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Unlocking Intradermal Permeability: The Efficacy of *Selaginella tamariscina*-derived Polysaccharides

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1. Introduction

In today's cosmetics and personal care market, consumers increasingly demand products that are both effective and safe. Active ingredients such as α-arbutin and ergothioneine are widely incorporated into skincare formulations due to their notable physiological benefits. α-Arbutin functions primarily by inhibiting tyrosinase activity, thereby reducing melanin synthesis and effectively lightening hyperpigmentation[1]. It is frequently used in brightening and anti-spot treatments. Ergothioneine, a naturally occurring antioxidant, exhibits multiple biological functions, including free radical scavenging, detoxification, support of DNA biosynthesis, promotion of normal cell growth, and enhancement of cellular immunity[2,3]. These properties contribute significantly to delaying skin aging and improving overall skin health[4]. However, the hydrophilic nature of these active ingredients limits their dermal permeability, thereby restricting their retention and bioavailability within the skin[1,2]. Enhancing the skin penetration of hydrophilic actives thus remains a critical challenge in cosmetic research and development[5].

To address this challenge, various penetration-enhancement strategies have been developed. Current approaches include chemical, physical, and biological enhancement techniques[6]. Chemical enhancers such as propylene glycol and azone are widely used due to their convenience and cost-effectiveness; nonetheless, they are often associated with adverse effects such as skin irritation and potential safety concerns[7]. Physical methods, including microneedles and ultrasound, can effectively increase skin permeability but are complex to operate and may cause mechanical damage to the skin[5,8,9]. In contrast, biological enhancers—derived from natural sources such as plant extracts—have gained attention in recent years due to their biocompatibility and low toxicity, making them attractive candidates for safer and more sustainable applications[10–12].

Plant-derived bioactive compounds, including polysaccharides, flavonoids, and alkaloids, are known for their antioxidant and anti-inflammatory properties, as well as their ability to modulate skin physiology[13]. Notably, polysaccharides can interact with keratin in the stratum corneum to increase hydration, leading to swelling of the

skin barrier and improved permeability[14]. Additionally, they can form a protective film on the skin surface, reducing the loss of active compounds and enhancing their retention[15]. Despite the promise of such compounds, research on plant-derived penetration enhancers is still in its infancy. Most existing studies focus on the screening and preliminary applications of single extracts, while comprehensive investigations into synergistic combinations and underlying enhancement mechanisms remain limited.

Several studies have demonstrated the potential of plant extracts to improve transdermal delivery. For instance, Liston et al. reported that aloe vera extract significantly enhances the skin permeability of water-soluble vitamin C, achieving efficacy comparable to chemical enhancers[16]. Liao et al. found that catechins in green tea extract increase the skin retention of niacinamide, boosting its anti-aging effects[17]. These findings provide strong theoretical support for the application of botanical extracts as transdermal enhancers in cosmetic formulations.

Nonetheless, several challenges remain. First, studies on the efficacy and mechanisms of different plant extracts are neither systematic nor comprehensive, with few comparative analyses available]. Second, existing research primarily focuses on enhancing the delivery of single active compounds, while little is known about the interactions and collective effects when multiple actives are present. Finally, integrating natural plant-based enhancers into modern cosmetic formulation technologies to create practically viable products remains a pressing issue.

Against this background, the present study aims to develop a natural, safe, and effective transdermal enhancer to improve the skin permeability and retention of hydrophilic active ingredients, thereby reducing reliance on chemical enhancers. Through screening various botanical extracts, *Selaginella tamariscina* extract was identified for its significant ability to enhance ergothioneine permeability. The study further optimizes its extraction process and investigates its enhancement mechanisms. Additionally, a synergistic formulation combining *Selaginella tamariscina* extract with *Alhagi sparsifolia* extract and β-glucan is developed to enable the efficient transdermal delivery of hydrophilic actives such as ergothioneine and α-arbutin. This work seeks to offer a novel, sustainable strategy for cosmetic penetration enhancement and to support the development of greener, safer, and more effective skincare solutions.

2. Materials and Methods

2.1 Materials

2.1.1 Plant materials

Dried whole‐plant aerial parts of *Selaginella tamariscina* (P. Beauv.) Spring were supplied by Anhui Bozhou QianCao National Pharmaceutical Co., Ltd. (Lot YC2211020; Linyi, Shandong, China). Dried herbs of *Alhagi sparsifolia* Shap., *Coreopsis tinctoria* Nutt. (snow chrysanthemum), and the red seaweed *Kappaphycus alvarezii* were purchased from commercial suppliers.

2.2 Preparation of Extracts

2.2.1 Preparation of Selaginella tamariscina Extracts

Sample STW: 100 g of powdered *S. tamariscina* was extracted with 70% ethanol at a solvent-to-material ratio of 15:1 (v/w) under reflux at 80 °C for 3 hours. This extraction was performed three times. The combined filtrates were concentrated under reduced pressure to obtain a crude extract, which was dispersed in distilled water and subsequently partitioned with ethyl acetate and *n*-butanol. The remaining aqueous phase was concentrated and vacuum-dried to yield sample STW.

Sample STA: 100 g of powdered *S. tamariscina* was extracted with 70% ethanol (15:1, v/w) at 80 °C for 3 hours, repeated three times. The combined filtrates were concentrated under reduced pressure to yield a crude extract. This extract was purified using AB-8 macroporous resin and eluted with 10% ethanol. The eluate was concentrated and dried under vacuum to obtain sample STA.

2.2.2 Preparation of Other Plant Extracts

Aqueous extracts of *Alhagi sparsifolia* Shap. (Sample AS), *Coreopsis tinctoria* Nutt. (Sample CT, commonly known as snow chrysanthemum), and *Kappaphycus alvarezii* (Sample KA, a red seaweed) were prepared using a standardized extraction protocol. Specifically, 200 g of powdered plant material was extracted with 2000 mL of purified water at 80 °C for 2 hours. The mixture was filtered, and the residue was subjected to a second extraction under identical conditions. The combined filtrates were concentrated to 200 mL, centrifuged, and the resulting supernatant was mixed with 800 mL of absolute ethanol. After standing for 12 hours, the precipitate was collected by filtration and dried at 60 °C to obtain the corresponding plant extract.

2.3 Determination of Total Carbohydrate Content and Reducing Sugar Content

2.3.1 Total Carbohydrate Content

Total carbohydrate content was determined using the phenol–sulfuric acid method. A 1 mg/mL glucose stock solution was prepared by dissolving 10 mg glucose in 10 mL distilled water, and working standards (0.0125–0.2 mg/mL) were prepared by serial dilution. To each test tube, 1.0 mL of standard or sample solution (0.1 mg/mL extract) was added with 0.5 mL 6% (w/v) phenol and 3.0 mL concentrated H_2SO_4. After vortexing, the tubes were heated in a boiling-water bath for 20 min, cooled, and 200 µL aliquots were transferred to a 96-well plate. Absorbance at 490 nm was recorded, and total sugar concentration was determined from the standard curve. The sugar content was calculated as:

$$W = \frac{(c \times v)}{m} \times F \times 100\%$$

where cc is the concentration (mg/mL), vv is the sample volume (mL), mm is the sample mass (g), FF is the dilution factor, and 0.9 is the glucose-to-polysaccharide conversion factor.

2.3.2 Determination of Monosaccharides and Polysaccharides by HPLC

For oligosaccharides and free sugars, the sample was weighed, diluted to 10 mL with water, and ultrasonicated for 30 min. After centrifugation, the supernatant was filtered and analyzed by HPLC using an Agilent amino column (250 × 4.6 mm, 5 μm) at 35°C, with a mobile phase of acetonitrile:water (70:30, v/v) at 1.0 mL/min. The injection volume was 10 μL, and detection was performed using a refractive index detector.
For reducing sugars, the sample was hydrolyzed with 72% sulfuric acid, followed by neutralization and derivatization with PMP at 70°C for 60 min. The reaction mixture was extracted with chloroform, and the aqueous phase was filtered before HPLC analysis. Chromatographic separation was performed on a C18 column (4.6 × 250 mm, 5 μm) at 25°C, with a gradient mobile phase consisting of 15% and 40% acetonitrile (in 0.05 mol/L KH_2PO_4, pH 6.8). The flow rate was 1.0 mL/min, with an injection volume of 20 μL and detection at 254 nm.

2.4 In Vitro Skin Permeation Study

Porcine ear skin (<6 months old) was procured from a local abattoir, stored at 4 °C, and used within 24 h. Surface hairs were gently shaved and residual fuzz removed with depilatory cream. Subcutaneous fat and connective tissue were excised; skin was rinsed with saline, cut into 1.54 cm² pieces, and stored at –20 °C.

Permeation experiments used Franz diffusion cells (donor and receptor chambers separated by skin; stratum corneum facing the donor; effective area 1.54 cm²). Receptor phase was PBS (pH 7.4), maintained at 32 °C and stirred at 350 rpm. Donor chambers received 1 mg/mL solutions of α-arbutin or ergothioneine. Aliquots (200 μL) of receptor fluid were withdrawn at 2, 4, 8, 12, and 24 h (replaced immediately with fresh PBS) .Chromatographic separation and quantification of α-arbutin and ergothioneine in the receptor samples were carried out on an HPLC system using a mobile phase of water and acetonitrile (38:62, v/v) at a constant flow rate of 1.0 mL/min; analyte detection was performed at 254 nm, with the column temperature maintained at 30 °C.
After 24 h, skin samples were embedded in 10% (w/v) CMC-Na, cryosectioned into stratum corneum (0–20 μm), epidermis (20–120 μm), and dermis (>120 μm). Each layer was weighed, homogenized by sonication in an appropriate solvent, and the extracted analytes quantified by HPLC.

3. Result

3.1 Retention of Ergothioneine in Skin

As shown in Figure 1 and Table 1, all plant extracts tested enhanced the skin retention of ergothioneine relative to the water control (2.577 ± 0.091 μg). Notably, the *Selaginella tamariscina* extract (STW) exhibited the strongest permeation-enhancing effect, increasing ergothioneine retention to 10.041 ± 1.75 μg—an approximate 290% gain over the control. The *Alhagi sparsifolia* extract (AS) also produced a significant improvement, raising retention to 5.478 ± 1.31 μg (112.6% increase). These results identify STW as the most potent enhancer of ergothioneine skin uptake, with AS showing a secondary but still significant effect.

Table 1. Retention of Ergothioneine in Skin Promoted by Various Plant Extracts (x ± SD, n = 3)

Sample	Skin retention amount（μg）	Relative retention growth rate (%)
Water	2.577±0.091	/
STW	10.041±1.75	289.63
AS	5.478±1.31	112.56
KA	4.659±0.75	80.80
CT	3.880±0.56	50.57

As shown in Figure 2, STW markedly outperformed both pure trehalose and the STA in promoting ergothioneine retention. STW increased skin retention to 10.04 ± 0.96 μg—an approximately 290% (Table 2) gain over the water control—whereas trehalose and STA yielded only 5.10 ± 0.61 μg (97.8% increase) and 5.51 ± 0.96 μg (113.9% increase), respectively. This demonstrates that STW's enhancement cannot be attributed solely to its trehalose content.

To probe the underlying cause, total sugar and r**educing sugar** analyses were performed (Table 3). STW exhibited the highest total glucose content, which correlated linearly with permeation enhancement and delivered a maximum relative retention. **Oligosaccharides** profiling revealed that trehalose accounted for only ~4% of STW's carbohydrate pool, and free glucose was initially undetectable—rising to ~68% only after acid hydrolysis—indicating that its carbohydrates exist predominantly as polysaccharides. In contrast, STA contained ~4% free glucose pre-hydrolysis and reached only ~49% glucose post-hydrolysis, reflecting a lower polysaccharide enrichment.

Taken together, these data confirm that the superior permeation-enhancing performance of STW derives from its high polysaccharide content. Consequently, the aqueous extraction protocol represented by STW affords an optimal *S. tamariscina* extract for improving the dermal delivery of hydrophilic actives.

Table 2. Retention of Ergothioneine in the Skin Promoted by Different Selaginella Extracts (x ± SD, n = 3)

Sample	Skin retention amount（μg）	Relative retention growth rate (%)
Water	2.577±0.091	/
Trehalose	5.097±0.61	97.77
STW	10.041±0.96	289.63
STA	5.512±0.96	113.88

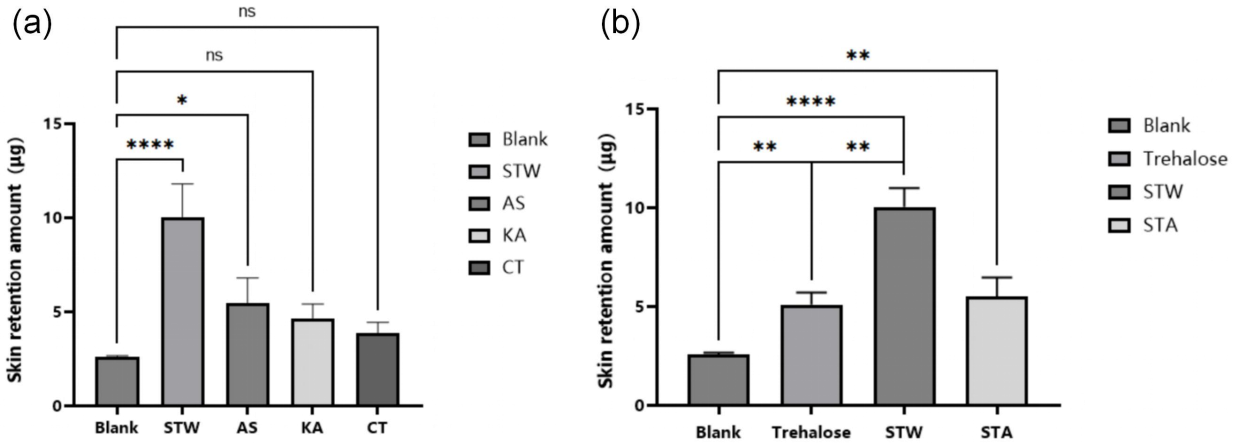

Figure 1. HPLC analysis of Skin retention amount of Ergothioneine after a 24-hour permeation test. (a)Various Plant Extracts, AS,(*Alhagi sparsifolia* Shap), CT,(*Coreopsis tinctoria* Nutt.), and KA,(*Kappaphycus alvarezii*); (b) Different Selaginella Extracts. The data are expressed as the means ± SDs (n ≥ 3). One-way ANOVA was used to determine statistical significance (*p < 0.05; **p < 0.01; no significant difference (ns) p > 0.05).

Table 3. Total carbohydrate content and individual carbohydrate composition of *Selaginella tamariscina* extracts obtained by different extraction methods.

Component		Unit	STW	STA
Total sugar		g/100g	**70.47**	**51.15**
Oligosaccharides	Trehalose	g/100g	4.72	4.54
	Stachyose	g/100g	NA	NA
	Raffinose	g/100g	NA	NA
Reducing sugar (Monosaccharides)	Guluronic acid	mg/kg	NA	NA
	Mannuronic acid	mg/kg	1490.98	7479.61
	Mannose	mg/kg	2361.57	798.43
	Glucosamine	mg/kg	1546.27	857.25
	Ribose	mg/kg	783.14	345.10
	Rhamnose	mg/kg	1565.49	1635.29
	Glucuronic acid	mg/kg	NA	NA
	Galacturonic acid	mg/kg	70616.47	52523.14
	Galactosamine	mg/kg	NA	NA
	Glucose	mg/kg	685990.59	496058.04
	N-Acetylgalactosamine	mg/kg	NA	NA
	Galactose	mg/kg	4260.00	1545.49
	Xylose	mg/kg	546.27	245.49
	Arabinose	mg/kg	4157.25	1887.84
	Fucose	mg/kg	230.59	NA
Free sugars	Fructose	g/100g	NA	NA

	Glucose	g/100g	NA	4.13
	Sucrose	g/100g	12.16	11.44
	Maltose	g/100g	NA	NA
	Lactose	g/100g	NA	NA

To further enhance the permeation‐boosting effect of the *S. tamariscina* extract (STW), we investigated ternary formulations combining STW with *A. sparsifolia* extract (AS) and β-glucan at varying ratios (Fig. 4). Three candidate blends were prepared, each containing 0.3% STW, with AS at 0.1, or 0.2%, and β-glucan fixed at 0.05%. In the permeation study of 0.1% ergothioneine , the formulation STW 0.3% + AS 0.2% + β-glucan 0.05% exhibited the greatest enhancement, elevating skin retention by 264% relative to the control (Water) group after 24 h (Appendix Table 1). When the ergothioneine concentration was increased to 0.3%, the same optimized blend delivered a 529% increase in skin retention and a 145.46% rise in cumulative permeation compared with control (Appendix Table 2). These results clearly demonstrate that the combination of STW, AS, and β-glucan acts synergistically, substantially improving both the retention and transdermal delivery of ergothioneine beyond what is achievable with any single enhancer.

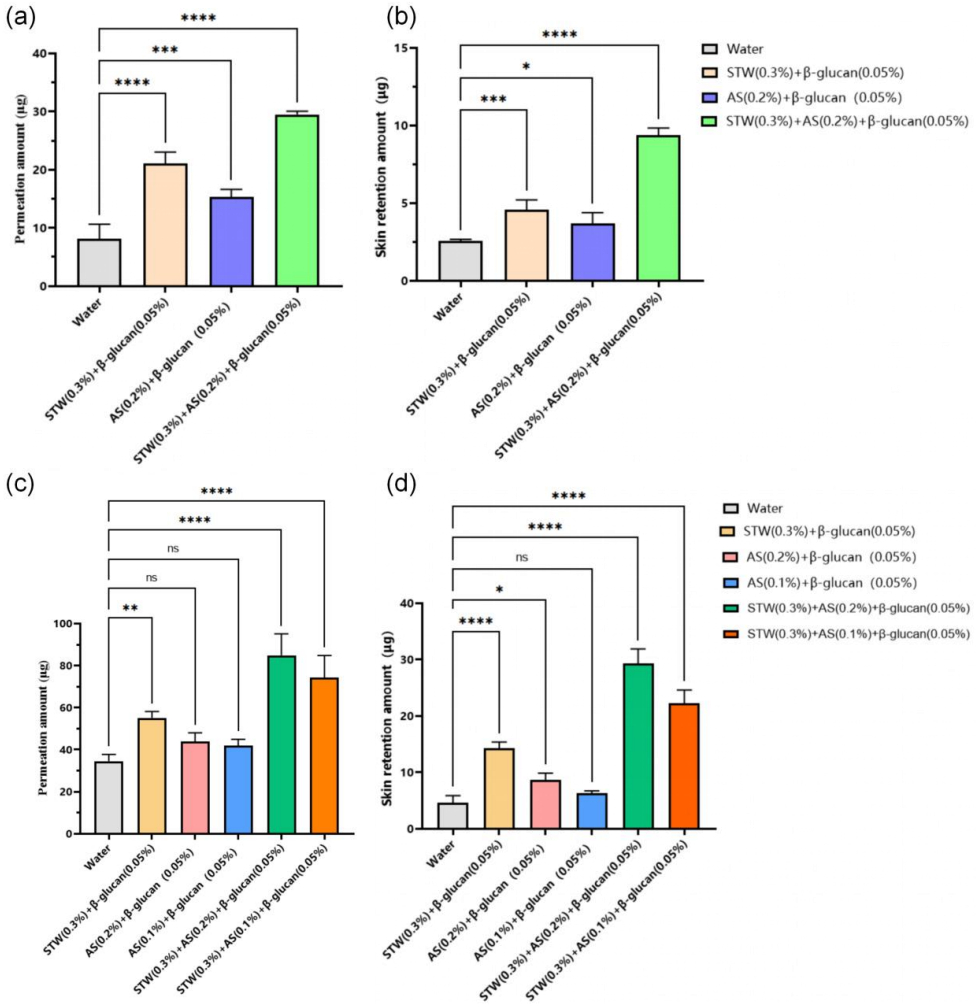

Figure 2. HPLC analysis of ergothioneine delivery after 24 h in various composite formulations. (a)Cumulative permeation of 0.1 % ergothioneine;(b) Skin retention of 0.1 % ergothioneine;(c)Cumulative permeation of 0.3 % ergothioneine;(d) Skin retention of 0.3 % ergothioneine. The data are expressed as the means ± SDs (n ≥ 3). One-way ANOVA was used to determine statistical significance (*p < 0.05; **p < 0.01; no significant difference (ns) p > 0.05).

3.2 Retention of α-Arbutin in skin

α-Arbutin, a widely used hydrophilic whitening agent, also suffers from poor dermal penetration. To address this, we evaluated the effects of STW, AS, and β-glucan combinations at various mass ratios on α-arbutin skin delivery. As shown in Figure 4, all ternary formulations significantly increased α-arbutin retention compared to the water control. Notably, the blend containing 0.2% STW + 0.2% AS + 0.1% β-glucan achieved the greatest enhancement, elevating skin retention by 42.40% after 24 h. Concurrently, HPLC analysis demonstrated that this optimized composition significantly reduced α-arbutin's cumulative permeation (Appendix Table 3), favoring its localization within the epidermal basal layer and potentially improving its bioavailability. These results indicate that the STW–AS–β-glucan ternary system can effectively modulate the skin distribution of α-arbutin, offering a promising strategy to enhance the topical efficacy of hydrophilic cosmetic actives.

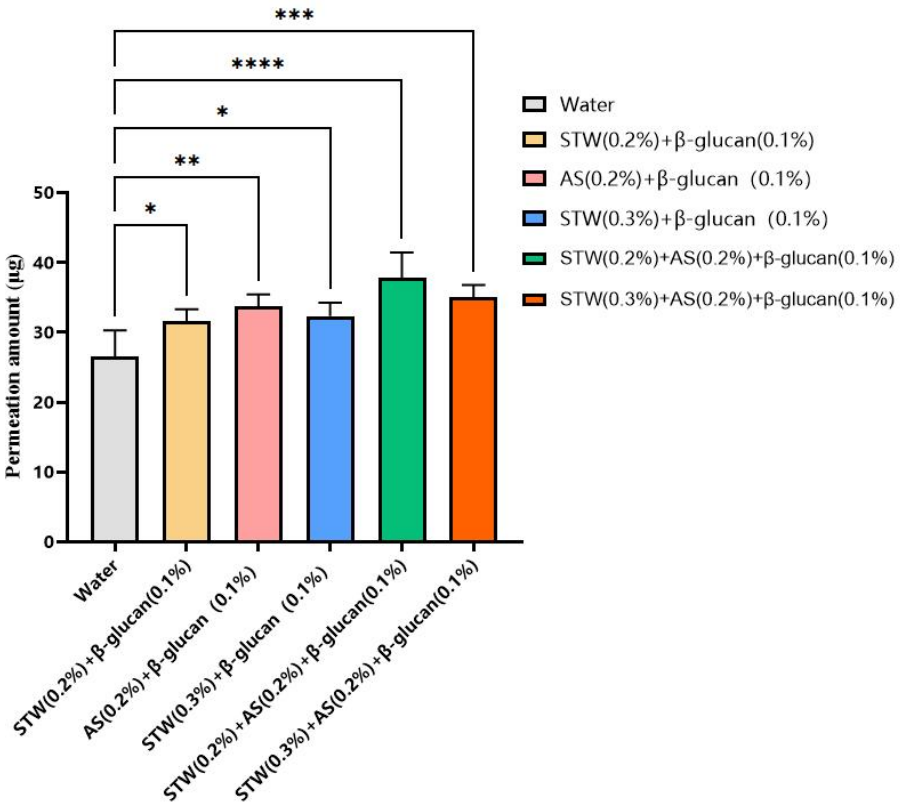

Figure 3. HPLC analysis of Skin retention amount of α-arbutin after 24 h in various composite formulations. The data are expressed as the means ± SDs (n ≥ 3). One-way ANOVA was used to determine statistical significance (*p < 0.05; **p < 0.01; no significant difference (ns) p > 0.05).

4. Discussion

Screening of various botanical extracts revealed that the aqueous *Selaginella tamariscina* extract (STW) significantly enhances the dermal retention of ergothioneine, achieving an approximately 290% increase over the water control. Monosaccharide profiling and total sugar quantification demonstrated that STW is highly enriched in polysaccharides, with free glucose only detectable after acid hydrolysis. This high polysaccharide content correlates strongly with permeation enhancement, likely by increasing stratum corneum hydration, forming a semi-occlusive film, and thereby facilitating the diffusion of hydrophilic actives. The negligible effect of pure trehalose compared to STW further confirms that the complex polymeric carbohydrates in STW, rather than any single sugar, drive its permeation‐boosting performance.

Building on this observation, ternary formulations combining STW, *Alhagi sparsifolia* extract (AS), and β-glucan were evaluated for synergistic effects. The optimized blend (0.3% STW + 0.2% AS + 0.05% β-glucan) increased ergothioneine skin retention by 529% and cumulative permeation by 145.5% in the 0.3% ergothioneine model, far surpassing single-component systems. AS likely contributes additional polysaccharides and proteinaceous constituents that interact with STW's polymers, while β-glucan enhances film formation and barrier modulation through its humectant properties. Importantly, in α-arbutin studies, this blend elevated epidermal retention by 42.4% while reducing transdermal losses, demonstrating that adjustments in enhancer type and ratio can precisely tune the balance between skin localization and permeation. Together, these findings establish that high-polysaccharide extracts and their synergistic combinations offer a customizable, natural approach to improving the topical delivery of hydrophilic cosmetic actives.

5. Conclusion

This work has developed a polysaccharide-based penetration enhancement strategy that effectively increases the skin retention and controlled permeation of hydrophilic actives such as ergothioneine and α-arbutin. The superior performance of the aqueous *S. tamariscina* extract underscores the pivotal role of high‐molecular-weight carbohydrates in modulating the skin barrier, while synergistic combinations with *A. sparsifolia* extract and β-glucan further amplify both retention and permeation control. By optimizing enhancer composition and ratio, we achieved up to a 529% increase in ergothioneine retention and a selective enhancement of α-arbutin localization. This natural, safe, and sustainable formulation approach promises to reduce reliance on chemical enhancers and to advance the development of greener, more effective cosmetic and transdermal products. Future studies will elucidate the molecular interactions underpinning these effects, validate in vivo efficacy, and explore broader applications across diverse skin types and active ingredients.

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Supplementary

Appendix Table 1. Retention of 0.1% ergothioneine in different skin layers and cumulative permeation for various composite formulations after 24 h (x ± SD, n = 4).

Sample	Corneum retention amount （μg）	Epidermis retention amount （μg）	Dermis retention amount （μg）	Whole skin retention amount （μg）	skin retention amount （μg）	Permeation amount（μg）	Relative retention growth rate (%)	Relative Permeation growth rate (%) （%）
Water	0.354±0.197	0.751±0.475	1.825±0.690	2.930±0.244	2.577±0.091	8.119±2.545	/	/
STW(0.3%)+β-glucan(0.05%)	0.401±0.083	1.021±0.235	3.554±0.392	4.98±0.71	4.58±0.63	21.121±1.942	77.73	160.14
AS(0.2%)+β-glucan（0.05%）	0.372±0.077	0.944±0.312	2.733±0.402	4.05±0.79	3.68±0.71	15.311±1.327	42.80	88.58
STW(0.3%)+AS(0.2%)+β-glucan(0.05%)	0.490±0.136	1.200±0.280	4.912±0.518	6.607±0.522	9.372±0.469	29.527±0.539	263.68	263.68

Appendix Table 2. Retention of 0.3% ergothioneine in different skin layers and cumulative permeation for various composite formulations after 24 h (x ± SD, n = 4).

Sample	Corneum retention amount （μg）	Epidermis retention amount （μg）	Dermis retention amount （μg）	Whole skin retention amount （μg）	skin retention amount （μg）	Permeation amount（μg）	Relative retention growth rate (%)	Relative Permeation growth rate (%) （%）
Water	0.447±0.343	0.569±0.088	4.093±1.318	5.108±0.887	4.662±1.229	34.577±15.212	/	/
STW(0.3%)+β-glucan(0.05%)	0.713±0.351	4.932±0.149	9.312±1.004	14.96±1.5	14.24±1.15	55.164±3.021	205.45	59.54
AS(0.2%)+β-glucan（0.05%）	0.592±0.216	2.514±0.073	6.132±1.142	9.24±1.43	8.65±1.22	43.912±4.114	85.54	27.00
AS(0.1%)+β-glucan（0.05%）	0.511±0.127	1.336±0.021	5.022±0.371	6.87±0.52	6.36±0.39	41.778±3.127	36.42	20.83
STW(0.3%)+AS(0.2%)+β-glucan(0.05%)	0.837±0.552	13.153±0.789	16.180±1.990	30.171±3.134	29.334±2.583	84.872±10.249	529.21	145.46
STW(0.3%)+AS(0.1%)+β-glucan(0.05%)	0.748±0.70	7.615±0.128	14.629±2.441	22.992±2.595	22.244±2.360	74.371±10.479	377.13	115.09

Appendix Table 3. Retention of 2% α-Arbutin in different skin layers and cumulative permeation for various composite formulations after 24 h (x ± SD, n = 4).

Sample	Corneum retention amount(μg)	Epidermis retention amount(μg)	Dermis retention amount（μg）	Whole skin retention amount（μg）	skin retention amount（μg）	Permeation amount（μg）	Relative retention growth rate (%)
Water	3.334±1.413	3.738±1.217	22.881±4.894	29.881±5.147	26.547±3.741	49.889±3.540	/
STW(0.2%)+β-glucan(0.1%)	0.931±0.411	3.611±0.512	27.996±1.193	32.54±2.12	31.61±1.71	47.779±1.231	19.07
AS(0.2%)+β-glucan（0.1%）	1.334±0.536	3.944±0.557	29.817±1.112	35.1±2.21	33.76±1.67	46.432±1.529	27.17
STW(0.3%)+β-glucan（0.1%）	2.115±0.421	2.136±0.415	30.162±1.522	34.41±2.36	32.3±1.94	49.548±1.272	21.67
STW(0.2%)+AS(0.2%)+β-glucan(0.1%)	1.118±0.555	2.562±0.623	35.241±4.058	38.921±4.058	37.803±3.637	45.822±7.439	42.40
STW(0.3%)+AS(0.2%)+β-glucan(0.1%)	0.825±0.146	3.267±0.333	31.717±2.120	35.809±1.786	34.984±1.792	25.845±10.189	31.78