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Studies on Skin Permeation with Polymer Micelles and the Cell Penetrating Peptide of Pyrus Serotina Var Stem Extracts

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Abstract – The stem extract from *Pyrus serotina var* has natural antioxidant ability, but the extraction method does not result in a soluble compound in cosmetic formulations. This study investigated the cosmetic efficacy of the *Pyrus serotina var* stem extract and its epidermis permeation ability when combined with polymer micelles and a cell penetrating peptide. The total concentration of polyphenol compounds was determined to be 103.1644 ± 1.38 mg/g in the ethanol extract and 78.97 ± 1.45 mg/g in the hydrothermal extract. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effects were 55.94 ± 0.22% in the ethanol extract at 1,000 mg/L. Superoxide dismutase (SOD) activity rates were 104.05 ± 3.28% in the ethanol extract at 62.5 mg/L. The elastase inhibition rate was 67.21 ± 2.72% in the ethanol extract at 1,000 mg/L. An antimicrobial effect was observed in the *Propionibacterium acnes* strain. In the epidermal permeability experiment, it was confirmed that formulation of the polymer micelle containing the *Pyrus serotina var* stem extract and cell penetrating peptide (R6, hexa-D-arginine) showed small particle size and much better skin permeability. The cumulative amount of total *Pyrus serotina var* stem extract that penetrated to the skin over time increased over 24 hours in three formulations. The three formulations showed 51.61 μg/cm^2 (Formulation 0), 75.97 μg/cm^2 (Formulation 1) and 95.23 μg/cm^2 (Formulation 2) skin penetration, respectively. Therefore, it was confirmed that the ethanol extracts of *Pyrus serotina var* stem showed good cosmetic efficacy and excellent epidermis permeation ability when combined with a polymer micelle and cell penetrating peptide. Thus, this extract has the potential to be used as a safe and natural cosmetic material in the future.

Key words: Antioxidant, Epidermal, Penetration, Polymer micelle, *Pyrus serotina var* stem

1. Introduction

Changes in cosmetics trends tend to increase proportionally with the interest of people with beautiful skin. In recent years, the cosmetics market has changed with the health and nature of cosmetics [1]. As the purchase of natural cosmetics increases, the consumption of cosmetics containing natural extracts is increasing, and there is an increasing interest in natural extracts that focus on the sustainability of maintaining clean land into the future. Among them, the pear *Pyrus serotina var* stem, is a fruit which can be consumed, has medicinal effects against sputum, cough and fever, and also contains a phenolic substance that is a natural antioxidant [2]. It is expected that the phenolic substance from *Pyrus serotina var* stem would be effective, but there is a lack of experiments testing this substance. If the *Pyrus serotina var* stem substance is investigated and found to be effective as a natural cosmetic material, the utilization of the *Pyrus serotina var* stem substance will increase.

Although the stem extract from *Pyrus serotina var* is likely to be used as a natural extract, there is a problem with the extraction method such that the substance is not completely solubilized in cosmetic formulations. Cosmetic formulations that are not fully solubilized have limited penetration, depending on the structural characteristics of the skin [3]. In order to overcome these limitations, interest and development studies are being conducted on a solubilization system in which poorly soluble components can be well dispersed in a hydrophilic solution. Among these development studies, the polymer micelle system is widely used [4].

Molecular micelles are characterized by an amphipathic polymer that exists as a large number of copolymers with different compositions for delivery of genes and molecular targeting drugs and has both hydrophobic and hydrophilic polymers in aqueous solution. The use of polymeric micelles has the advantage that they can effectively dissolve insoluble components. One of the polymer micelles, 'PCL-PEG,' is a copolymer of hydrophobic PCL (polycaprolactone) and hydrophilic PEG (polyethyleneglycol), which can form micelles in a hydrophilic solution to collect hydrophobic substances. This can be achieved by controlling the compounding ratio of PCL and PEG to solubilize a variety of poorly soluble materials and is advantageous as a biocompatible polymer [5,6]. Using this PCL-PEG copolymer, it is expected that not only the insoluble natural materials used in cosmetic formulations would increase, but also the trans epidermal permeability would be increased due to the reduction of the particle size.

The stratum corneum, which is the outermost layer of the epidermis and the core of the trans epidermal drug delivery, acts as a barrier by forming a lamellar structure between the keratinocyte lipids and

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proteins. This interferes with the permeation of external substances and thus the percutaneous permeation of the functional substance becomes difficult [7]. To solve these problems, peptide cosmetics are attracting attention as functional cosmetic ingredients. The peptide acts as a chemical messenger in the body, and the cell permeable peptide is easily accessible to the cell membrane due to the 'arginine' in the peptide [8,9]. The cell membrane and epidermal keratinocyte lipids have a similar lipid bilayer structure. Therefore, if the arginine oligomer, a key amino acid sequence of a cell-penetrating peptide, is applied to a cosmetic product together with a functional substance, it may be possible to maximize the efficacy of the functional substance by enhancing epidermal penetration.

In this study, we investigated the stem extract of *Pyrus serotina var* for the following: as an antioxidant, antibacterial, anti-wrinkle, and whitening activities. We also confirmed the stability of the formulation through the preparation of a polymer micelle containing the pear extract and then measured skin permeation.

2. Experimental Section

2-1. Instruments and reagents

Stems from *Pyrus serotina var* were collected from Gangrim-meoyn, Hoengseong-eup, and Gangwon-do. The equipment and reagents used for each experiment are as follows. The equipment used in the experiment included: an absorption spectrophotometer (SYNERGY HTX multi-mode reader, Bio Tek, Korea), a centrifugal separator (Supra-25K, Hanil Scientific Inc., Korea), a thermostat (Changshin, Korea), a particle size analyzer (Nanoctrac Flex, DREAM, Korea), a particle metrix (Stabino® Particle Charge Mapping, DREAM CORP, Korea), and Franz Diffusion Cells and Systems (PermeGear, USA). The solutions used for the polyphenol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and elastase tests used in the antioxidant and antibacterial tests were obtained from Sigma Aldrich (Korea). The PCL-PEG copolymer (Mn=2,500, Mw=2,500, ratio of PCL to PEG = 1:1) was purchased from Sigma Aldrich (USA) and R6 (hexa-D-arginine) was obtained from the Dermafirm Co. (Korea).

2-2. Extraction procedure

Stems from *Pyrus serotina var* were extracted with purified water and 80% ethanol, respectively. In the hydrothermal extraction method, purified water was added to the *Pyrus serotina var* stem powder and extracted for 4 h in a thermostat at 80 °C, filtered and freeze dried. Ethanol extracts were prepared by adding 80% ethanol to the *Pyrus serotina var* stem powder. The extracts were extracted for three days, filtered, and then concentrated.

2-3. Total polyphenol content measurement

The amount of polyphenol was quantified using the Folin-danis method [10]. To 100 μL of the Folin-Ciocalteu phenol reagent, 100 μL of the diluted sample solution was added and reacted at room temperature for 3 minutes. Next, 100 μL of the Na_2CO_3 solution was added and the absorbance was measured at 760 nm with an ELISA reader. The average value of the polyphenol concentration was calculated. The calibration curves were quantitatively analyzed using garlic acid as a standard at 25-125 mg/L.

2-4. Antioxidant activity measurement

2-4-1. Measurement of DPPH radical scavenging ability

The antioxidant effect of DPPH radical scavenging was measured using the Blois method [11]. To 100 μL of the extract solution, 120 μL of 0.45 mM 2,2-diphenyl-1-picrylhydrazyl solution was added and reacted in the dark for 30 minutes. The absorbance was measured at 530 nm with an ELISA reader.

DPPH radical scavenging activity (%) = (Ac-At) / Ac × 100

where, At was the absorbance of samples and Ac, the absorbance of the DPPH solution.

2-4-2. Measurement of elastase

Elastase inhibitory activity was measured as in Cannell [12]. The experiment was conducted using the EnzCheck® elastase assay kit (E-12056). Reaction buffer (1X) was used to dilute the *Pyrus serotina var* stem extract sample, which was then incubated in a 96-well black plate using 100 mg/L DQ elastin solution and 0.2 U/mL elastase for 30 minutes at room temperature. The absorbance was measured at 480 nm (excitation) and 535 nm (emission fluorescence) with an ELISA reader.

Inhibition rate of elastase (%)
= [1-(absorbance in the sample addition group / absorbance in the no additives)] × 100

2-5. Measurement of tyrosinase activity

Tyrosinase activity was measured using a modification of the Kubo method [13]. Twenty microliters of 0.1 M sodium phosphate buffer, 20 μL of each extract solution, 40 μL of 150 mM L-tyrosine, and 20 μL of 2000 U tyrosinase were added and incubated at 37 °C for 13 minutes.

The absorbance was measured at 490 nm with an ELISA reader.

Inhibition rate of tyrosinase (%) = [100-((b-b')/(a-a')) × 100]

a: Absorbance after reaction of blank
b: Absorbance after sample liquid reaction
a',b': Absorbance measured by replacing with buffer solution

2-6. Measurement of Superoxide Dismutase (SOD)

SOD-like activity was measured using a modification of Marklund's method [14]. The experiment was conducted using the SOD Assay Kit (BCBV5418). Twenty microliters of buffer solution and 20 μL of enzyme working solution were added to 20 μL of each sample solution, and then incubated at 37 °C for 20 minutes. The absorbance at 420 nm was measured by an ELISA reader.

SOD activity (%)
= [1-(absorbance in the sample addition group / absorbance in the no additives)] × 100

Table 1. List of strains and cultivation condition used for antimicrobial experiments

Strains	Media	Temperature (°C)	Time (h)
Staphylococcus aureus (ATCC6538)	MH	37	24
Escherichia coli (ATCC23726)	MH	37	24
Bacillus subtilis (ATCC19659)	MH	37	24
Propionibacterium acnes (ATCC6919)	RC	37	72

Media was used by MH (Muller-Hinton medium) and RC (Reinforced Closridial medium)

2-7. Antibacterial experiment

A disc diffusion test was performed to determine the antimicrobial activity of the stem extract of *Pyrus serotina var* [15]. *Staphylococcus aureus*, *Escherichia coli, Bacillus subtilis*, and *Propionibacterium acnes* were purchased from KCM and KCTC. The strains *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* were cultured in Muller-Hinton medium at 37 °C for 24 hours, re-cultured once, and then the absorbance at 600 nm was measured using a spectrophotometer. *Propionibacterium acnes* was incubated in a sealed container for 72 hours, re-incubated once, and then the absorbance at 600 nm was measured using a spectrophotometer. The culture conditions are shown in Table 1.

2-8. Polymer micelle creation

Through previous experiments, polymer micelle formulations were made using the ethanol extracts of the stems of *Pyrus serotina var*, which was relatively effective, compared to use of the hydrothermal extract. To create the polymer micelle, 1% total *Pyrus serotina var* stem ethanol extract was added to 99.9% ethanol, mixed and incubated at 65 °C. PCL-PEG was added at 5% of the total amount, followed by evaporation of the ethanol and a small amount of purified water. The conditions for producing PCL-PEG are shown in Table 2.

2-9. Skin penetration experiment

2-9-1. Ingredients of *Pyrus serotina var* stem extract with HPLC analysis

In order to investigate the composition of the *Pyrus serotina var* stem, various components were screened, and tannic acid was confirmed in the *Pyrus serotina var* stem. Therefore, in this experiment, we conducted HPLC analysis of tannic acid, which was regarded as an index component of *Pyrus serotina var* stem, and a permeation penetration experiment was conducted.

2-9-2. Epidermal penetration

Trans epidermal permeability was measured using Franz Diffusion Cells and Systems (PermeGear, USA). The artificial skin was placed on the receptor chamber with the stratum corneum facing up, and the donor chamber was fixed on the stratum corneum. The temperature was maintained at 37 °C in a constant-temperature water bath, and the sample was applied to the skin after stabilization for 30 minutes. The permeated sample was uniformly mixed, then, the receptor medium in which the sample was dissolved was sampled at a fixed time, and the same amount of receptor medium was supplemented. Finally, we compared the tannic acid content among the three formulations. Skin permeability conditions are shown in Table 3.

Table 3. Conditions of skin penetration experiment

Skin	Neoderm®-E (Tegoscience, Korea)
Receptor medium	PBS (SigmaAldrich, USA) 8.5 ml (add 5.0% Tween 80)
Sampling aliquot	500 μL
Donor chamber area	1.326665 cm^2
Stir bar speed	500
Sampling time	4,8,12,16,20,24
Temperature	37 °C

2-9-3. Formulations

We made three formulations for skin permeability experiments. Formulations for future skin penetration experiments were based on the *Pyrus serotina var* stem ethanol extract, which showed good results in previous antioxidant and antibacterial experiments. The first formulation consisted of *Pyrus serotina var* stem ethanol extract dissolved in 99.9% ethanol and then purified in water at a ratio of 5:5. The second formulation consisted of polymer micelles containing 1% *Pyrus serotina var* stem ethanol extract. The third formulation consisted of the polymer micelle containing 1% R6. Each condition is indicated in Table 4.

2-9-3. Measurement of particle size

We measured the particle size of the three formulations at three times using the particle metrix (Stabino® Particle Charge Mapping, DREAM Co.,, Korea).

Table 2. Composition of *Pyrus serotina var* stem extraction formulation for polymer micelle

Classification	Phase	Component	w/w %
Formulation 1	Ethanol	99.9 ethanol	25.0
	Active	*Pyrus serotina var* stem ethanol extract	1.0
	Polymer micelle	PCL-PEG[a]	5.0
	Water	Distilled water	up to 100
Formulation 2	Protein	R6[b] (cell permeable peptide)	1.0 of total polymer micelle.

PCL-PEG[a] : Ratio of PCL to PEG = 50 : 50, R6[b]: cell permeable peptide, hexa-D-arginine

Table 4. Conditions of formulation

Formulation	Composition
Formulation 0 (*Pyrus serotina var* stem ethanol extract)	10 mg/L *Pyrus serotina var* stem ethanol extract was dissolved in 99.9% ethanol and purified water at a ratio of 5 : 5
Formulation 1 (Polymer micelle)	1% active (*Pyrus serotina var* stem ethanol extract) in water
Formulation 2 (Polymer micelle + R6)	0.1% R6 in Formulation 1(Polymer micelle)

All formulations were prepared based on 10 ml.

2-10. Statistical processing

All experiments were repeated three times. All values were expressed as mean and standard deviation. The difference between the values was analyzed by t-test and one-way analysis of variance (ANOVA) with Fisher's least significant difference post hoc test, respectively.

3. Results and discussion

3-1. Yield

Pyrus serotina var stem was extracted with 80% ethanol and hydrothermal. Each yields were 1.59% in PSE (PSE: *Pyrus serotina var* stem ethanol extract) and 3.02% in PSH (PSH: *Pyrus serotina var* stem hydrothermal extract).

3-2. Total polyphenol content

The total polyphenol content of the Pyrus *serotina var* stem extract and the comparison of the extraction process of Pyrus *serotina var* stem extract are shown in Table 5. From 250 mg/L, 103.1644 ± 1.38 mg/g of polyphenol was extracted from the ethanol extraction and 78.97 ± 1.45 mg/g of polyphenol was extracted from the hydrothermal extraction.

3-3. Antioxidant efficacy of *Pyrus serotina var stem*

3-3-1. DPPH radical scavenging ability

The DPPH radical is a method of measuring the activity of a hydrogen donor. When the DPPH radical obtains an electron from phenolic compounds or aromatic amines, the color changes from purple to yellow by the action of proton-radical scavengers [16]. The antioxidant activity of the extracts was determined to be between 62.5-1,000 mg/L. The highest radical scavenging activity (55.94 ± 0.22% at 1,000 mg/L) was shown in the ethanol extract (Table 6).

3-3-2. Elastase inhibitory effect

Elastase, which is present in the dermis of the skin, is an enzyme capable of degrading various proteins including collagen and elastin and helps to maintain elasticity of the skin in the dermis [17]. The elastase inhibitory effect is effective for improving wrinkles in the skin and was measured according to the concentration, dependent at 62.5-1,000 mg/L. The highest inhibition rate was shown in the ethanol extract at 1,000 mg/L (Table 7).

3-4. Tyrosinase Inhibitory effect

Melanin is a pigment produced by melanomas, one of the cell organelles. Melanin is produced by the action of various enzymes such as tyrosinase, tyrosinase-related protein 1 (TRP1) and tyrosinase-related protein 2 (TRP2) in melanomas [18]. Among them, tyrosinase is a major regulatory enzyme that plays a role in the oxidation of tyrosine to dihydroxyphenylalanine (DOPA) quinone after being hydrolyzed with DOPA and is related to melanin. There was no inhibition of tyrosinase in the *Pyrus serotina var* stem extract, suggesting that there

Table 5. Total polyphenols of extracts from *Pyrus serotina var* stem

Samples	Method	Total polyphenols (mg/g)
PSE	Ethanol extract	103.1644 ± 1.38
PSH	Hydrothermal extract	78.97 ± 1.45

Values represent the mean ± SD of three independent experiments, PSE: *Pyrus serotina var* stem ethanol extract, PSH: *Pyrus serotina var* stem hydrothermal extract.

Table 6. Scavenging effect of *Pyrus serotina var* stem on DPPH assays

Concentration (ppm)	Extract	M	SD	p
62.5	PSE	26.71	± 0.46	0.000***
	PSH	20.83	± 0.68	
125.0	PSE	35.62	± 0.92	0.000***
	PSH	28.85	± 0.21	
250.0	PSE	44.87	± 1.13	0.002***
	PSH	38.85	± 0.94	
500.0	PSE	49.43	± 1.36	0.006***
	PSH	44.51	± 0.87	
1,000.0	PSE	55.94	± 0.22	0.092*
	PSH	54.46	± 0.70	

Values represent the mean ± SD of three independent experiments. Positive control : Ascorbic acid 200 μg/mL to 97.42%. *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$, PSE: *Pyrus serotina var* stem ethanol extract, PSH: *Pyrus serotina var* stem hydrothermal extract.

Table 7. Scavenging effect of *Pyrus serotina var* stem on elastase assays

Concentration (ppm)	Extract	M	SD	p
62.5	PSE	36.01	± 1.52	0.018**
	PSH	28.11	± 3.19	
125.0	PSE	48.94	± 1.24	0.000***
	PSH	36.16	± 1.71	
250.0	PSE	57.35	± 0.45	0.006***
	PSH	42.71	± 2.22	
500.0	PSE	63.23	± 0.45	0.008***
	PSH	51.16	± 2.16	
1,000.0	PSE	67.21	± 2.72	0.003***
	PSH	57.06	± 0.75	

Inhibitory effect of ASE, AWE and AEE against elastase. Results are expressed as mean ± S.D. of data obtained from three independent experiments. Positive control : N-Succinyl-Ala-Ala-Ala-p-nitroanilide 10 μg/mL to 23.9%. *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$, PSE: *Pyrus serotina var* stem ethanol extract, PSH: *Pyrus serotina var* stem hydrothermal extract.

Table 8. Scavenging effect of *Pyrus serotina var* stem on SOD assays

Concentration (ppm)	Extract	M	SD	p
3.90	PSE	49.37	± 0.40	0.030**
	PSH	42.48	± 2.22	
7.81	PSE	68.02	± 6.19	0.048**
	PSH	57.89	± 0.32	
15.62	PSE	89.10	± 3.23	0.046**
	PSH	82.83	± 1.95	
31.25	PSE	100.63	± 3.70	0.058*
	PSH	92.30	± 0.41	
62.50	PSE	104.05	± 3.28	0.078*
	PSH	97.76	± 0.36	

Values represent the mean ± SD of three independent experiments. Positive control : Ascorbic acid 500 μg/mL to 106.27%. *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$, PSE: *Pyrus serotina var* stem ethanol extract, PSH: *Pyrus serotina var* stem hydrothermal extract.

is no indicator component in the *Pyrus serotina var* stem that expresses a whitening effect.

3-5. Superoxide Dismutase (SOD) activity

The SOD activity assay is an antioxidant activity assay using color development of automatic oxidation [19]. The substances that inhibit superoxide in the samples used in the experiment can inhibit oxidation in the presence of SOD or SOD-like active substances. The highest SOD-like activity of 104.05 ± 3.28% was found in the ethanol extract at 62.5 mg/L (Table 8).

3-6. Antibacterial effect

The antimicrobial test was conducted three times using the paper disc method [20]. The results of the clear zone measurement are shown in Table 9. The antimicrobial effect was confirmed for only the *Propionibacterium acnes* strain where the largest zone of clearance (11.3 ± 0.58 mm) was found at the concentration of 10 mg/L ethanol extract.

3-7. Skin penetration effect

3-7-1. Ingredients of *Pyrus serotina var stem* with HPLC analysis

Tannic acid as an indicator component of *Pyrus serotina var* stem was quantified using HPLC (Agilent, USA). The mobile phase was made with 50% methanol and 50% water. The flow rate was set to 1 mL/min and 20 μL of each solution was injected into the chromatograph. The employed stationary phase was Shiseido C18 (3.2×250 mm, 5 μm). The UV detector was employed at the wavelength of 270 nm. In later epidermal permeability experiments, the confirmation of the

Table 9. Effect of *Pyrus serotina var* stem extraction amount on area of clear zone

Strain	Clear zone (mm^2)									
Concentration (mg/L)	Positive control 20.00	10.00		5.00		2.50		1.25		
Staphylococcus aureus	9.0	No effect								
Escherichia coli	10.0	No effect								
Bacillus subtilis	9.0	No effect								
Propionibacterium acnes	10.0	E	H	E	H	E	H	E	H	
		11.3±0.58	-	9.0±0.58	-	8.3±0.58	-	8.3±0.58	-	

Positive control: *Staphylococcus aureus, Escherichia coli, Bacillus subtilis* are used methyl paraben, *Propionibacterium acnes* is used salicylic acid, PSE: *Pyrus serotina var* stem ethanol extract, PSH: *Pyrus serotina var* stem hydrothermal extract.

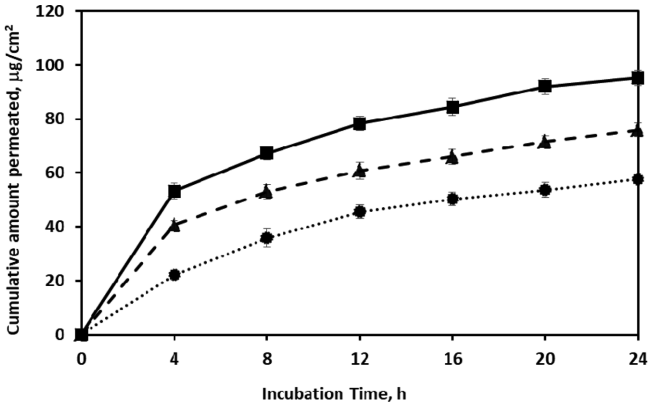


Fig. 1. *In vitro* skin penetration profiles of *Pyrus serotina var* stem extract, Polymer micelle Polymer micelle + R6 through epidermal skin and penetration cumulative amount: ●, *Pyrus serotina var* stem ethanol extract; ▲, Polymer micelle; ■, Polymer micelle + R6.

increase in epidermal permeability was measured with tannic acid as an indicator component.

3-7-2. Epidermal permeability

Figure 1 shows the permeation rate of the *Pyrus serotina var* stem extract over time for a given area of skin. The cumulative amount of total *Pyrus serotina var* stem extract that penetrated into the skin over time increased over 24 hours in all three formulations. When permeation was observed, the amount of permeation was in the order of Formulation 2, Formulation 1, and Formulation 0, suggesting that Formulation 2 had the highest permeation and polymeric micelles and cell permeable peptides can increase skin penetration. The results of the trans epidermal permeability experiment were as follows. 'Formulation 2' was the most penetrating when the three formulations showed trans epidermal permeability and it had 95.23 μg/cm^2, as shown in Table 10.

3-8. Measurement of particle size

We measured the particle size of the three formulations and focused on the intensity of the particle size. In this study, 'Formulation 2' had the smallest size among the three formulations. This is because it is thought that the shape of the polymer micelle helps the dispersion of the extract to reduce the particle size. Particle size results are shown in Table 11.

4. Conclusions

The purpose of this study was to investigate the efficacy of *Pyrus serotina var* stem as an active ingredient in cosmetics and to measure the epidermal penetration of the *Pyrus serotina var* stem extract through the use of PCL-PEG, one of the polymer micelles.

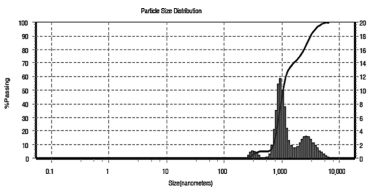
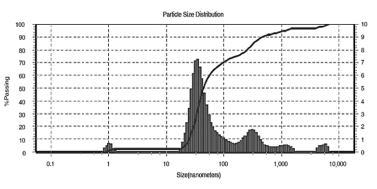
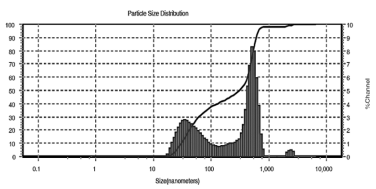
First, the total polyphenol amounts in the *Pyrus serotina var* stem extracts were determined to be 103.1644 ± 1.38 mg/g in the ethanol

Table 10. Epidermal penetration experiment's result using franz cell according to formulation with *Pyrus serotina var* stem

Classification		Formulation			
		Formulation 0 (*Pyrus serotina var stem* ethanol *extract*)	Formulation 1 (Polymer micelle)	Formulation 2 (Polymer micelle + R6)	*F*(p)
4h	M (SD)	22.10^a (1.72)	40.62^b (1.55)	53.27^c (2.86)	26.466 (0.001)***
	post hoc		LSD = c > b > a		
8h	M (SD)	35.78^a (3.29)	53.02^b (2.64)	67.29^c (2.52)	92.739 (0.000)***
	post hoc		LSD = c > b > a		
12h	M (SD)	45.60^a (2.46)	60.82^b (3.27)	78.30^c (2.58)	102.895 (0.000)***
	post hoc		LSD = c > b > a		
16h	M (SD)	50.34^a (2.45)	66.08^b (2.79)	84.44^c (3.20)	109.228 (0.000)***
	post hoc		LSD = c > b > a		
20h	M (SD)	53.69^a (2.89)	71.64^b (2.04)	92.05^c (2.85)	160.274 (0.000)***
	post hoc		LSD = c > b > a		
24h	M (SD)	57.61^a (0.72)	75.97^b (2.57)	95.23^c (2.76)	215.431 (0.000)***
	post hoc		LSD = c > b > a		

Each value represents the mean and S.D. Statistical analysis was performed using the one-way ANOVA. *$p < 0.1$, **$p < 0.05$, ***$p < 0.01$.

Table 11. Particle size of *Pyrus serotina var* stem ethanol extract, Polymer micelle, Polymer micelle + R6

	Formulation 0 *Pyrus serotina var* stem ethanol extract	Formulation 1 Polymer micelle	Formulation 2 Polymer micelle + R6
Particle size			
M	2206.872 nm	300.658 nm	245.381 nm
SD	±899.28	±174.39	±177.26

extract. In addition, the antioxidant test confirmed the efficacy of the *Pyrus serotina var* stem as an active ingredient in cosmetics. In the DPPH radical scavenging experiment, the radical scavenging ability of the ethanol extract of *Pyrus serotina var* stem was analyzed and shown to be 55.94 ± 0.22% at the concentration of 1,000 mg/L and a higher radical scavenging ability was confirmed compared to the hydrothermal extract of pear trunk at the same concentration. The inhibition rate of 67.21 ± 2.72% was confirmed at 1,000 mg/L of the stem ethanol extract of *Pyrus serotina var*. As a result of the SOD experiment, the ethanol extract of the pear stem showed a higher similarity to that of the hydrothermal extract of the *Pyrus serotina var* stem of 104.05 ± 3.28% at 62.5 mg/g. The efficacy was not confirmed by a tyrosinase experiment showing the whitening effect. It was concluded that the effective ingredient of *Pyrus serotina var* stem was not a tyrosinase inhibitor. The above data suggest that the results of the antioxidant, anti-wrinkle and whitening tests were dependent on the concentration. The stem ethanol extract from *Pyrus serotina var* showed higher antioxidative effect than the hydrothermal extract of the *Pyrus serotina var* stem.

The antibacterial efficacy of the *Pyrus serotina var* stem extract was confirmed in the *Propionibacterium acnes* strain. A clear zone of 11.3 ± 0.58 mm was obtained at a concentration of 50 mg/L of pear ethanol extract. This indicated that the ethanol extracts of the *Pyrus serotina var* stem can be an ingredient in acne cosmetics. Antioxidant and antimicrobial tests showed that the amount of tannin in the *Pyrus serotina var* stem extract was increased in the ethanol extract and reflected the antimicrobial activity and antibacterial power of tannic acid. For the skin penetration test, the total amount of tannic acid, which is an index component of the *Pyrus serotina var* stem extract, that permeated the skin was in the order of formulation 2 > formulation 1 > formulation 0. Thus, the cell permeable peptide (R6) was beneficial in passing the active ingredient through the stratum corneum. This also showed a similar trend in particle size. In other words, *Pyrus serotina var* stem extract, which was dissolved in ethanol in the previous experiment, was completely solubilized in water without polymer in the form of the polymer micelle and showed better results in terms of particle size and penetration of the skin.

Through this study, we confirmed the antioxidant, antibacterial, anti-wrinkle and whitening effects of the *Pyrus serotina var* stem extract. Through the production of the macromolecular micelle containing *Pyrus serotina var* stem extract, which increased penetration of the extract into the skin, this extract could be used as a raw material in the cosmetics industry as a natural extract ingredient. Future studies will investigate the relationship between zeta potential and the particle size of each formulation by measuring the zeta potential of the three formulations.

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