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ORIGINAL RESEARCH

The Treatment and Evaluation of Dandruff and Scalp Erythema with Fermented *Salix purpurea* Bark Extract

Oliwia Kalinowska[1,*], Julia Maślana[1,*], Andrea Gilreath[2]

[1]J.S. Hamilton Poland Sp. Z o.o. Testing Laboratory, Gdynia, Poland; [2]Actera Ingredients Inc, Newtown, PA, USA

*These authors contributed equally to this work

Correspondence: Andrea Gilreath, Actera Ingredients Inc, 66 Walker Ln, Newtown, PA, 18940, USA, Email agilreath@acteraingredients.com

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Purpose: The aim of this study was to gain knowledge of scalp and hair tolerance and efficacy of two investigational shampoo formulations with different concentrations of fermented *Salix purpurea* (purple willow) bark extract tested on dandruff sufferers.

Methods: This non-invasive study included a trichologist assessment of tolerance, clinical evaluation of efficacy, and photo documentation by macrophotography with Aramo ASW 300F. Statistical analysis was conducted using paired sample t-tests or Wilcoxon signed-rank tests to assess within-group changes, and unpaired t-tests or Mann–Whitney *U*-tests to compare results between groups. A significance level of $p < 0.05$ was used to determine the efficacy of the treatments. A split-panel of 21 participants with varying scalp conditions, including visible dandruff, used the shampoos (Salixin Shampoo 2% or Salixin Shampoo 4%) for 56 consecutive days. Regular assessments were performed under trichological and dermatological supervision, including evaluation of scalp appearance, itching, redness, dandruff visibility, and subject-reported side effects.

Results: Results indicated that both formulations were well tolerated. Evaluations demonstrated a reduction in dandruff symptoms for both shampoos, with Salixin Shampoo 4% showing a more pronounced effect, although not statistically significant.

Conclusion: These findings suggest that fermented *Salix purpurea* bark extract is effective in reducing dandruff and improving scalp condition, with higher concentrations offering enhanced efficacy.

Keywords: dandruff, scalp health, *Salix purpurea* bark extract, clinical evaluation, trichologist assessment, dermatological control

Introduction

Dandruff is a common scalp condition characterized by excessive shedding of dead skin cells, often accompanied by itching and irritation. This condition affects a significant portion of the global population and can be caused by various factors, including fungal infections, scalp dryness, or excessive oil production.[1] Dandruff sufferers can control symptoms by using daily medicated shampoos. However, these shampoo formulations often rely on active ingredients that have toxicological concerns; for example, since 2022 the EU has banned the use of one of the most common anti-dandruff actives, zinc pyrithione, for this reason. The demand for safer and effective anti-dandruff treatments has led to the development of various formulations, often incorporating natural extracts with known therapeutic benefits. Among these, *Salix purpurea* (purple willow) bark extract has gained attention for its anti-inflammatory, antifungal,[2] and exfoliating properties, primarily due to its salicylate content.[3]

This study focuses on evaluating the clinical efficacy of two formulations containing different concentrations of fermented *Salix purpurea* bark extract. The primary aim is to assess the impact of these formulations on dandruff reduction and overall scalp health, as well as their tolerability among users. The study was conducted under the supervision of a trichologist and control of a dermatologist to ensure accurate and comprehensive evaluations.

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Materials and Methods

The study was designed to clinically evaluate the effectiveness of the anti-dandruff effect of the investigational formulations and to compare results between the formulations following 56 consecutive days of use by a panel of 21 subjects divided into 2 groups depending on sample – Salixin Shampoo 2% or Salixin Shampoo 4%. The investigational formulations were applied under normal conditions of use by the test subjects at home and in accordance with the declared method of use. The formulations were used under trichologist supervision as well as under dermatological control.

The experimental conditions adopted for the tested formulations were representative of normal conditions for a typical shampoo formula and included application area, quantity of investigational products applied, and frequency of application. The observance of the experimental conditions by the test subjects was assessed at the end of the study. The user experience of the test subjects was considered as it is representative of the target consumer. The skin examination was performed by a qualified investigator.

The results of the study were visually documented by macrophotography of the surface of the scalp and hair in zoom 60-times by using Aramo ASW 300F, manufactured by ARAM HUVIS Co. The investigational formulations were of known quality and composition. Microbiological purity testing and dermatological irritation testing (patch testing) were performed prior to study start. Box 1 identifies the composition of the investigational formulations, with the only variance being the percent of *Salix purpurea* bark extract (2% or 4%).

While the aim of this study was to compare the efficacy of the test formulations on dandruff visibility and scalp health, it was also important to consider the entire formulation of the shampoos. The shampoo base serves as a matrix for the active ingredient, *Salix purpurea* bark extract, and ensures the other beneficial properties of the shampoo. The following is a brief discussion of the role of each ingredient.

Deionized Water

Deionized water is purified water, free from minerals, salts, and other impurities. It serves as a base for cosmetics, ensuring that other ingredients can dissolve better and work more effectively.

Polyquaternium-10

This is a synthetic compound belonging to the polymer group and is widely used in hair care products as a conditioning agent. Polyquaternium-10 forms a protective layer on the hair, smoothing it, making it easier to detangle, reducing frizz, and adding shine. It also has antistatic properties, helping to prevent hair from becoming static.[4]

Box 1 INCI Composition of Investigational Formulations

Deionized Water
Polyquaternium-10
Caprylyl Glyceryl Ether (and) Caprylhydroxamic Acid (and) Propanediol
Cocamidopropyl Hydroxysultaine (and) Sodium Cocoamphohydroxypropylsulfonate (and) Cocamide DIPA (and) Maltodextrin Laurate
Sodium Cocoyl Glutamate
Lactobacillus/*Salix purpurea* Bark Ferment Extract (and) Glycerin
Citric Acid 50% Solution
Sodium Chloride

Caprylyl Glyceryl Ether and Caprylhydroxamic Acid and Propanediol

- Caprylyl Glyceryl Ether – A conditioning, moisturizing, and softening ingredient. It improves the structure of hair and skin, leaving it soft.[5]
- Caprylhydroxamic Acid – A natural preservative with antibacterial and antifungal properties. It is gentle on the skin and hair, preventing the growth of bacteria and fungi in the product.[6]
- Propanediol – A natural humectant that moisturizes the skin and hair and can improve the penetration of other ingredients. It acts as a solvent, improving the solubility of other compounds.[7]

Cocamidopropyl Hydroxysultaine and Sodium Cocoamphohydroxypropylsulfonate and Cocamide DIPA and Maltodextrin Laurate

- Cocamidopropyl Hydroxysultaine – A gentle surfactant (detergent) that effectively cleanses while being mild on the skin. Derived from coconut oil, it also has moisturizing and nourishing properties.[8]
- Sodium Cocoamphohydroxypropylsulfonate – A mild plant-derived surfactant that enhances foaming while reducing potential skin irritation.[9]
- Cocamide DIPA – A foam stabilizer and emulsifier. It helps improve the quality of the foam and also softens the hair.[10]
- Maltodextrin Laurate – A stabilizing agent that improves the product's consistency and enhances smoothing properties for the hair.[11]

Sodium Cocoyl Glutamate

A gentle plant-derived detergent made from coconut fatty acids and glutamic acid. It gently cleanses hair and skin without causing irritation. It is often used in products for sensitive skin and has moisturizing properties.[12]

Lactobacillus/Salix purpurea Bark Ferment Extract and Glycerin

- Lactobacillus/*Salix purpurea* Bark Ferment Extract – This is a fermented extract from the bark of purple willow. Willow contains salicylic acid and other beneficial salicylates, polyphenols, and flavonoids which together impart cleansing, anti-inflammatory, and anti-dandruff properties. The fermentation process can improve the bioavailability of active ingredients and offer probiotic benefits, supporting the healthy microbiome of the scalp.[13]
- Glycerin – A strong humectant that binds water, moisturizing the skin and hair. It reduces water loss, leaving hair softer and more flexible.[14]

Citric Acid 50% Solution

A citric acid solution acts as a pH regulator, helping maintain the correct pH of the shampoo. Maintaining an acidic pH is essential for sealing the hair cuticle, making hair smoother and shinier. Citric acid also has cleansing properties, which can help remove impurities and product buildup.[15]

Sodium Chloride

Sodium chloride (salt) acts as a thickening agent in shampoos, increasing viscosity. It also has cleansing properties, although in large amounts it can dry out the hair and skin.[16]

The composition of the shampoos suggests that it is a gentle, moisturizing, and conditioning formula, suitable for people with sensitive scalps. The use of mild surfactants reduces the risk of irritation. Conditioning ingredients help improve the appearance and texture of hair, making it easier to detangle and reduce frizz. The fermented willow bark extract has anti-inflammatory and potential anti-dandruff effects, while glycerin ensures the product is lightly moisturizing.

Study Protocol

The study was performed in compliance with the procedures of the investigating center, J.S. Hamilton Poland Sp. z o.o. Testing Laboratory, and was established according to the regulations in force. The investigator in charge of the performance of the study ensured the quality of the work of the technical staff, particularly concerning adherence to protocol and its appendices, the

collection of raw data, and the management of the investigational products. The personal and medical information concerning each subject required for recruitment and inclusion was confidential. Each test subject was coded when included in the study to preserve anonymity. The information about the study was given orally and as a written document to each test subject before the start of the study. This information was accessible, understandable, and suitable for each test subject.

Exclusions for use of the products were acute inflammation of the place of application, ongoing pharmacological treatment, and allergy or hypersensitivity to any of the test formulation ingredients.

The study panel was comprised of 21 female and male volunteers, with the age range of 18+, of all scalp and hair types, sensitive and non-sensitive, with visible dandruff. The panel was split in half, with one-half using the Salixin Shampoo 2% and one-half using the Salixin Shampoo 4%.

The subjects received the test formulations, specially developed self-assessment questionnaire, and daily log. They were obliged to:

- Use the test formulation regularly according to the method of use
- Discontinue use of other dandruff products
- Complete the provided questionnaire
- Note any discomfort or medication taken in the daily log
- Discontinue use of the test formulation and consult the specialist at J.S. Hamilton, Poland, in the case of any side effects

Skin reactivity, history of atopy, and contraception use were documented by the investigator in the case report form (CRF). No medication likely to interfere with the study was allowed during the study. If the health of the subject justified medication (particularly anti-inflammatory drugs), any information relating to this concomitant medication was carefully documented in the CRF. The investigator had to exclude the test subjects taking concomitant medication likely to interfere with the study and the interpretation of the results.

The study included visits on days 0, 7, 14, 28, 42 and final visit on day 56. At each visit, the daily logs were collected and checked in case of adverse events, the trichologist examined the study zone, measurements were collected of the studied zone by macrophotography of the surface of the scalp and hair in zoom 60-times, and any adverse events were recorded.

Ethical Standards

Ethics approval for this study was obtained by the J.S. Hamilton Internal Bioethics Committee. The committee is comprised of a Good Clinical Practice (GCP) specialist, lawyer, dermatologist, project manager, and cosmetologist who collectively review study protocol and oversee study procedures. According to the procedure of the investigating center and bioethics committee, informed consent was obtained along with pre-clinical information concerning the investigational formulations. The committee ensured the project met the conditions of optimal scientific rigor, assessed its general relevance, determined the suitability between the aim of the study and the means implemented, and provided an assessment of the protection of the test subjects.

The described study was conducted in accordance with the Good Clinical Practice defined by the ICH Topic E6 "Note for Guidance and Good Clinical Practice" (CPMP/ICH/135/95) and the Helsinki Declaration (1964, WMA) and its successive updates. The study was conducted according to Standard Operating Procedures and to the study protocol defined by the sponsor. All study events recorded during the study were reported. Controls on data veracity and conformity with the protocol were confirmed.

SCOPE OF TESTS COMPLIANT WITH:

- Regulation of the European Parliament and of the Council (EC) No. 1223/2009 of 30 November 2009 on cosmetic products
- Cosmetics Europe – The Personal Care Association (previously COLIPA) Guidelines "Product Test Guidelines for the Assessment of Human Skin Compatibility 1997"
- Cosmetics Europe – The Personal Care Association Guidelines for the Evaluation of the Efficacy of Cosmetic Products 2008

- Cosmetics Europe – The Personal Care Association Guidelines for Cosmetic Product Claim Substantiation Revising and Expanding the COLIPA Guidelines on Efficacy

Unlike pharmaceuticals, governing law for cosmetics does not require registered clinical trials for studies focused on the investigation of performance claims, nor does it require registered clinical trials to place cosmetic products on the market. Rather, formal safety assessments, which are done in a variety of ways, are the primary requirement for placing products on the market. Cosmetic performance claims investigation requires only ethical consideration that follows the principles of the Helsinki guidelines and additional regulations outlined above, which is the case for the performance claims study summarized in this work. For these reasons, this study was not registered as a clinical trial.

Use Test

The use test was conducted at home, under trichologist supervision, and under dermatological control. The study assessed the impact of cosmetic formulations on tolerance and efficacy at the application site as a result of regular, repetitive application of the product, according to the purpose and use of the specified time (repetitive test).

Trichologist Assessment and Clinical Evaluation of Formulation Efficacy

Assessment scales were based on the work of Tronnier et al and Bhattacharyya et al.[17,18] The trichologist assessed the type (dry, normal, oily) and condition (dermal efflorescence) of the scalp before and after product application using a 2-point scale (0–1). This scale refers to the presence or absence of the features evaluated, where 0 represents absence and 1 represents presence of the feature.

Assessment of the scalp appearance and symptoms (redness, pain, burning, itching, severity of dandruff – hair is covered with white and yellow flakes, seborrhoea) was recorded with severity assessed by a 4-point scale (0–3). Here, 0 represents none, 1-slight, 2-moderate, and 3-strong. The direct efficacy of the tested formulations on impacting the severity of scalp appearance and symptoms was determined by these ratings.

The assessment of each parameter was performed before the product application (D0), after 7 days (D7), after 14 days (D14), after 28 days (D28), after 42 days (D42) and after 56 days (D56) of regular use. Efficacy was indicated by a significant decrease in the average value difference at each time point compared to baseline (Δ%).

Instrumental Evaluation

Macrophotography of the surface of the scalp and hair in zoom 60-times was conducted using the Aramo ASW 300F device, manufactured by ARAM HUVIS Co. Instrumental evaluation was carried out on all subjects. The macrophotography of scalp and hair was performed at the specified zone before product application (D0), after 7 days (D7), after 14 days (D14), after 28 days (D28), after 42 days (D42) and after 56 days (D56) of regular use. The study was carried out in temperature-controlled conditions at 20±2°C and relative humidity 50±10%.

Statistical Analysis

The results were statistically analysed with STATISTICA 13. Paired sample *t*-test or Wilcoxon signed rank test (according to the result of the previously applied normality test) was used to assess differences in comparison to measurements. Additionally, the unpaired two-sample *t*-test or Mann–Whitney *U*-test (according to the result of the previously applied normality test and Levene's test) was used to assess differences between the two products. The level of significance was set $p < 0.05$.

Results

This study was finished by 20 subjects. Both investigational formulations were very well tolerated at the site of application. For all subjects who finished the study, during the regular application in the interview (subjective evaluation reported in daily logs) there were no negative symptoms that might indicate an intolerance to any component of the products, such as irritation, burning sensation, redness, or itching.

Assessments of scalp type and dermal efflorescence by a trichologist during the study are presented in Table 1. The trichologist recorded the number of subjects with reported changes (improvement or deterioration) in the following

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Table 1 The Number of Subjects With Reported Changes in the Parameters According to Trichologist Evaluation. NC – No Changes Reported

			Day 7		Day 14		Day 28		Day 42		Day 56	
			Salixin Shampoo 2%	Salixin Shampoo 4%	Salixin Shampoo 2%	Salixin Shampoo 4%	Salixin Shampoo 2%	Salixin Shampoo 4%	Salixin Shampoo 2%	Salixin Shampoo 4%	Salixin Shampoo 2%	Salixin Shampoo 4%
Scalp Type	Dry Scalp	Improvement	0	1	1	2	3	2	3	5	4	6
		Deterioration	2	0	0	0	0	0	0	0	0	0
	Normal Scalp	Improvement	NC	2	1	2	3	2	3	5	6	6
		Deterioration	NC	0	3	0	3	0	3	0	3	0
	Scalp with Dandruff	Improvement	NC	2	2	2	4	2	2	3	8	9
		Deterioration	NC	0	0	0	0	0	0	0	0	0
Sensitive Skin		Improvement	1	NC	2	NC	1	0	2	0	2	NC
		Deterioration	1	NC	0	NC	1	1	0	1	0	NC
Scalp Appearance and Symptoms	Redness	Improvement	NC	1	1	1	0	2	1	2	2	2
		Deterioration	NC	0	0	0	1	1	0	0	0	0
	Burning	Improvement	NC	NC	0	NC	NC	NC	NC	NC	NC	NC
		Deterioration	NC	NC	1	NC	NC	NC	NC	NC	NC	NC
	Itching	Improvement	2	NC	2	NC	3	NC	3	5	3	5
		Deterioration	0	NC	0	NC	0	NC	0	0	0	0

parameters: dry scalp, normal scalp, presence of dandruff, skin sensitivity, scalp redness, scalp burning, and scalp itching. At several timepoints, no changes were observed. There is a strong directional improvement for several of the parameters in subjects using both test formulations, with a larger number of subjects seeing improvements at day 42 and day 56. A slightly more dramatic improvement was observed in several parameters for subjects using Salixin Shampoo 4%.

The results of the clinical evaluation are summarized in Table 2 and Table 3. It was determined that a significant decrease in the Δ% value indicates formulation efficacy for reducing dandruff. The portion of subjects with an observed improvement in dandruff was captured by quantifying the percent of subjects with a positive effect on the visibility of dandruff.

The differences in efficacy concerning the visibility of dandruff when comparing Salixin Shampoo 2% and Salixin Shampoo 4% are presented in Table 4. Significance was captured for each time point throughout the duration of the study.

Table 2 Clinical Evaluation of the Average Visibility of Dandruff in Subjects Using Salixin Shampoo 2% According to the Scale

	Baseline (Day 0)	Day 7	Day 14	Day 28	Day 42	Day 56	Difference (D7-D0)	Difference (D14-D0)	Difference (D28-D0)	Difference (D42-D0)	Difference (D56-D0)
Mean	1.6	1.5	1.1	0.7	0.5	0.6	−0.2	−0.5	−1.0	−1.2	−1.1
Min	1.0	1.0	0.0	0.0	0.0	0.0	−1.0	−2.0	−3.0	−2.0	−3.0
Max	3.0	3.0	2.0	2.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0
SD	0.7	0.7	0.5	0.7	0.5	0.5	0.4	0.7	1.1	0.6	0.9
Median	2.0	1.0	1.0	1.0	0.5	1.0	0.0	0.0	−1.0	−1.0	−1.0
p-value							NA	0.0431	0.0150	0.0077	0.0117
Test type							NA	Wilcoxon	*t*-test	Wilcoxon	Wilcoxon
Significance							NA	Yes	Yes	Yes	Yes
Δ %							−11%	−33%	−61%	−73%	−67%
% of subjects with positive effect							18%	45%	80%	90%	80%

Table 3 Clinical Evaluation of the Average Visibility of Dandruff in Subjects Using Salixin Shampoo 4% According to the Scale

	Baseline (Day 0)	Day 7	Day 14	Day 28	Day 42	Day 56	Difference (D7-D0)	Difference (D14-D0)	Difference (D28-D0)	Difference (D42-D0)	Difference (D56-D0)
Mean	1.7	1.3	1.0	0.9	0.5	0.4	−0.4	−0.7	−0.8	−1.2	−1.3
Min	1.0	1.0	0.0	0.0	0.0	0.0	−1.0	−2.0	−2.0	−2.0	−2.0
Max	2.0	2.0	2.0	2.0	2.0	2.0	0.0	0.0	0.0	0.0	0.0
SD	0.5	0.5	0.7	0.6	0.7	0.7	0.5	0.8	0.8	0.8	0.8
Median	2.0	1.0	1.0	1.0	0.0	0.0	0.0	−0.5	−1.0	−1.0	−1.5
p-value							0.0679	0.0431	0.0277	0.0117	0.0117
Test type							Wilcoxon	Wilcoxon	Wilcoxon	Wilcoxon	Wilcoxon
Significance							No	Yes	Yes	Yes	Yes
Δ %							−24%	−41%	−47%	−71%	−76%
% of subjects with positive effect							40%	50%	60%	80%	80%

Table 4 The Difference in the Visibility of Dandruff Results Between Salixin Shampoo 2% and Salixin Shampoo 4% at Each Time Point

	After 7 Days	After 14 Days	After 28 Days	After 42 Days	After 56 Days
ΔΔ %	13%	8%	−14%	−2%	9%
p-value	0.3012	0.7261	0.6232	0.9699	0.4963
Test type	Mann–Whitney *U*-test	Mann–Whitney *U*-test	Mann–Whitney *U*-test	Mann–Whitney *U*-test	Mann–Whitney *U*-test
Significance	No	No	No	No	No

Macrophotography of the surface of the scalp and hair was used for the visual documentation of changes in dandruff visibility. Representative images showing the reduction in dandruff and changes in scalp redness in subjects over the duration of the study using both Salixin Shampoo 2% and Salixin Shampoo 4% are presented in Figures 1 and 2.

Comparative analysis of the performance of both test formulations at each time point is presented in Figure 3. Statistical significance was captured for each time point with significance determined by $p < 0.05$.

Discussion

Improvements in several parameters, especially the main parameter of interest, visibility of dandruff, were observed for both test formulations throughout the duration of the study. It was determined that Salixin Shampoo 4% demonstrates faster and stronger results across most scalp conditions, especially for dry scalp, severity of dandruff, and itching and showed noticeable improvements earlier on and more widespread benefits by day 56. Salixin Shampoo 2%, while clearly effective, shows more gradual improvements, with certain conditions like normal scalp and sensitive skin seeing more fluctuations, including some early-stage deterioration before improvements become evident.

The Salixin Shampoo 4% formulation is generally more potent but can cause deterioration in sensitive skin and redness in a few cases, likely due to its higher concentration. Dandruff severity and itching respond well to both

Figure 1 Aramo ASW 300F images of the surface of the scalp and hair in zoom 60-times before application and at each time point showing a reduction in flaking in subjects using Salixin Shampoo 2%.

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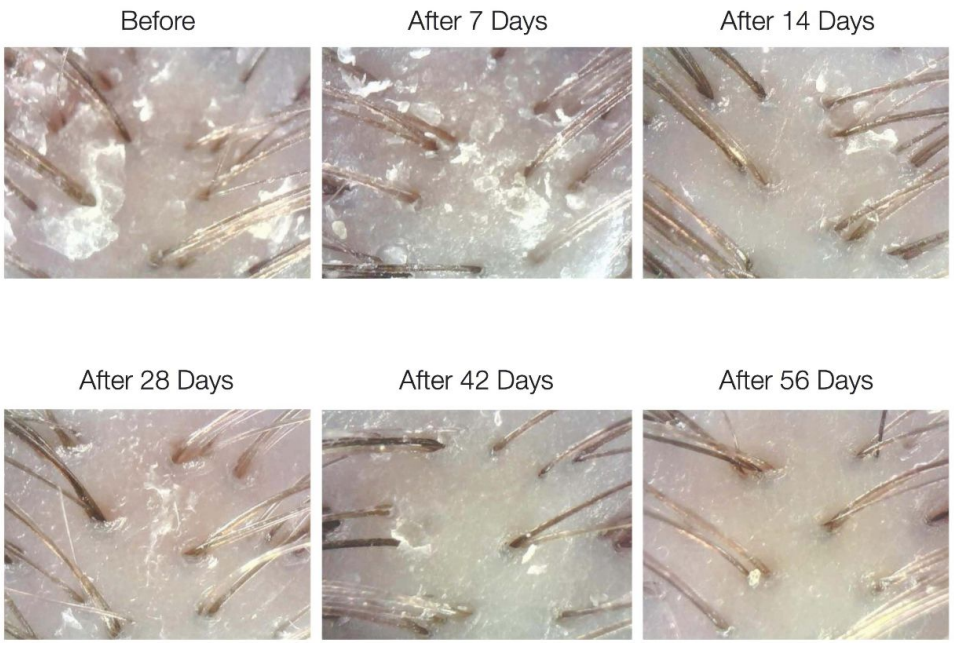

Figure 2 Aramo ASW 300F images of the surface of the scalp and hair in zoom 60-times before application and at each time point showing a reduction in flaking in subjects using Salixin Shampoo 4%.

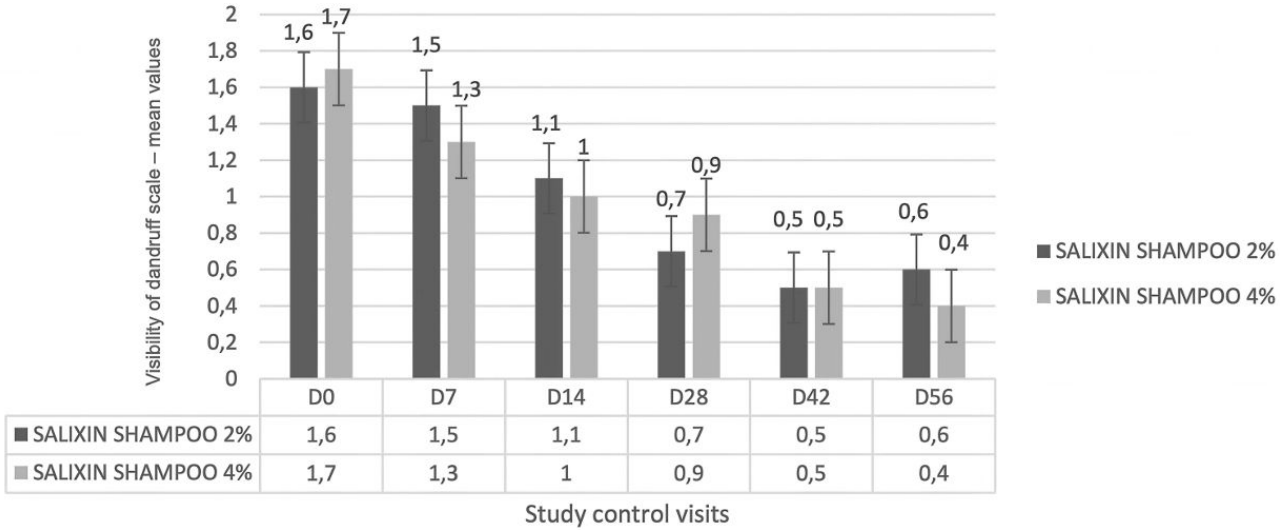

Figure 3 A comparison of mean values for both formulations: Salixin Shampoo 2% and Salixin Shampoo 4%, representing visibility of dandruff clinically assessed in accordance with the scale.

formulations, but the 4% version produces quicker and more substantial results. Redness and sensitive skin appear to be better managed with the 2% formulation, which might be gentler on sensitive skin types.

Salixin Shampoo 4% is more effective for users seeking faster relief and more significant improvements, while Salixin Shampoo 2% may be better suited for individuals with sensitive skin or those prone to initial irritation. It is important to note that while the differences were clearly captured, they were not statistically significant.

Future research could include additional studies on larger populations, the inclusion of a placebo formulation, testing against a traditional dandruff shampoo containing an OTC anti-dandruff active ingredient, and testing in various climates. These strategies could confirm efficacy benefits and improve statistical significance.

Conclusions

The Salixin Shampoo at both the 2% and 4% dose have been shown to substantially reduce dandruff symptoms and the appearance of flaked scalp in dandruff sufferers. The 4% dose shows a faster and more significant reduction in dandruff visibility compared to the 2% concentration, especially noticeable in the first two weeks. However, by day 42, both concentrations achieve similar effectiveness, though the 4% concentration maintains a slightly better long-term control by day 56. This suggests that the higher concentration is more effective in the short term, but both are comparable in long-term dandruff control. These findings indicate that regular use of gentle shampoo containing fermented purple willow bark extract can support scalp health and contribute to a reduction in dandruff symptoms.

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Disclosure

Ms Andrea Gilreath is affiliated with Actera Ingredients Inc. The authors report no other conflicts of interest in this work.

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