Original Article

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Encapsulation of *Clitoria ternatea* L. Blue Flower Petal Extract in Lipid Vesicles for Enhancing Hair Growth-Promoting Activity

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Abstract:

Objective: This study aimed to develop hair lotions containing butterfly pea (*Clitoria ternatea*) blue flower petal extract-loaded phytosomes (BP phytosomes) and to evaluate their hair growth-promoting activity.

Material and Methods: A standardized aqueous butterfly pea petal extract (BP extract) was encapsulated into phosphatidylcholine-based lipid vesicles via a complexation method and incorporated into a Sepigel® 305 lotion base. Hair growth-promoting activity was evaluated in female Institute of Cancer Research (ICR) mice following once-daily topical application to a shaved dorsal area for 28 days. Hair length, thickness, and bulb size were measured weekly, and histological examination and hair follicle counting were performed after treatment.

Results: The optimized phytosome formulation exhibited an entrapment efficiency of 57.1±2.9%, vesicle size of 170.3±4.2 nm, and zeta potential of –9.9±0.5 mV in citrate buffer (pH 5.5). Treatment with BP extract and BP phytosome significantly increased hair length compared with the no-treatment and vehicle groups (p-value<0.05). Notably, the BP phytosome group showed significantly greater hair length and higher hair follicle counts than the minoxidil-treated group (p-value<0.05), with no skin irritation throughout the study period.

Conclusion: The encapsulation of BP extract into phytosomes enhances hair growth-promoting activity, highlighting its potential as a natural-based strategy for hair growth formulations.

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Introduction

Hair loss and hair thinning are common conditions affecting both men and women, with prevalence rising with increasing age. Although not life-threatening, these conditions can significantly impair self-esteem, psychological well-being, and overall quality of life. Hair loss is multifactorial, involving genetic, endocrine, immune, lifestyle, and nutritional factors. Current treatment options primarily include pharmacological agents such as minoxidil and finasteride, as well as surgical procedures like hair transplantation. However, despite their clinical efficacy, these approaches are associated with several limitations and potential adverse effects. Topical minoxidil (2–5% w/v) promotes hair growth through vasodilation and stimulation of follicular epithelial cell proliferation, but may cause itching, erythema, irritation, dizziness, and tachycardia. Finasteride, an oral 5α-reductase inhibitor widely used for androgenetic alopecia in men, has been associated with adverse effects such as sexual dysfunction and testicular pain[1]. Consequently, increasing attention has been directed toward alternative approaches, particularly natural products (e.g., amino acids, vitamins, minerals, and herbal extracts) and emerging technologies, including nanotechnology, gene therapy, stem cell technology, and microneedling[1–6].

Clitoria ternatea Linn., commonly known as butterfly pea, is a medicinal plant of the Fabaceae family, widely used in Thailand and other Asian countries for hair care. Traditionally, fresh flowers are crushed and applied to the scalp to promote hair growth and impart a deep blue coloration[7,8]. The petals are rich in anthocyanins and flavonoids, which exhibit antioxidant, anti-inflammatory, circulation-enhancing, and tyrosinase-stimulating activities[9,10]. Previous studies have demonstrated that ethanolic butterfly pea flower petal (BP) extract possesses strong 5α-reductase inhibitory activity (15.39±0.67 mg finasteride equivalent/g crude extract) and promotes hair growth in animal models[7]. In Wistar rats, topical application of a serum containing 2% w/w aqueous BP extract resulted in earlier onset of hair growth and increased hair length compared with the control group[8]. However, despite these beneficial properties, the cosmetic application of BP extract remains limited due to the poor skin permeation of its hydrophilic constituents and the instability of anthocyanins under environmental conditions such as pH, temperature, and light[11].

Phytosomes are molecular complexes between plant extracts and phospholipids, typically phosphatidylcholine, in which phytoconstituents interact with the phospholipid at defined ratios (e.g., 1:1 or 2:1), depending on the characteristics of the active compounds[12]. This delivery system enhances the stability and absorption of bioactive phytoconstituents, thereby improving their bioavailability and biological activity compared with conventional formulations[13,14]. Although phytosome technology has demonstrated significant potential for enhancing the delivery of plant-derived compounds, its application to BP extract for hair growth promotion remains underexplored. Therefore, this study aimed to develop hair lotion formulations incorporating butterfly pea petal extract-loaded phytosomes (BP phytosomes) and to evaluate their hair growth–promoting efficacy in an *in vivo* model.

Material and Methods

2, 2-Diphenyl-picrylhydrazyl (DPPH), aluminum chloride, L-α-phosphatidylcholine from soybean (type II-S), and quercetin were obtained from Sigma-Aldrich

(Missouri, USA). Folin–Ciocalteu's phenol reagent and gallic acid were purchased from Merck (Darmstadt, Germany). L(+)–Ascorbic acid was obtained from Poch S.A. (Gliwice, Poland). Tween 80 was supplied by P.C. Drug Center Co., Ltd. (Bangkok, Thailand). Sepigel® 305 (polyacrylamide, C13–14 isoparaffin, and laureth–7) was obtained from Adinop Co., Ltd. (Bangkok, Thailand). A 2% (w/v) minoxidil topical solution was purchased from Umeda Co., Ltd. (Bangkok, Thailand). All chemicals were of analytical grade and used as received.

Plant material

Fresh butterfly pea (*Clitoria ternatea* L.) flowers were obtained from a local fresh market in Hat Yai, Songkhla, Thailand. The plant material was authenticated, and a voucher specimen (No. SKP 098.03.20.01) was deposited at the Herbarium of the Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand.

Animals

Female Institute of Cancer Research (ICR) mice (5 weeks old) were obtained from the Laboratory Animal Center, Prince of Songkla University, Songkhla, Thailand. The mice were housed under standard laboratory conditions with ad libitum access to food and water and maintained on a 12 h light/dark cycle. All experimental procedures were reviewed and approved by the Animal Ethics Committee of Prince of Songkla University (Approval No. MOE 0521.11/238).

Preparation and standardization of butterfly pea blue petal extract

Fresh butterfly pea (*Clitoria ternatea* L.) petals were homogenized in Milli–Q water and filtered twice through four layers of cheesecloth. The clarified filtrate was lyophilized using a freeze–dryer (Kinetic Flexi–Dry, USA) to obtain dried BP extract.

Total phenolic content (TPC) of the BP extract was quantified using a 96–well microplate Folin–Ciocalteu assay, according to Sultana et al. (2012)[15]. Absorbance was measured at 765 nm, and the results were expressed as gallic acid equivalents (GAE/g dry extract).

Total flavonoid content (TFC) was determined using the aluminium chloride method (Herald et al. 2012)[16]. Absorbance was measured at 510 nm, and results were expressed as quercetin equivalents (QE/g dry extract).

Total monomeric anthocyanin content was determined using the pH differential method (Pinsuwan et al. 2010)[17]. Results were expressed as delphinidin–3–glucoside equivalents (Dpd–3–glu), one of the predominant anthocyanins in BP[18], and calculated using Equation (1).

$$\text{Monomeric anthocyanin (mg/g)} = \frac{(A \times MW \times DF \times 1000) / (\varepsilon \times 1)}{W} \quad (1)$$

Where: A is the absorbance of diluted sample; $(A_{543} - A_{700})_{pH\ 1.0} - (A_{543} - A_{700})_{pH\ 4.5}$), MW is the molecular weight of Dpd–3–glu (518.5 g/mol), DF is the dilution factor, ε is the molar absorptivity of Dpd–3–glu (29,000 mol/L), 1 is the path length (1 cm), and W is the weight of the dried extract (g).

Determination of antioxidant activity

Antioxidant activity was determined using the DPPH radical scavenging assay according to the procedure described by Yupanqui et al. (2024)[19]. A DPPH solution (6×10^{-5} M) in 95% ethanol was mixed with an equal volume (100 µL) of BP extract or ascorbic acid standard. The mixtures were incubated at 30 °C for 30 min in the dark, and absorbance was measured at 517 nm using a microplate reader (PowerWaveX, BioTek, USA). Radical scavenging activity (%) was calculated using Equation (2).

$$\text{DPPH scavenging activity (\%)} = 1 - \frac{\text{Absorbance of in the presence of extract}}{\text{Absorbance in the absence of extract}} \times 100 \quad (2)$$

Preparation of BP phytosome complexes

BP phytosome complexes were prepared according to Sandhya et al. (2010)[20] using varying weight ratios of BP extract and phosphatidylcholine, with Tween 80 as an edge activator. Phosphatidylcholine and Tween 80 were dissolved in 95% ethanol under heating until completely solubilized. The BP extract was then incorporated and sonicated for 2 h to facilitate complex formation. Ethanol was removed under reduced pressure at 50 °C to obtain the BP extract–phospholipid complex. The complex was stored at 4 °C and, prior to use, dispersed in a citrate buffer (pH 5.5) by sonication for 1 h.

Characterization of BP phytosomes

BP phytosomes dispersed in citrate buffer (pH 5.5) were characterized for particle size, polydispersity index (PDI), and zeta potential using dynamic light scattering (Zetasizer Nano ZS, Malvern Instruments, Malvern, UK). For entrapment efficiency determination, the dispersion was centrifuged at 15,000 rpm for 30 min at 4 °C to separate the unentrapped extract. The supernatant was collected to determine the amount of unentrapped extract in terms of total phenolic content, as previously described. The total amount of extract in the phytosome dispersion was determined after disrupting the phytosomes with an equal volume of 10% Triton X-100. Entrapment efficiency (EE%) was calculated using Equation (3):

$$\text{Entrapment efficiency } (\%) = \frac{A_T - A_F}{A_T} \times 100 \qquad (3)$$

where A_T is the total biomarker content in the phytosome dispersion and A_F is the amount of free (non-entrapped) biomarker.

The optimized BP phytosome formulation was selected based on particle size below 200 nm and high entrapment efficiency. Vesicle morphology was characterized using transmission electron microscopy (TEM) (JEM-2010, JEOL Ltd., USA). Fourier-transform infrared (FTIR) spectra were recorded using a PerkinElmer SpectrumOne spectrophotometer, Germany. KBr discs were prepared by compressing the sample under a hydraulic pressure of 10 tons, and spectra were acquired over the range of 450–4000 cm^{-1} at a resolution of 4 cm^{-1} with 16 scans.

Preparation of hair growth-promoting lotion formulations

Hair growth-promoting lotion formulations containing 1% (w/v) BP extract (powder or phytosome) were prepared using ethanol as a penetration enhancer and preservative, with Sepigel® 305 (0.3–0.7%, w/v) as the thickening agent. The optimized phytosome formulation was selected based on cosmetic acceptability parameters, including spreadability, smoothness, and non-greasy texture, evaluated using a 5-point scoring system (0=not acceptable; 1=poor; 2=fair; 3=good; 4=excellent). The assessment involved visual evaluation of the formulations after application onto a mirror surface. Stability studies were conducted at 4 °C and 30 °C for 2 months and under heating–cooling cycles (4 °C/45 °C, 24 h per cycle) for four cycles. Physicochemical properties were assessed before and after stability testing.

In vivo evaluation of hair growth-promoting activity

Hair growth-promoting activity was evaluated according to Kumar et al. (2012)[7] using five-week-old female ICR mice. The animals were randomly divided into five groups (n=4 per group): (1) untreated control (no treatment), (2) vehicle control (lotion base), (3) positive control treated with 2% (w/v) minoxidil topical solution, (4) lotion containing 1% (w/v) BP extract, and (5) lotion containing 1% (w/v) BP extract in phytosome form.

A 3×3 cm area on the dorsal region of each mouse was shaved prior to treatment. Test formulations (100 µL) were applied topically to the shaved area once daily for

28 consecutive days. Hair growth was evaluated on days 1, 7, 14, 21, and 28 by measuring the percentage of hair regrowth. Terminal hair growth was further assessed using a semi-quantitative scoring system: 0 (no growth), 1 (<20%), 2 (20–40%), 3 (40–60%), 4 (60–80%), and 5 (80–100% hair coverage).

Measurement of hair growth parameters (length, thickness, and bulb size)

Hair growth parameters, including hair length, thickness, and bulb size, were measured according to Yoon et al. (2010)[21]. On days 7, 14, 21, and 28, ten hairs were randomly collected from the treated dorsal area of each mouse for analysis. Hair length was determined from microscopic images captured using a BX61 microscope (Olympus Europa SE & Co. KG, Germany). Hair thickness and bulb size were measured manually at the end of the 28-day treatment period.

Histological evaluation of hair follicles

Histological evaluation of hair follicles was performed on day 28. Mice were sacrificed, and dorsal skin samples were collected and fixed in 10% neutral buffered formalin, followed by paraffin embedding. Tissue sections were prepared in both longitudinal and transverse orientations and stained with hematoxylin and eosin (H&E). Representative microscopic images were captured at 100× magnification using a light microscope. Hair follicle density was quantified by manual counting within a defined area (500 pixels in width).

Statistical analysis

All *in vitro* experiments were performed in three independent replicates. Data are expressed as mean ± standard deviation (S.D.). Statistical analysis was conducted using one-way analysis of variance (ANOVA), with a p-value<0.05 considered statistically significant.

Results

Phytochemical content and antioxidant activity of BP extract

The BP extract was obtained as a dark blue powder with an extraction yield of 4.7%. The extract exhibited total phenolic, total flavonoid, and total monomeric anthocyanin contents of 60.5±0.5 mg GAE/g dry extract, 58.6±0.7 mg QE/g dry extract, and 68.2±2.4 mg Dpd-3-glu equivalent/g dry extract, respectively. In the DPPH assay, the extract demonstrated antioxidant activity with an EC_{50} value of 102±13 µg/mL, compared with 6±1 µg/mL for ascorbic acid.

Physicochemical properties of BP phytosomes

FTIR analysis of BP extract revealed characteristic absorption bands at 1636.4 cm^{-1} and 1409.1 cm^{-1}, corresponding to C=O and aromatic C=C stretching vibrations, respectively. Phosphatidylcholine exhibited peaks at 2923.2 cm^{-1} (C-H stretching) and 1738.8 cm^{-1} (C=O stretching). In the BP phytosome spectrum, peak shifts were observed at 1734.4 cm^{-1} and 1640.8 cm^{-1}, indicating intermolecular interactions between BP extract and phosphatidylcholine (Figure 1).

BP phytosomes dispersed in citrate buffer produced a stable blue colloidal system. Table 1 presents their physicochemical properties, including vesicle size, PDI, zeta potential, and entrapment efficiency. Variation in BP extract-to-phospholipid ratios significantly affected vesicle size and entrapment efficiency, yielding particle sizes between 170 and 277 nm. Formulation C-2 was selected for further modification with Tween 80 due to its small vesicle size (<200 nm) and relatively uniform distribution. The optimized formulation, C-7 (0.75:1:0.19), demonstrated the most favorable characteristics. Tween 80 incorporation reduced vesicle size and increased entrapment efficiency to 57.1% compared with formulations without surfactant. TEM imaging (Figure 2) revealed predominantly spherical large unilamellar vesicles with variable diameters.

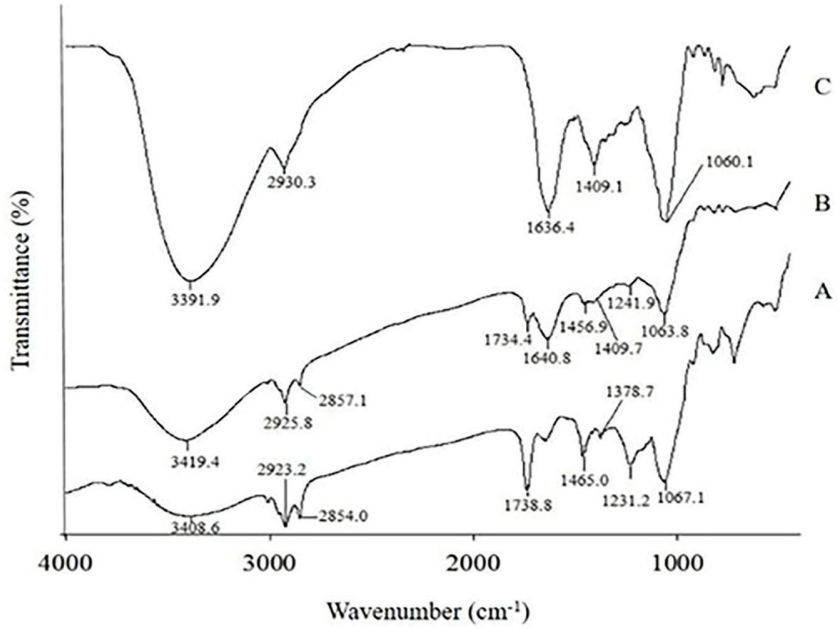


Figure 1 (A) FTIR spectra of phosphatidylcholine, (B) butterfly pea petal extract-loaded phytosomes at a ratio of BP: PC=0.75:1, (C) standardized butterfly pea petal extract

Table 1 Characteristics of BP phytosomes

No.	Weight ratio BP:PC:TW80	Vesicle size (nm)	PDI	Zeta potential (mV)	Entrapment efficiency (%)*
C-1	0.5:1:0	185.0±3.2	0.56±0.50	-29.2±5.5	43.2±3.3
C-2	0.75:1:0	186.5±4.0	0.42±0.03	-32.4±0.1	48.4±0.1
C-3	1:1:0	234.4±5.5	0.42±0.01	-31.5±0.2	51.5±2.0
C-4	1.5:1:0	276.6±7.2	0.81±0.30	-28.3±0.1	55.0±2.1
C-5	2:1:0	223.3±3.9	0.42±0.06	-27.8±0.7	28.3±5.3
C-6	2.5:1:0	ND	ND	ND	ND
C-7	0.75:1:0.19	170.3±4.2	0.67±0.02	-9.9±0.5	57.1±2.9

*total phenolic content, BP=butterfly pea petal extract, PC=phosphatidylcholine, TW80=Tween 80, PDI=polydispersity index, ND=not determined due to precipitation, All values presented as mean±S.D. (n=3)

Preparation of hair growth-promoting lotions

Hair growth-promoting lotions containing BP phytosome (formulation C-7), equivalent to 1% w/v BP extract, were prepared using Sepigel® 305 (0.5% w/v) and 95% ethanol (15% v/v), with citrate buffer (pH 5.5) used for volume adjustment. The formulation exhibited satisfactory cosmetic characteristics, including good spreadability, smooth texture, and minimal greasiness (total score=12). Stability findings are summarized in Table 2. The formulation remained physically and chemically stable, with biomarker

retention exceeding 90%, pH maintained at 4.5–6.0, and viscosity variation below 20% after 2 months at both 4 °C and 30 °C. In contrast, accelerated stability testing showed a 34% reduction in total anthocyanin content.

Hair growth-promoting activity

Throughout the 28-day study period, no signs of skin irritation were detected in any treatment group. The hair growth-promoting activity of the formulations is summarized in Table 3 and Figure 3. Visible hair regrowth was observed after 14 days of treatment. At this time point, both BP extract and BP phytosome groups demonstrated >40% hair regrowth of the shaved area, while the vehicle-treated and minoxidil groups remained below 40%. No statistically significant difference was found between the BP extract and BP phytosome groups. By day 28, full hair regrowth was observed across all treatment groups.

Hair length, thickness, and bulb size were subsequently analyzed as summarized in Table 3. After 21 days of treatment, the BP phytosome group exhibited

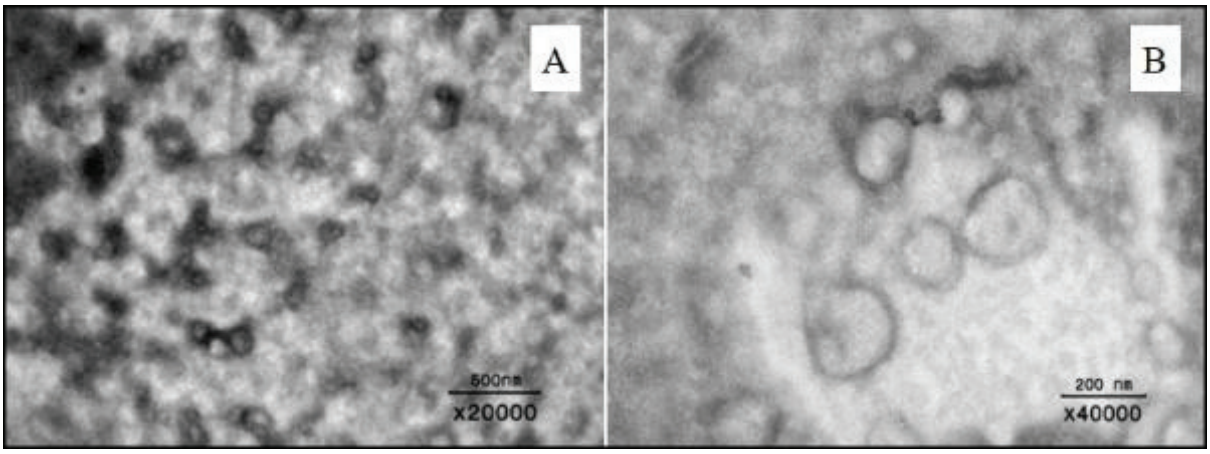


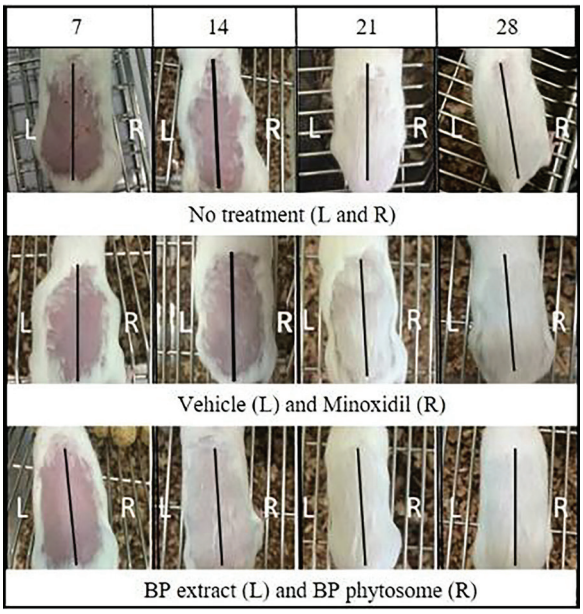
Figure 2 Transmission electron microscopy (TEM) images of BP phytosome formulation C-7 at magnifications of 20,000x (A) and 40,000x (B)

Table 2 Stability of hair growth-promoting lotions during storage under various conditions

Stability characteristics	Freshly prepared	Heating-Cooling	2 months-storage	
			4 °C	30 °C
Appearance	Blue colloid	Blue colloid	Blue colloid	Blue colloid
pH	5.91±0.01	4.95±0.01	5.71±0.00	5.70±0.00
Viscosity (cP)	5.78±0.00	4.68±0.00	5.20±0.07	4.84±0.07
% Remaining				
-Total phenolic	100.0	92.6±4.4	96.6±2.5	92.6±1.7
-Total flavonoid	100.0	88.1±6.9	101.1±2.3	96.2±1.3
-Total anthocyanin	100.0	66.4±2.3	100.5±1.4	98.4±1.9

significantly greater hair length compared with the no-treatment, vehicle, and minoxidil groups (p-value<0.05). Hair thickness was significantly higher in the BP phytosome group than in the no-treatment group (p-value<0.05). Furthermore, both BP extract and BP phytosome treatments significantly increased hair bulb size relative to the no-treatment and vehicle groups (p-value<0.05). No significant differences were detected between the BP phytosome and BP extract groups across all parameters.

Histological evaluation of longitudinal sections revealed a significantly increased number of hair follicles in the BP phytosome group relative to the minoxidil group (p-value<0.05). Consistently, transverse section analysis demonstrated that the BP phytosome group had the highest follicle density among all groups (Table 3 and Figure 5). Collectively, these findings indicate that BP phytosomes may enhance hair growth by stimulating follicular development during the anagen phase.



L=left, R=right

Figure 3 Representative images illustrating the hair growth-promoting effects at days 7, 14, 21, and 28 following treatment with different formulations

Table 3 Hair growth promoting effect of various lotion formulations in mice

Treatment	No treat	Vehicle	Minoxidil	BP extract	BP phytosome
Hair growth score					
– 7 Days	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0	1.0±0.0
– 14 Days	3.0±0.0	2.5±0.6	2.8±0.5	3.5±0.6[b]	3.5±0.6[b]
– 21 Days	4.5±0.6	4.8±0.5	4.8±0.5	4.8±0.5	5.0±0.0
– 28 Days	5.0±0.0	5.0±0.0	5.0±0.0	5.0±0.0	5.0±0.0
Hair length (mm)					
– 14 Days	2.0±0.4	1.3±0.6	1.7±0.6	2.3±0.8	2.6±0.8
– 21 Days	5.3±0.4	5.5±0.5	5.6±0.6	6.6±0.6[a,b]	7.0±0.7[a,b,c]
– 28 Days	6.5±0.1	7.3±0.2[a]	7.7±0.4[a]	9.2±1.1[a,b]	9.9±0.3[a,b,c]
Hair thickness (μm)	19.9±0.6	20.4±0.8	20.6±1.0	21.3±1.4	22.1±1.3[a]
Hair bulb (μm)	24.3±1.6	23.8±2.1	26.7±2.1[b]	28.7±3.5[a,b]	33.1±7.6[a,b]
Hair follicle					
– Longitudinal	7.3±0.5	6.0±1.8	6.0±0.8	7.5±0.6	7.8±0.1[b,c]
– Transverse	10.8±1.7	10.5±1.9	10.8±2.5	11.8±1.7	17.5±2.1[a,b,c,d]

[a-d]p-value<0.05 versus the no treatment, vehicle, minoxidil, and BP extract-treated groups, respectively
Values are presented as mean±S.D. (n=4). Hair growth scoring was defined as follows: 0=no growth observed, 1= <20% growth, 2=20–40% growth, 3=40–60% growth, 4=60–80% growth, 5=80–100% growth

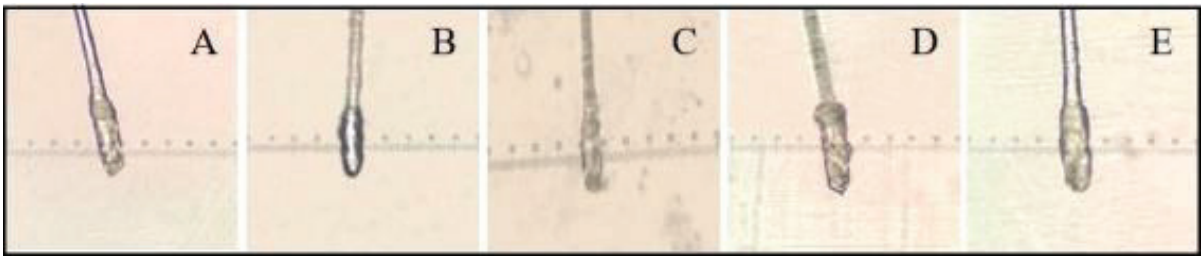


Figure 4 Representative microscopic images of hair bulbs following 28 days of treatment: (A) no treatment, (B) vehicle control, (C) minoxidil, (D) BP extract, and (E) BP phytosome at 400x magnification

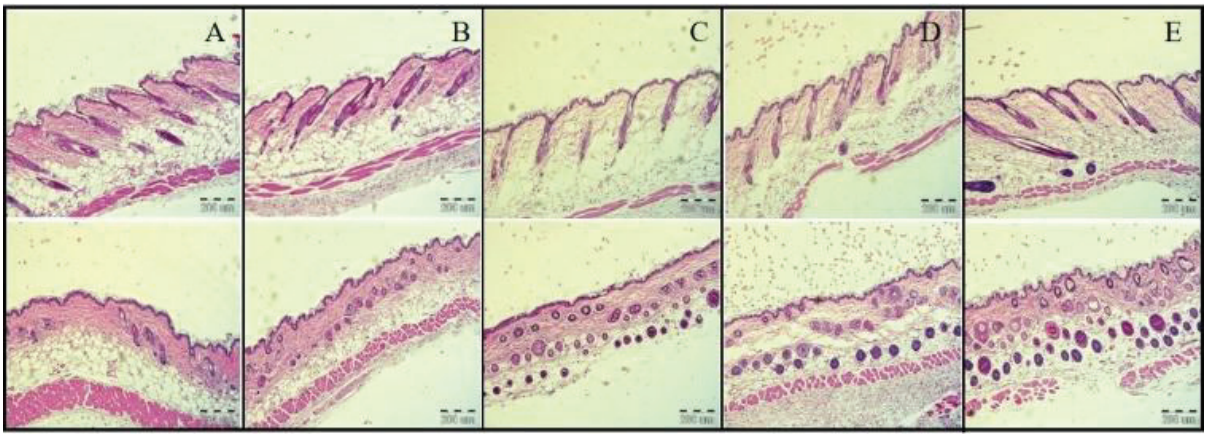


Figure 5 Representative hematoxylin–eosin–stained histological micrographs of dorsal skin following 28 days of treatment. Longitudinal sections are shown in the upper row and transverse sections in the lower row. Treatment groups: (A) no treatment, (B) vehicle control, (C) minoxidil, (D) BP extract, and (E) BP phytosome (100x magnification)

Discussion

In this study, BP extract was successfully incorporated into phytosome vesicles to enhance its stability and hair growth–promoting potential. The phytosome system exhibited an average particle size of ~200 nm, suggesting its suitability as a nanoscale carrier for topical application. Entrapment efficiency was influenced by interactions between BP extract and phosphatidylcholine, as evidenced by shifts in FTIR characteristic peaks relative to the individual constituents. These findings are consistent with previous reports demonstrating that phytosome formation is accompanied by characteristic FTIR spectral shifts[22].

The inclusion of Tween 80 in the phytosome formulation significantly influenced both vesicle size and entrapment efficiency. As a widely used edge activator, Tween 80 enhances vesicle bilayer flexibility and deformability, thereby improving the encapsulation efficiency of bioactive compounds and their physicochemical stability and solubility[23]. The optimized formulation containing Tween 80 demonstrated the smallest vesicle size and highest

entrapment efficiency, confirming the beneficial role of the surfactant in modulating vesicle properties.

The BP extract exhibited moderate antioxidant activity, likely attributable to its high content of phenolic compounds, flavonoids, and anthocyanins[24]. These bioactive constituents are known to protect hair and scalp tissues from oxidative stress and environmental damage. A lotion formulation incorporating Sepigel® 305 as a thickening agent was developed for the topical delivery of BP phytosomes. Ethanol was included as both a penetration enhancer and preservative[25]. Previous studies have shown that ethanol-containing vesicular systems can enhance skin permeation and improve phytosome stability during storage[26]. Citrate buffer (pH 5.5) was selected as the vehicle to maintain extract stability, as anthocyanins are more stable under mildly acidic conditions within the physiological pH range suitable for scalp applications (pH 4.5–6.0).

A noticeable reduction in total anthocyanin content was observed during accelerated stability testing under heating-cooling cycles, despite the absence of visible physical changes. This decrease is likely attributable to the inherent instability of anthocyanins, which are highly sensitive to environmental factors such as oxidation, temperature, and light[27]. Elevated temperature may induce hydrolysis of glycosidic bonds, resulting in the cleavage of glycosyl moieties and subsequent structural degradation of anthocyanins. In addition, cyanidin-3-glycosides are relatively stable under anaerobic conditions but undergo rapid degradation in the presence of oxygen[28]. These mechanisms likely contributed to the observed anthocyanin loss under accelerated conditions. Nevertheless, the lotion formulation demonstrated acceptable stability, retaining a high proportion of bioactive compounds after storage at 4 °C and 30 °C for 2 months. Storage below 30 °C in light-resistant containers is therefore recommended to minimize thermal and photodegradation. The BP phytosome exhibited an entrapment efficiency of 57%, indicating that a considerable fraction of the extract remained unencapsulated. Further optimization to enhance vesicular loading may improve the protection of bioactive compounds. Additionally, incorporating antioxidants such as sodium metabisulfite may help mitigate oxidative degradation[25,29].

The *in vivo* results demonstrated that both BP extract and BP phytosome formulations promoted hair growth in ICR mice. The anthocyanins and flavonoids present in BP extract have been reported to possess antioxidant and vasodilatory activities, which may enhance blood circulation around hair follicles and subsequently stimulate hair growth[30]. Kumar et al. (2012)[7] reported that a 1% w/w ethanolic BP extract formulated in a propylene glycol-water-ethanol (5:3:2) vehicle inhibited 5α-reductase activity and promoted hair growth more effectively than a 2% w/w minoxidil solution in experimental models. In addition, a recent study demonstrated that a liposomal formulation containing BP extract and clove oil enhanced eyebrow thickness and pigmentation[31]. Collectively, these findings support the potential of BP extract as a promising natural ingredient for hair care applications.

The comparable hair growth-promoting activity of BP phytosome and BP extract-based lotions may be attributed to the penetration-enhancing properties of ethanol present in both systems. Ethanol has been reported to increase the thermodynamic activity of active compounds after evaporation and to transiently disrupt stratum corneum lipids, thereby enhancing both trans-appendageal and transepidermal permeation pathways. However, excessive ethanol may extract cutaneous lipids, causing dryness or irritation[32]. In the present study, the absence of irritation may be explained by the relatively low ethanol concentration (15% v/v) used in the formulations.

In contrast, only the BP phytosome lotion produced significantly greater increases in hair length and follicle

density compared with minoxidil, highlighting the added benefit of the phytosome-based delivery system. This result aligns with previous findings on ethanol-containing vesicular systems, such as ethosomes, where ethanol enhances vesicle fluidity and promotes dermal penetration[33]. Ethanol concentrations of 10–15% (v/v) have been reported to optimize liposomal performance by maintaining vesicle size, encapsulation capacity, and pH gradient stability[34]. Additionally, phosphatidylcholine, the principal component of phytosomes, functions as an emollient that may mitigate scalp dryness associated with ethanol exposure.

The findings of this study demonstrate that phytosome technology enhances both the delivery and hair growth-promoting efficacy of BP extract. The optimized BP phytosome formulation exhibited favorable physicochemical characteristics and significant *in vivo* hair growth-promoting activity, highlighting its potential as a natural alternative to conventional treatments such as minoxidil.

Conclusion

The study successfully developed BP extract-loaded phytosomes with favorable physicochemical properties and enhanced biological efficacy. Treatment with the optimized formulation resulted in significant improvements in hair growth, hair thickness, and follicular development. These findings underscore the potential of phytosome-based delivery systems for natural hair care formulations.

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Conflict of interest

The authors declare no conflicts of interest.

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