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Cosmetic effects of *Prunus padus* bark extract

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Abstract−*Prunus padus* bark extract was tested for use as a natural cosmetic ingredient. *P. padus* bark extract was obtained by hot water extraction and succeeding maturing processes. Total polyphenol and flavonoid concentrations were measured, and safety test (cell toxicity test), efficacy tests (antioxidation, antiwrinkle, whitening), and temperature stability tests were conducted in experiments. Total polyphenol and flavonoid concentrations were 714.7±0.5 mg/g and 72.1±2.2 mg/g, respectively. Compared with other natural antioxidants, polyphenol concentration in *P. padus* bark extract was extremely high. *P. padus* bark extract showed lower cell toxicity in 100-500 μg/ml concentration by MTT assay. *P. padus* bark extract indicated 71% DPPH free radical scavenging activity (antioxidation), 36% elastase inhibition (antiwrinkle), and 38% tyrosinase inhibition (whitening) at 350 μg/ml, respectively. W/O/W lotion formulation containing 1% *P. padus* extract was prepared and stability tests were done to see variations in cosmetic properties. Viscosity, pH, particle size, and appearance of lotion containing 1% *P. padus* extract maintained stable condition for 28 days. Particle size of lotions showed homogeneous 362-426 nm ranges during stability tests. From this study, *P. padus* bark extract displayed strong possibility as a natural antioxidative cosmetic agent.

Keywords: *Prunus padus*, Cosmetic Agent, Antioxidant, W/O/W Emulsion, Stability

INTRODUCTION

Skin care cosmetics, sometimes called basic cosmetics, mainly consist of water phase, oil phase, surfactants, and other components. Water phase ingredients frequently used in cosmetics include distilled water, glycerol, propylene glycol, sorbitol, sodium 2-pyrrolidone-5-carboxylate, and sodium hyaluronate among others [1]. Typical oil phase contains wide variety of chemicals such as plant oils (olive oil, castor oil, etc.), wax esters (carnauba wax, bee wax, lanolin), hydrocarbons (paraffin, petrolatum, squalane), higher fatty acids (lauric acid, stearic acid), higher alcohols (cetyl alcohol), and esters (isopropyl myristate) [2]. Since water and oil phase do not form a homogeneous solution, surfactants must be used for stability in cosmetics. Suitable surfactants are used to make certain types of cosmetics like lotion, cream, and solution. Cosmetic surfactants are classified into ionic (anionic, cationic, amphoteric), nonionic (Tween, Span), and natural surfactants (lecithin, saponins) [3].

Besides water phase, oil phase, and surfactants, cosmetics require numerous chemicals to maintain formulation as commercial products. Antioxidants (vitamin E, dibutylhydroxytoluene, gallic acid, etc.), thickening agents (xanthan gum, carboxyvinyl polymer), chelating agents (Na-EDTA), color (dye, pigment), preservatives (parabens, phenoxyethanol), and fragrance (natural, artificial) are added in normal skin care cosmetics [4]. Ordinary skin care cosmetics contain at least 20 ingredients.

It's a marked trend in cosmetics worldwide to use natural sources as cosmetic ingredients, especially plant origins. As mentioned, most cosmetic materials are synthetic chemicals. Major cosmetic firms are looking for new natural ingredients substituting organic chemicals such as surfactants and preservatives. An institution like ECOCERT regulates natural cosmetics and requires up to 95% natural and organically grown plant ingredients [5].

Cosmetics like medicine are tightly regulated to protect national health by government institution. Several cosmetic assays in Korea are enlisted by Korea Ministry of Food and Drug Safety [6]. Basic cosmetic ingredient researches consist of safety, efficacy, formulation, and stability tests. The European Union has banned animal tests as safety tests since 2013 and alternative assays are being developed. Cosmetic efficacy tests contain antiwrinkle, whitening, antioxidation, antimicrobial, and UV blocking assays in general [7]. Stability tests measure physical characteristics (viscosity, pH, particle size, appearance) for temperature variations in a certain period of time using cosmetic formulations (lotion, cream, solution, etc.).

Prunus padus, known as Bird Cherry, is a species of cherry native to northern Europe and northern Asia. It is a deciduous small tree or large shrub, 8-16 m tall. Numerous, showy flowers are arranged in elongate, cylindrical, and terminal racemes [8]. *Prunus padus* Linne (bark, fruit, and leaf) has been widely used as a traditional medicine such as Compendium of Medical Herbs 1596, compiled by Li Shizhen, with beneficial effects on anti-inflammatory and antinociceptive activities in numerous diseases including stroke, neuralgia, and hepatitis [9].

In fruits of *P. padus*, high concentration of anthocyanins (cyanidin-3-rutinoside and cyanidin-3-glucoside) was determined by chromatographic and spectroscopic methods [10]. Generally, anthocyanins have shown strong antioxidant capacity for bioactive materials [11], which proves potential for cosmetic agents. Essential oils from fruits, stems, leaves, barks, and trunk cores of *P. padus* Linne contain

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large amounts of benzoic acid and benzaldehyde [12]. *P. padus* bark was reported to contain tannins, two cerebrosides, and six phenolic compounds [13]. Since *P. padus* bark is known to include various bioactive compounds, it can be a good candidate for cosmetic materials.

We tested extract from *P. padus* bark for a natural cosmetic agent. *P. padus* bark extract was obtained from hot water extraction and succeeding maturing processes. Total concentrations of polyphenol and flavonoid were measured and cell toxicity was evaluated. As the result of efficacy tests, antioxidation, antiwrinkle, and whitening effects were determined. W/O/W lotion formulation including 1% *P. padus* bark extract was developed and pH, viscosity, and particle size of lotion were measured for 28 days in 4 °C, 25 °C and 47 °C through stability tests.

MATERIALS AND METHODS

1. *Prunus padus* bark Extract and Reagents

P. padus extract was obtained from Biometics Co. (Gimhae, Korea). *P. padus* bark collected in Yeongdeok-gun, Gyeongbuk, Korea was dried and extracted with hot water. The extract was matured for a month, and reverse osmosis and ultrasonic extraction were applied. Whole solution was freeze-dried (ILSHIN BioBase, Korea) after filtration.

Sodium carbonate, Folin-Denis' reagent, gallic acid, diethylene glycol, sodium hydroxide, rutin hydrate, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), mushroom-tyrosinase, L-tyrosine, 3,4-dihydroxy-L-phenylalanine (DOPA), elastase (pancreatic solution), *N*-succinyl-$(Ala)_3$-*p*-nitroanilide,adenosine, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma (USA). Mouse melanoma cells (B16F10) were purchased from Korea Cell Line Bank. Cells were cultured at 37 °C under 5% CO_2 in an incubator (MCO-15AC, Sanyo, Japan) using Dulbecco's modified eagle's medium (DMEM, Lonza, USA), which contained 10% (v/v) FBS (fetal bovine serum) and 1% (v/v) penicillin.

2. Total Polyphenol and Flavonoid Content Measurement

The total polyphenol content was determined using Folin-Denis' reagent [14]. 200 µl of extract (500-1,000 µg/ml) and 200 µl of Folin-Denis' reagent were mixed and kept for 3 min at room temperature. 400 µl of 2 M sodium carbonate solution and 200 µl of distilled water were added in the solution. The mixture was allowed to stand for 30 min at room temperature and absorbance was measured at 725 nm in a microplate reader (Power Wave XS2, BIOTEK, USA). A standard curve was constructed using gallic acid (0-500 µg/ml).

The total flavonoid content was determined as follows [15]: 100 µl of extract (500-1,000 µg/ml) and 1,000 µl of diethylene glycol were mixed and 100 µl of 1 N sodium hydroxide was added. This mixture was kept for 1 h at 37 °C. The absorbance was measured at 420 nm in a microplate reader. Rutin hydrate was chosen as a standard.

3. Cosmetic Assays

To evaluate cell toxicity, MTT assay was conducted as follows [16]: After seeding mouse melanoma cells (B16F10) on 24-well plate in 1×10^5 cell/ml concentration, cells were cultured for 24 h in CO_2 incubator. *P. padus* extract was mixed into new medium and cultured for 24 h. MTT solution (5 mg/ml) was added and kept for 2 h in 37 °C, 5% CO_2 incubator. Formazan was dissolved in DMSO (dimethyl sulfoxide) and transferred to 96-well plate. Absorbance was measured at 570 nm by microplate reader and cell viability was calculated as follows:

$$\text{Cell viability (\%)}=[(\text{Exp.}-\text{Blank})/\text{Control}]\times100 \quad (1)$$

Exp: Absorbance of extract with cell
Blank: Absorbance of extract without cell
Control: Absorbance of water with cell

Antioxidant activity was measured by DPPH free radical scavenging assay [17]. 100 µl of DPPH (α,α-diphenyl-β-picrylhydrazyl) was reacted with 200 µl of *P. padus* extract for 10 min at 25 °C and absorbance was measured at 517 nm by microplate reader. The free radical scavenging activity was calculated as follows:

$$\text{DPPH scavenging activity (\%)}=[1-(\text{Exp.}-\text{Blank})/\text{Control}]\times100 \quad (2)$$

Exp: Absorbance of extract with DPPH
Blank: Absorbance of extract with ethanol
Control: Absorbance of water with DPPH

Antiwrinkle effect was measured by elastase inhibition assay [18]. *N*-succinyl-$(Ala)_3$-ρ-nitroanilide (as substrate) was dissolved in 0.2 M tris-HCl buffer (pH 8.0) to make 1.0 mM concentration. 80 µl of *P. padus* extract and 20 µl of 2.5 U/ml elastase were added to 200 µl of tris-HCl buffer including substrate, and reacted for 10 min at 25 °C. Absorbance was measured at 410 nm and elastase inhibition activity was calculated as follows:

$$\text{Inhibition ratio (\%)}=[1-(\text{Exp.}-\text{Blank})/\text{Control}]\times100 \quad (3)$$

Exp: Absorbance of extract with elastase
Blank: Absorbance of extract without elastase
Control: Absorbance of water with elastase

Tyrosinase inhibition activity was performed as follows [18]: 200 µl of 0.1 M sodium phosphate buffer (pH 6.5), 20 µl of 1.5 mM L-tyrosine, and 60 µl of *P. padus* extract were mixed. 20 µl of mushroom tyrosinase (1,000 U/ml, Sigma, USA) were reacted for 5 min at 37 °C. Absorbance was measured at 490 nm in a microplate reader.

Water-in-oil (W/O/W) emulsions are multi-compartmentalized systems in which minute water particles are dispersed inside oil phase, which are dispersed in turn inside a continuous aqueous phase, as shown in Fig. 1. Due to their properties of entrapping and protecting various substances and releasing them in a controlled manner, emulsions of this kind have been used for micro-encapsulation in pharmaceuticals, cosmetics, and other industrial applications [19,20].

In cosmetics, advantages of W/O/W multiple emulsion are in stabilization of active ingredients and unique texture. There are many

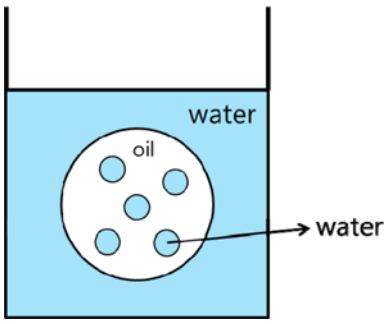

Fig. 1. W/O/W emulsion (multiple emulsion).

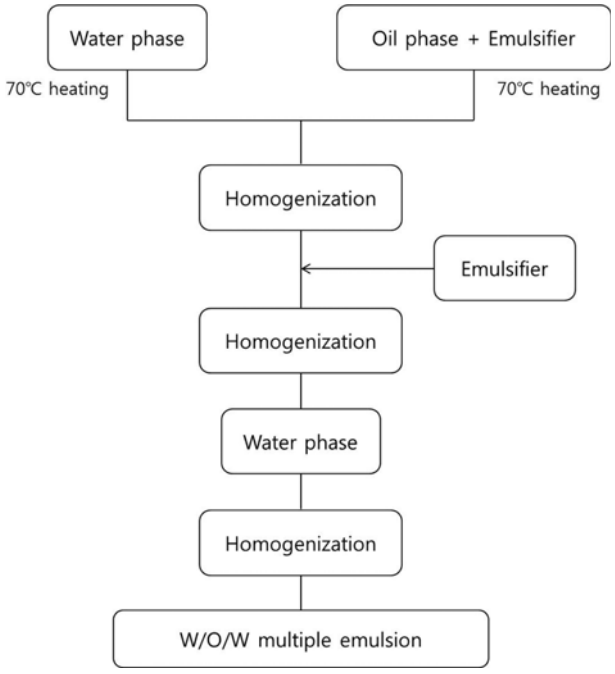

Fig. 2. Preparation process of the W/O/W multiple emulsion containing *P. padus* bark extract.

cosmetic materials which have different physicochemical properties [21]. HLB (Hydrophile-Lipophile Balance) means the balance between the hydrophilic and the lipophilic portions of the nonionic surfactants. Generally, the emulsifier with low HLB is used in W/O emulsion, while the emulsifier with higher HLB value is used in O/W emulsion [22]. The HLB value of sorbitan isostearate and polysorbate 20 used in this study is 4.7 and 13.3, respectively.

W/O/W emulsion was prepared following a two-step emulsification process [19]. The preparation process is shown in Fig. 2. First, water in oil (W/O) emulsion was prepared with W/O emulsifier (sorbitan isostearate) using a mixer (T 18 basic ULTRA-TURAX, IKA, Germany). Next, second emulsifier (polysorbate20) was added to the W/O emulsion. Finally, the W/O emulsion and water phase were homogenized and whole emulsion was left to cool at room temperature**.** The W/O/W emulsion was checked by microscope (BA210, Motic, Hong Kong). Lotion containing 1% *P. padus* bark extract and control (no extract) was prepared as shown in Table 1.

Table 1. W/O/W Lotion formulation containing 1% *Prunus padus* bark exract and control (without extract)

Component	Content (%)	
	Control lotion	Extract lotion
Deionized water	Up to 100	Up to 100
Jojoba oil	15	15
Emulsifier (sorbitan isostearate, polysorbate20)	5-10	5-10
Hyaluronic acid	3	3
Grapefruit seed extract	1	1
P. padus extract	-	1

As a stability test, viscosity (Brookfield viscometer DV-1, USA), pH, particle size (90 Plus BIC, USA), and appearance of lotions were measured at 4 ℃, 25 ℃ and 47 ℃ for 28 days. All the tests were analyzed by the SPSS program (version 20.0). The experiment was repeated three times and data were expressed as mean±S.D.

RESULTS AND DISCUSSION

1. Total Polyphenol and Flavonoid Content

Polyphenols including flavonoids are known to have strong antioxidation effect as cosmetic agents. Polyphenol and flavonoid concentrations of *P. padus* extract were measured to see a possibility as cosmetic antioxidants. The phenolic content of *P. padus* bark extract was 714.7±0.5 mg/g of extract, and the flavonoid content was 72.1 ±2.2 mg/g of extract. The phenolic content was very high compared with some known antioxidant plants such as thistle, mate, slippery elm bark, and pine needles [23]. Our data confirms high concentration of anthocyanins in *P. padus* as reported in literature [10]. Na et al. [24] reported two cerebrosides (pinelloside, soyacebroside), and six phenolic compounds (quercetin-3-O-β-D-galactopyranoside, nudiposide, isolarisiresinol 9-O-β-D-xylopyranoside, khaephuoside A and icariside) in *P. padus*. Polyphenols are reported to favorably supplement sunscreen protection, and may be useful for skin diseases associated with solar UV radiation-induced inflammation, oxidative stress, and DNA damage [25,26]. This result implies good potential of *P. padus* extract as a cosmetic antioxidation agent.

2. Safety Test

As a safety test, MTT assay was performed in 100-1,000 μg/ml concentration using mouse melanoma cell (B16F10) as shown in Fig. 3. Cell viability of *P. padus* extract was 80% at 350 μg/ml and compared with vitamin C as a standard. Cell viability of *P. padus* extract was similar to that of plant extracts including *Saururus chinensis* extract, *Morus bombycis* stem extract, and *Morus papyrifera* stem extract [27].

3. Efficacy Test

Antioxidant activity was measured by DPPH free radical scavenging assay as shown in Fig. 4. Vitamin C (L-ascorbic acid), well known for an antioxidant, was used as positive control. Antioxidative effect of *P. padus* bark extract was 71% at 350 μg/ml and higher than 70% at all concentrations. Most cosmetic formulations require

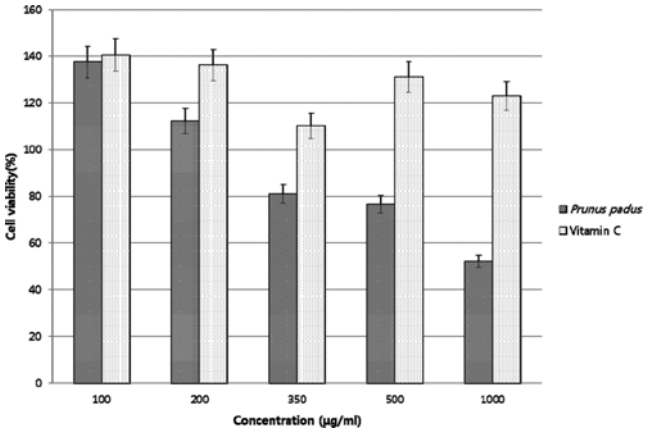

Fig. 3. Cell toxicity of *Prunus padus* bark extract by MTT assay.

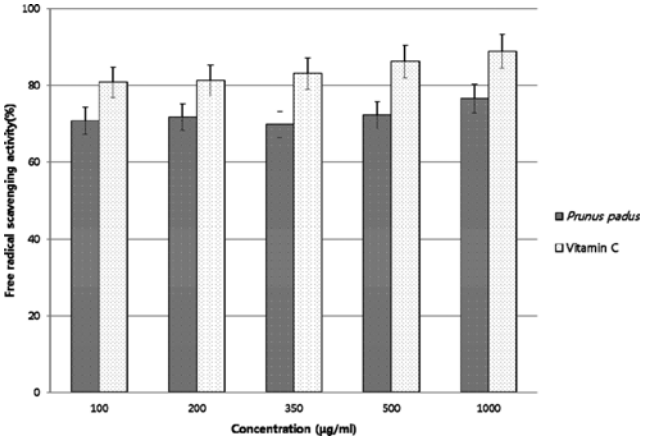

Fig. 4. DPPH free radical scavenging activity of *Prunus padus* bark extract.

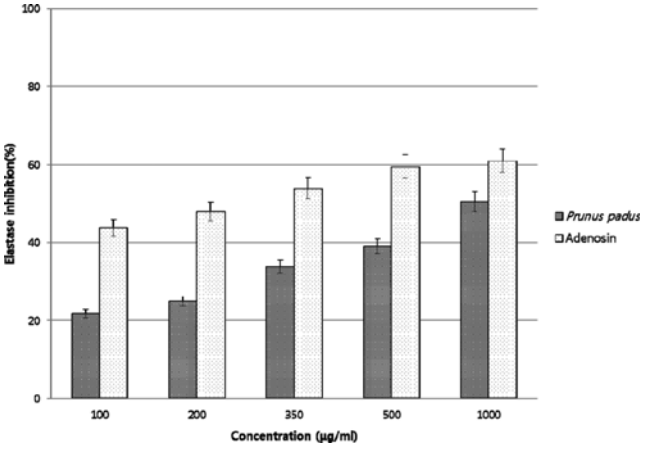

Fig. 5. Elastase inhibition activity of *Prunus padus* bark extract.

certain amounts of antioxidants because they can be decayed fast when cosmetics are exposed to the air and skin. A recent trend in cosmetics uses natural ingredients substituting chemical agents. Vitamin C is a potent antioxidant, though it is quickly dissolved when it is applied as a cosmetic agent [28]. *P. padus* extract shows strong potential as a natural antioxidative cosmetic agent.

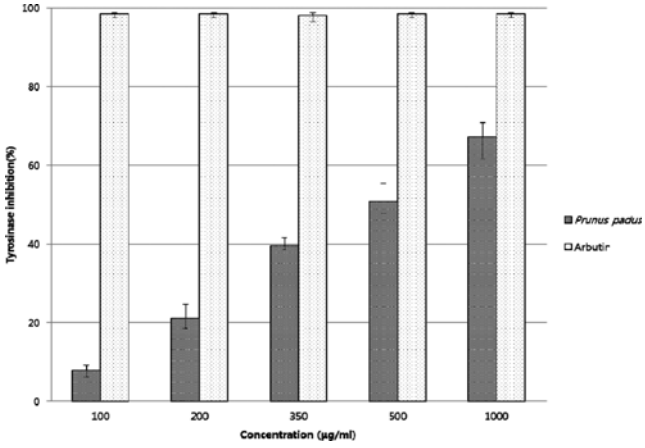

Fig. 6. Tyrosinase inhibition activity of *Prunus padus* bark extract.

Antiwrinkle effect of *P. padus* extract was conducted by elastase inhibition assay as shown in Fig. 5. Adenosine, officially recognized as an antiwrinkle cosmetic agent by Korea Ministry of Food and Drug Safety was applied as reference. Elastase inhibition was increased linearly as concentration of *P. padus* extract increased from 100 to 1,000 μg/ml. Elastase inhibition was 36% at 350 μg/ml. Compared with other natural antiwrinkle agents, *P. padus* extract indicated moderate elastase inhibition effects [29,30].

Whitening effect of *P. padus* extract was carried out by tyrosinase inhibition assay as shown in Fig. 6. Arbutin, officially certified as a whitening agent by Korea Ministry of Food and Drug Safety, was served as a standard. Tyrosinase inhibition effect of *P. padus* extract was 38% at 350 μg/ml, and it increased monotonically as it mounted from 100 to 1,000 μg/ml. Though native plant extracts showing strong whitening effect indicated 40-70% inhibition at 200 μg/ml, *P. padus* extract shows mild whitening effect [31,32].

4. Stability Test

The W/O/W emulsion prepared was checked by microscope and shown in Fig. 7. In 400 times magnification, W/O/W emulsion is clearly seen in the photographs. Lotion formulations containing 1%

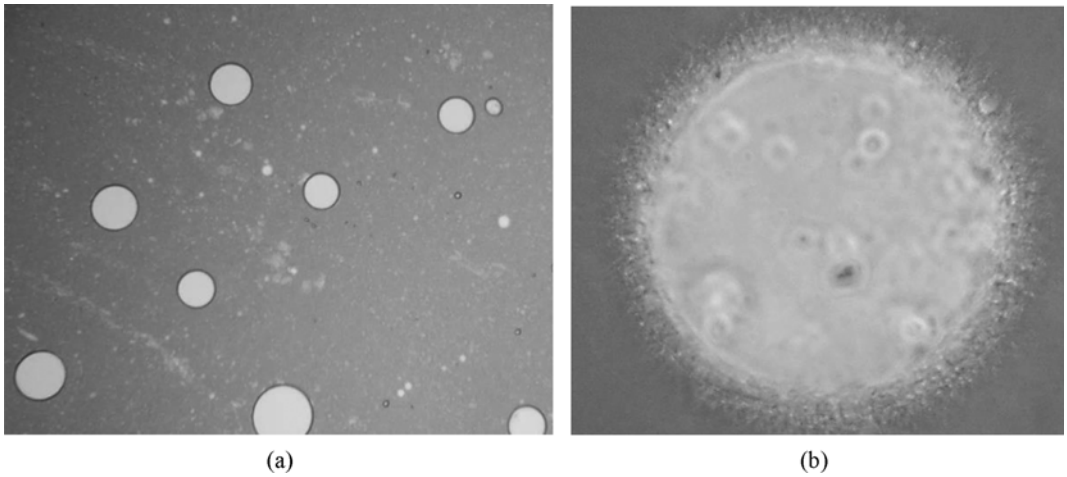

Fig. 7. Microscopic views of W/O/W emulsions including 1% *Prunus padus* bark extract. (a) ×100 magnification and (b) ×400 magnification.

Table 2. Stability test of lotion including 1% *Prunus padus* bark extract and control (without extract) at 4 ºC, 25 ºC and 47 ºC for 28 days

Temp.			0 Day	7 Day	14 Day	21 Day	28 Day
4 ºC	pH	Test	5.7	5.83	5.52	5.61	5.42
		Control	5.92	5.24	5.47	5.52	5.4
	Viscosity (cP)	Test	4540	4460	4500	4480	4490
		Control	4520	4670	4710	4690	4710
	Particle size (nm)	Test	421.1	406.5	368.4	404.4	381.2
		Control	425.1	418.9	379.2	362.1	372.1
	Appearance	Test	white, stable	white, stable	white, stable	white, stable	white, stable
		Control	white, stable	white, stable	white, stable	white, stable	white, stable
25 ºC	pH	Test	5.7	5.61	5.34	5.4	5.48
		Control	5.92	5.59	5.67	5.55	5.66
	Viscosity (cP)	Test	4540	4650	4570	4610	4630
		Control	4520	4570	4730	4620	4640
	Particle size (nm)	Test	421.1	417.2	380.6	392	414.9
		Control	425.1	383.4	420.8	413.9	401.1
	Appearance	Test	white, stable	white, stable	white, stable	white, stable	white, stable
		Control	white, stable	white, stable	white, stable	white, stable	white, stable
47 ºC	pH	Test	5.7	5.45	5.25	5.23	5.38
		Control	5.92	5.41	5.49	5.49	5.51
	Viscosity (cP)	Test	4540	4520	4320	4300	4340
		Control	4520	4540	4670	4610	4630
	Particle size (nm)	Test	421.1	392.1	367.2	361.6	390.5
		Control	425.1	404.5	419.4	384.6	397.3
	Appearance	Test	white, stable	white, stable	white, stable	white, stable	white, stable
		Control	white, stable	white, stable	white, stable	white, stable	white, stable

P. padus bark extract and control (no extract) were prepared to examine stability of the extract as cosmetics. Viscosity, pH, particle size, and appearance of lotions were measured at 4 ºC, 25 ºC and 47 ºC for 28 days as shown in Table 2. Viscosity, pH, and particle size of lotion containing 1% *P. padus* extract and control maintained stable condition for 28 days without phase separation. Particle size of lotions prepared maintained steady 362-426 nm levels, which is suitable for skin penetration. Thus, *P. padus* extract can be in good harmony with other cosmetic ingredients. Multiple emulsions generally maintained longer and more stable formulation than simple W/O or O/W emulsion in our lab even though extracts were different [27,32].

CONCLUSIONS

Prunus padus bark extract was tested to use as a natural cosmetic agent. *P. padus* bark extract was obtained by hot water extraction, maturing processes and reverse osmosis. The total polyphenol and flavonoid concentrations were 714.7±0.5 mg/g and 72.1±2.2 mg/g. Compared with other natural antioxidants, polyphenol concentration in *P. padus* bark extract was very high. *P. padus* bark extract showed lower cell toxicity in 100-500 μg/ml concentration by MTT assay. Antioxidative ability of *P. padus* bark extract was strong as DPPH free radical scavenging activity was higher than 70% at 100-1,000 μg/ml. Elastase inhibition (antiwrinkle effect) and tyrosinase inhibition (whitening effect) were mild as indicated by 36% elastase inhibition and 38% tyrosinase inhibition at 350 μg/ml, respectively. W/O/W lotion formulation containing 1% *P. padus* bark extract maintained stable condition in viscosity, pH, particle size, and appearance for 28 days. Particle size of lotions retained homogeneous 362-426 nm ranges throughout the experiment. From this study, *P. padus* bark extract indicated strong potential for a natural antioxidative cosmetic agent.

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