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Article

Eco-Friendly Scalp Purifying Serum Made from *Zanthoxylum ailanthoides*

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Abstract: Modern lifestyles impact scalp health as UV radiation, pollution, and stress are increasing. Such factors lead to sensitivity, inflammation, hair loss, and premature graying. To address this, we formulated a plant-based follicle care product to promote scalp and hair health using *Zanthoxylum ailanthoides* (ZL) extract. Its efficacy was evaluated using cell assays, microbial testing, and stability assessments. HaCaT cells were used for viability and inflammation tests, while B16F10 cells were used for melanin production tests. Stability tests were conducted to measure pH, color, and microbial safety. ZL extracts showed no cytotoxicity, increased melanin production, and reduced inflammation. Therefore, a dual-phase product containing ZL was developed, featuring an upper frozen gel layer and a lower serum layer in the scalp serum formulation. The upper frozen gel helps prevent oxidation, preserves the efficacy of active ingredients, and enhances the moisturizing and reparative effects. The product passed temperature stability tests, long-term storage assessments, and microbial activity as a stable and safe formulation. The scalp serum containing *ZL* can be used to address scalp issues, enhance overall scalp health and be improved through future innovations in scalp care and treatments.

Keywords: Scalp health; *Zanthoxylum ailanthoides*; Melanin promotion

1. Introduction

Zanthoxylum ailanthoides (ZL) also known as ailanthus-like prickly ash, belongs to the Rutaceae family and is renowned for its aromatic leaves. It is distributed across China, South Korea, Japan, the Ryukyu Islands, the Philippines, and low-altitude forests in Taiwan. Indigenous Taiwanese communities, such as the Bunun and Atayal, use the fragrant leaves in traditional cuisine. This deciduous tree has thorns on its stems and crowns. Indigenous cultivation such as pruning promotes shoot growth and makes leaf harvesting easy. The cultivated variety has a shrub-like appearance, with pricklier and more pungent leaves. In addition to its culinary use, ZL is used in medicine. The root and stem bark are used to treat rheumatism, viral infections, and colds, containing bioactive compounds like alkaloids, lignans, flavonoids, and triterpenoids [1–3]. The scalp maintains healthy hair by providing a supportive environment for hair follicles. However, an unhealthy scalp, impacted by oxidative stress, pollution, hormonal imbalances, and poor hygiene causes dandruff, seborrheic dermatitis, psoriasis, and hair loss. Scalp aging is driven by intrinsic factors including genetics, which contribute to hair thinning and graying, and extrinsic factors such as UV radiation and environmental damage. Over time, the scalp's capacity to retain moisture, collagen, and proper blood flow diminishes, resulting in hair graying and structural damage. Medicated shampoos, antioxidant-rich serums, moisturizing oils, and supplements are used for restoring scalp health and reducing the effects of aging [4–7]. Oily scalp, dandruff, and hair loss are common issues closely associated with scalp health. The scalp's invisible microbial ecosystem contributes to such hair-related problems. Currently, there are limited plant-based products formulated for scalp care. Therefore, we have developed a scalp serum containing ZL extract, to improve scalp conditions and promote hair darkening and overall scalp health.

2. Materials and Methods

2.1. ZL Extraction

The extract of ZL is obtained using two methods. In water extraction, the stems and deionized water are mixed at a 1:8 to 1:10 weight ratio and extracted ultrasonically at a power of 300–600W at 4°C for 90 min. In ethanol extraction, 100 g of stems are soaked in ethanol of five times more volume at low temperature, followed by ultrasonic extraction. In both methods, the supernatant is obtained through high-speed centrifugation and vacuum filtration and can be concentrated.

2.2. Cytotoxicity of ZL Extract on Skin Cell Lines

Based on the safety evaluation of the water extract (ZLSW) and ethanol extract (ZLSE) from ZL stems, human keratinocyte cell line (HaCaT) and murine melanoma cells (B16F10) were used to measure cell viability at different concentrations. The tested concentrations were 20, 50, 100, 250, 500, and 1000 μg/ml, and cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay after 24 hours of treatment.

2.3. Melanin Content in B16F10 Cells Treated with ZLSE and α-MSH

B16F10 melanoma cells were seeded in 96-well culture plates and incubated overnight to allow cell attachment. The cells were then exposed to different concentrations of ZLSE (100, 250, and 500 μg/ml) for 72 h, with or without 100 nM α-melanocyte-stimulating hormone (α-MSH). At the end of the treatment, the supernatant was collected, and the absorbance at 400 nm was measured using an enzyme-linked immunosorbent assay (ELISA) reader.

2.4. Inflammatory Cytokines of ZL Extract on Skin Cell Lines

The secretion of TNF-α and IL-1β was measured using ELISA, following the manufacturer's protocols (BD OptEIA™ Human TNF-α ELISA set and Human IL-1β ELISA set, BD Biosciences, San Jose, CA, USA). Briefly, a 96-well plate was coated with capture antibodies at 4°C overnight, washed, and blocked. Samples and standards were pipetted into the wells in duplicates and incubated for 2 h at room temperature. After incubation, the plates were washed, and detection antibodies along with appropriate solutions were applied. The signal was developed, and absorbance was measured at 450 nm with a 570 nm correction. The total release of TNF-α and IL-1β in the culture media was quantified.

2.5. Preparation of ZL Shine Scalp Purifying Serum

The ZL Shine Scalp Purifying Serum was made in this study (Fig. 1). Its upper layer consists of dimethicone, cyclopentasiloxane, dimethicone/vinyldimethicone crosspolymer, squalane, and isononyl isononanoate. The lower layer contains water, glycerin, ZLSW, 1,3-butylene glycol, sodium hyaluronate, and xanthan gum. After separately preparing the two layers, they are mixed into a bottle. When inverted or lightly shaken without external force, the oil and liquid layers do not mix. The upper layer, containing silicone polymers, not only protects the plant extracts in the lower layer from degradation or contamination but also forms a thin film on the skin when applied. This film protects the skin, prevents moisture loss, and provides nourishing and reparative benefits.

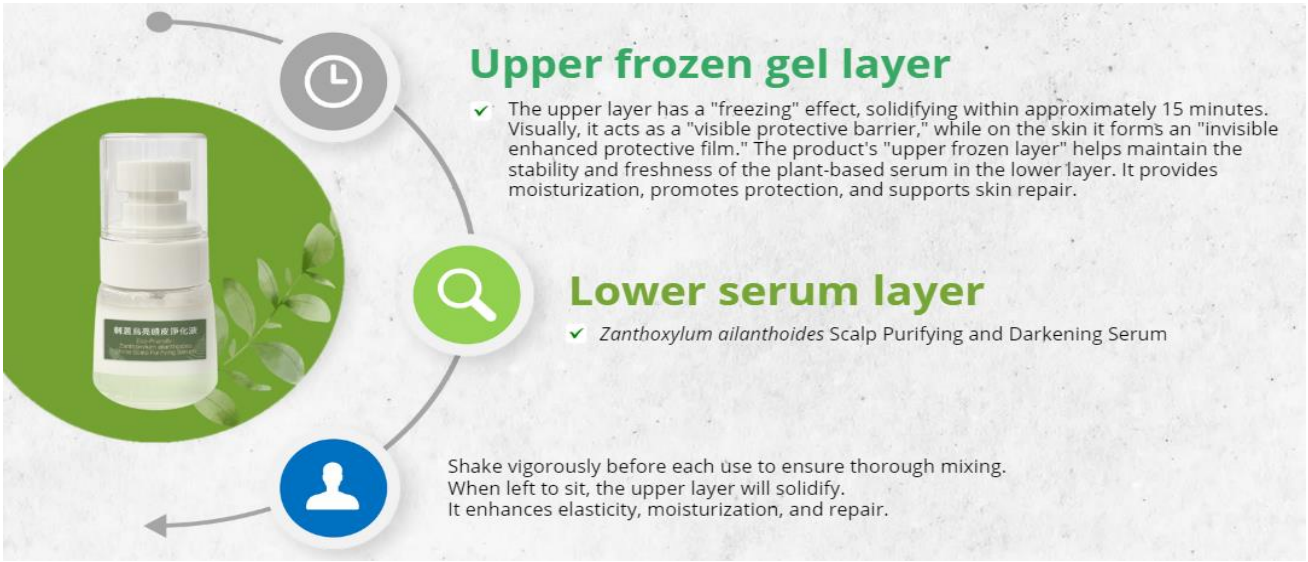

Figure 1. ZL shine scalp purifying serum.

2.6. Physical Parameters and Stability

The physical stability of the ZLSW scalp serum was assessed under various storage conditions. The serum was stored at 25 ± 2 °C for 6 months, as well as at 4 ± 2 °C and 45 ± 2 °C for 30 days. Key parameters such as pH, color, odor, and consistency were monitored throughout the period to detect any signs of instability or degradation. In addition, microbial testing was conducted regularly during the period to ensure the serum remained free from contamination, confirming the overall stability of the formulation. Microbial assessments were conducted for total aerobic plate count, and the absence of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans.*

3. Results

3.1. Extraction Yields of ZLSW and ZLSE

After washing the stems of ZL with clean water, they were air-dried in the shade. Dried samples of 100 g each from the stems and leaves were separately subjected to ZLSW and ZLSE. The resulting extracts were concentrated by vacuum evaporation to remove excess solvent, followed by freeze-drying for 48 h. The dried extracts were then weighed, and the extraction yield was calculated by dividing the weight of the dried extract by the original dry weight. The extraction yield of ZLSE was 3.2%, while the yield with the extraction yield of ZLSW was 6.6%. These results indicated that deionized water extraction consistently yielded more product than ethanol extraction, suggesting that the compounds in ZL are water-soluble.

3.2. Cell Viability of ZL

The cell viability assay was conducted using human keratinocyte cells (HaCaT) and mouse melanoma cells (B16F10). HaCaT cells were exposed to different concentrations of the extracts (20, 50, 100, 250, 500, and 1000 μg/ml) for 24 h, while B16F10 cells were treated for 72 h. Cell viability was measured using the MTT assay. When treated with ZLSW at a concentration of 20 μg/ml, both HaCaT and B16F10 cells maintained a viability rate above 80%, with HaCaT cells showing viability close to 100%, similar to the control group (Fig. 2(A)). In contrast, when treated with ZLSE at concentrations higher than 250 μg/ml, the viability of both HaCaT and B16F10 cells dropped below 80%, indicating cytotoxicity. Based on the overall cytotoxicity results, ZLSW demonstrated tolerance in HaCaT and B16F10 cells, whereas ZLSE showed significant cytotoxic effects at high concentrations (>250 μg/ml).

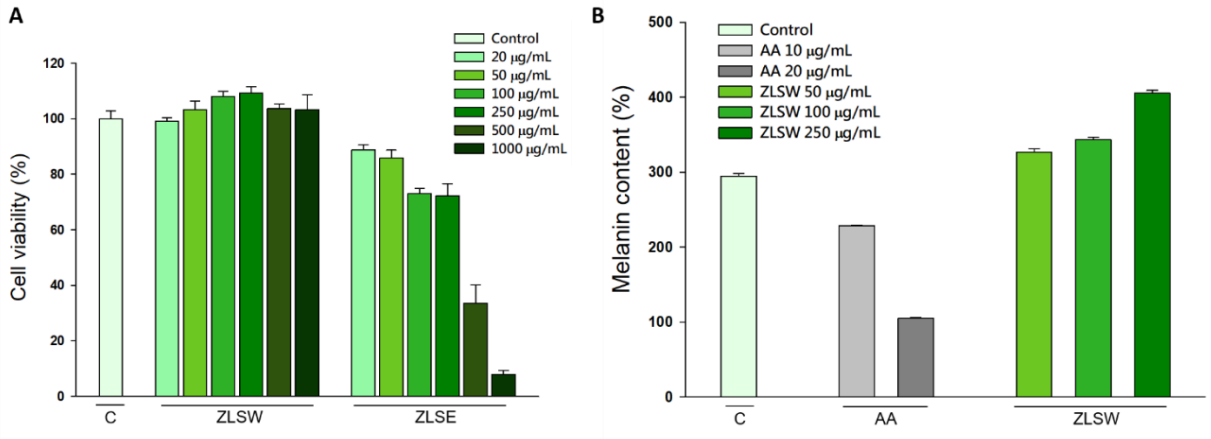

Figure 2. The effect of ZLSW and ZLSE on cell viability and melanin synthesis in skin cells. (**A**) Cell viability of HaCaT keratinocytes after 24 hours of treatment with varying concentrations of the extracts (20, 50, 100, 250, 500, and 1000 μg/ml), was evaluated using the MTT assay. (**B**) Melanin synthesis in B16F10 melanoma cells after 72 hours of exposure to ZLSW (50, 100, and 250 μg/ml) and ascorbic acid (AA), measured by the melanin content assay. Data are presented as mean ± SD from three independent experiments.

3.3. Melanin Content of ZLSW Treatment on B16F10 Cells

B16F10 mouse melanoma cells were treated with α-MSH (100 nM) for 24 hours, followed by the addition of ZLSW at concentrations of 50, 100, 250, and 500 μg/ml for 72 h. After treatment, intracellular melanin content was measured. As shown in

Fig. 2(B), ZLSW significantly increased melanin content, with an increase of 405.6% at the concentration of 250 μg/ml compared to the α-MSH-treated group alone, suggesting that ZLSW promotes melanin production.

3.4. Lipopolysaccharide (LPS)-Induced TNF-α and IL-1β Expression in HaCaT Cells Treated with ZLSW

Upon LPS stimulation, the expression of TNF-α in HaCaT cells significantly increased, reaching 161.41%, while IL-1β expression levels also rose to 128.57%. These results indicate that LPS effectively promotes the release of inflammatory cytokines in HaCaT cells, strongly activating the cellular inflammatory response. However, when the cells were treated with 250 μg/ml of ZLSW, the expression of TNF-α decreased to 138.04%, and IL-1β to 111.90%, compared with the LPS-treated group. These findings demonstrated that ZLSW at a concentration of 250 μg/ml significantly suppressed the inflammatory response induced by LPS, particularly by inhibiting the release of the key inflammatory cytokines TNF-α and IL-1β. Overall, ZLSW exhibited potent anti-inflammatory potential, modulating the LPS-induced inflammatory response by reducing the release of these pro-inflammatory cytokines.

3.5. Stability and Microbial Assessment of ZLSW Scalp Serum

The physicochemical properties of the ZLSW scalp serum were evaluated (Table 1), showing that the appearance consisted of an upper layer with a white haze and a lower layer with a light green color. The serum had a fruity odor, and the pH was measured at 5–7. Stability tests were conducted under various conditions: storage at 25°C for 6 months, 4°C for 1 month, and 45°C for 1 month. In all cases, no changes were observed in the formulation, indicating physical stability. Microbial testing included assessing for total aerobic plate count, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida albicans. All microbial test results were negative, confirming that the product remained free of microbial contamination. Such results demonstrated that the ZLSW scalp serum maintained stability and resisted microbial contamination in a variety of conditions, ensuring both safety and efficacy.

Table 1. Specifications of ZL Shine Scalp Purifying Serum

Item		Result
Physicochemical Properties	Appearance	Upper layer - white haze / Lower layer - light green
	Odor	Fruity
	pH	5~7
Stability Testing	25°C for 6 months 4°C for 1 month 45°C for 1 month	No change in appearance
Microbial Testing	Total Aerobic Plate Count	<1000 cfu/mL
	Escherichia coli	Negative
	Pseudomonas aeruginosa	Negative
	Staphylococcus aureus	Negative
	Candida albicans	Negative

4. Discussion

ZL is one of the *Zanthoxylum* genus, known for its medicinal and pharmacological applications. Phytochemicals from *Zanthoxylu*m species, such as alkaloids, flavonoids, terpenoids, and lignans have the potential for various biological activities, including anti-cancer, antimicrobial, antifungal, and antiviral properties. These compounds are secondary metabolites, which plants produce in response to environmental stresses or pathogens [8]. For skin, ZL's compounds including flavonoids and phenolic acids have anti-inflammatory and antioxidant properties. These compounds reduce oxidative stress, a key factor in skin aging, and maintain the skin's health by protecting it from free radical damage. Several species within this genus exhibit antimicrobial properties which prevent skin infections [9]. Plant-derived bioactive compounds have significant potential to improve scalp health by promoting hair growth and modulating scalp conditions. Healthy scalp conditions are maintained through proper hydration, reduced inflammation, and a balanced microbiome, creating an environment where hair follicles thrive. Compounds such as polyphenols, flavonoids, and terpenoids, commonly found in green tea, chamomile, rosemary, and tea trees, are known for their anti-inflammatory, antioxidant, and antimicrobial properties. Flavonoids, for instance, reduce oxidative stress, which is a key factor in scalp aging and hair loss [10]. These compounds also sustain scalp health by soothing irritation, reducing redness, and controlling dandruff. Bioactive compounds also interact with microorganisms including Malassezia which causes dandruff and seborrheic dermatitis. These compounds disrupt microbial cell walls, inhibit biofilm formation, and interfere with microbial signaling

pathways, ultimately promoting a healthier scalp environment. Furthermore, terpenoids, present in essential oils, regulate the scalp microbiome, inhibiting harmful microbial growth and promoting hair follicle health. Other compounds such as alkaloids, found in plants such as Zanthoxylum, offer antimicrobial and anti-inflammatory that prevent infections and scalp irritation, and enhance hair follicle health. Saponins, present in ginseng, stimulate blood circulation, ensuring adequate nutrient delivery to hair follicles, leading to stronger and healthier hair [11,12].

The developed dual-phase scalp serum containing ZL extract in this study addresses common scalp health issues such as sensitivity, inflammation, and hair loss. The ZL extract exhibited significant anti-inflammatory and melanin-promoting effects without cytotoxicity, while the innovative dual-phase formulation enhanced stability and preservative benefits. Its proven stability and microbial safety allow for long-term use. The ZLSW-based scalp serum is useful in scalp care as it promotes overall scalp health as a plant-based scalp treatment solution. Future studies, including clinical trials, are essential to confirm the efficacy of the serum.

5. Conclusions

The developed ZL shine scalp purifying serum can be used to improve both scalp and hair health. The extraction process using deionized water was more effective than using ethanol. ZLSW showed no cytotoxicity, increased melanin production, and reduced inflammatory cytokine levels in cell-based assays. The dual-phase formulation with an upper frozen gel layer and a lower serum layer not only preserves the efficacy of the active ingredients but also enhances the product's moisturizing and reparative properties. Furthermore, the ZL scalp serum maintains its safety and effectiveness in various storage conditions. The serum is a promising plant-based solution for scalp care and can be used for future innovations in treating scalp conditions to enhance overall scalp health.

6. Patents

1. Republic of China (Taiwan) Invention Patent. Certificate Number: I789749. Title of Invention: *Zanthoxylum ailanthoides* Extract for Antioxidant, Anti-inflammatory, and Melanin Synthesis Promotion.

2. Republic of China (Taiwan) Invention Patent. Certificate Number: I818215. Title of Invention: Elastic Gel Composition with Protective Function and Preparation Methods for the Same.

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Conflicts of Interest: The authors declare no conflict of interest.

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