents an important factor in shampoo evaluation as well correlated with the

Table 7: Minimal inhibitory concentrations (MICs) of *Salix babylonica*, *Ziziphus spina-christi*, and *Glycyrrhiza glabra* compared to the polyherbal shampoo against the indicator pathogens

Pathogens	<i>G. glabra</i>	<i>S. babylonica</i>	<i>Z. spina-christi</i>	Herbal shampoo	Vancomycin	Ketoconazole
Gram positive						
<i>Staphylococcus aureus</i> (ATCC 43300)	-	-	-	250	65	-
<i>Bacillus cereus</i>	125	-	-	65	65	-
Gram negative						
<i>Proteus</i> spp.	-	250	250	65	65	-
<i>Escherichia coli</i> (ATCC 25922)	-	-	-	-	65	-
Yeast						
<i>Candida albicans</i> (NRRL Y-477)	125	-	-	65	-	65

Table 8: Physicochemical evaluation of different herbal shampoo formulations and a commercial product

Formulation	F0	F1	F2	F3	F4	Commercial product
Color	Colorless	Dark brown	Dark brown	Dark brown	Dark brown	Light green
Transparency	Transparent	Transparent	Transparent	Transparent	Transparent	Transparent
Odor	Good	Good	Good	Good	Good	Good
Foam-producing ability	Mild foam	Mild foam	Good foam	No foam	Good foam	Mild foam
pH	6.15±0.03	5.4±0.04	5±0.02	5.9±0.01	5.5±0.02	6.8±0.01
Solid content (%)	20.05±0.04	14.01±0.03	16.41±0.06	19.52±0.04	28.8±0.03	16.7±0.01
Surface tension measurement (dyne/cm)	33.11±0.06	34.40±0.02	34.09±0.62	37.15±0.12	31.65±0.32	31.95±0.25
Wetting time (seconds)	8±0.07	5±0.02	8±0.01	15±0.05	2±0.01	3±0.02

Results are Mean ± standard deviation (SD), n = 3

Table 9: Foam stability of different herbal shampoo formulations and a commercial product

Time (min)	Foam volume (mL)					
	F0	F1	F2	F3	F4	Commercial product
1	150	140	170	No foam	200	160
2	148	138	167	No foam	198	156
3	146	137	165	No foam	196	154
4	145	135	163	No foam	195	153

consumer acceptance.^[42] All four herbal shampoo formulations had the ability to produce persistent foam, except F3 (*S. babylonica*), because of its lack of saponin content [Table 9].

Dirt dispersion performance

Dirt dispersion reflects the cleansing power of shampoo formulations. The persistence of dirt in the foam is related to a difficulty to rinse it away from hair.^[55] All the formulated and marketed shampoos, except F3, exhibited concentrated ink in the water portion, ensuring their satisfactory cleansing ability.

Wetting time

The wetting ability of a shampoo depends mainly on the surfactant concentration in its composition; thus, this test is commonly performed to test the efficacy of the surfactant. The shorter the time taken by the disc to sink, the higher the wetting efficiency of the tested shampoo. The wetting times for all the prepared formulations were compared with that of the commercial shampoo

Table 10: Average scores given by the woman volunteers, reflecting their opinions on the conditioning of the tresses after treatment with the optimized formulated shampoo and a commercial shampoo (n = 25)

Score	Polyherbal shampoo (F4)	Commercial shampoo	No washing
*	2	1	21
**	3	4	4
***	13	15	0
****	7	5	0
Average	3	2.96	1.16

* = poor, ** = satisfactory, *** = good, **** = excellent

[Table 8]. The polyherbal shampoo formulation (F4) exhibited the shortest wetting time (2s), followed by the herbal shampoo formulation (F2). This observation may be explained by the presence of a large amount of saponin among the components of these formulations.

On the basis of these results, the polyherbal shampoo formulation (F4), which combines *Z. spina-christi*, *G. glabra*, and *S. babylonica*, appeared to be an optimized

herbal shampoo formulation because it exhibited good foaming ability and stability, ideal solid content percentage, lowest wetting time, and lowest surface tension, reflecting the cleansing ability of this formulation because of the presence of active natural foam-forming agents (saponins).

Estimation of conditioning performance

The conditioning performance of the polyherbal shampoo (F4) and marketed shampoo based on the mean scores of the women testers is presented in Table 10. A majority of the volunteers rated the

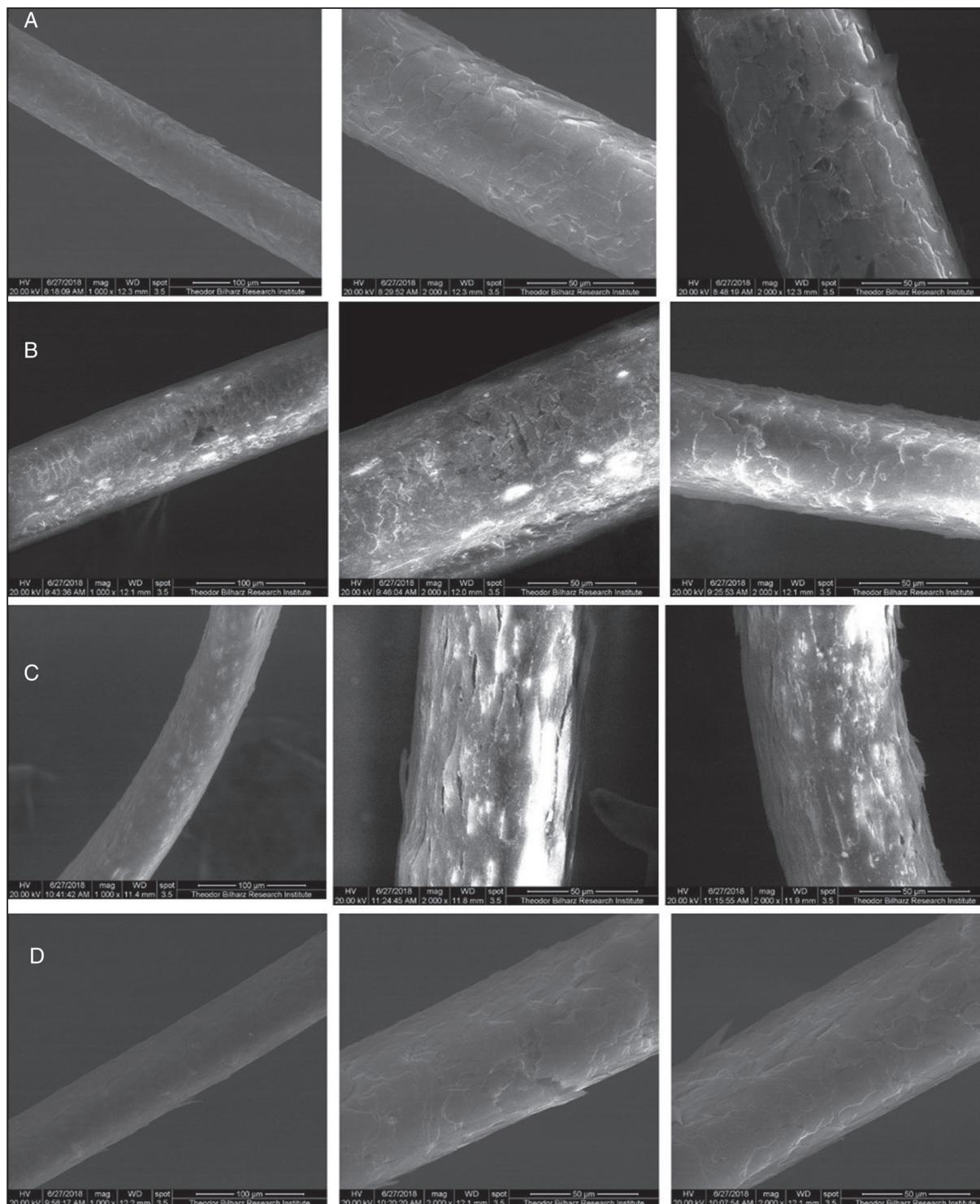


Figure 1: Scanning electron microscope micrographs of different hair samples. (A) Clean hair. (B) Hair with sebum. (C) Hair with sebum washed with commercial shampoo. (D) Hair with sebum washed with polyherbal shampoo (F4)

truss that was washed five times with the formulated polyherbal shampoo (F4) as having the best conditioning performance, and as expected, the control truss (unwashed) received the lowest score.

Surface characterization of hair using scanning electron microscope

Figure 1 shows digital images of different hair samples obtained by SEM using different magnifications (1000× and 2000×). SEM was successfully used to study the effects of treatment with shampoo on hair microstructure, and it indicated a clear difference between treated and untreated hairs. In this study, SEM was used to compare the differences between normal clean hair, hair covered with sebum, hair treated with the polyherbal shampoo, and that treated with the commercial product [Figure 1A–D].

The untreated hair sample had a shiny shaft because of the sebum, and the scale edges appeared raised and highly nonuniform [Figure 1B]. On studying the effect of the commercial shampoo [Figure 1C], the shaft continued to appear shiny because of poor cleaning, and the scale edges remained highly jagged. Our polyherbal shampoo [Figure 1D] showed a strong conditioning effect; the hair scales were flat, regularly oriented, and characterized by homogenous borders with smooth cuticles, and no damage or groove was observed along the long axis of the hair.^[45,56] The saponin content provided a demulcent effect, whereas the phenolic content was responsible for the radical scavenging ability and reducing the potential alterations in hair morphology.

Shampoo antimicrobial activity and minimum inhibitory concentration

The antimicrobial activity of the formulated shampoo after combining the three extracts increased and gave significant positive activity against all tested microorganisms [Table 6]. The MIC values of the formulated shampoo were comparable to the standard antibiotic and antifungal used with a value of 65 µg/mL, whereas its activity was slightly lower against *S. aureus* with MIC 250 µg/mL [Table 7].

CONCLUSION

The completely polyherbal shampoo formulated with *Z. spina-christi*, *G. glabra*, and *S. babylonica* proved to be a multipurpose shampoo. The high phenolic and mineral content of this formulation led to strong antioxidant activity and provided natural preservative action, whereas the saponins in both *Z. spina-christi* and *G. glabra* acted as natural foaming agents, eliminating

the use of SLS in addition to having a significant conditioning effect, as observed by SEM. The good homogeneity and appearance of our formulation acted as a natural colorant, with no need for a stabilizer in the final formulation. Therefore, this product can help to promote hair healthy, providing satisfaction with few side effects.

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Conflicts of interest

There are no conflicts of interest.

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