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Nelumbo nucifera extracts as whitening and anti-wrinkle cosmetic agent

Tagon Kim*, Hee Jin Kim*, Sung Ki Cho*, Whan Yul Kang, Hyun Baek**, Hye Young Jeon**, Boyoung Kim**, and Donguk Kim*,†**

*Department of Pharmaceutical Engineering, Inje University, Gimhae, Gyeongnam 621-749, Korea
**Amaranth Cosmetics, 1534-2, Songjung-dong, Gangseo-gu, Busan 618-817, Korea
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Abstract−Water extract from *Nelumbo nucifera* was tested for possible functional cosmetic agent. Whitening effect was measured by tyrosinase inhibition assay and DOPA-oxidase inhibition assay, and anti-wrinkle effect was checked by elastase inhibition assay. DOPA-oxidase inhibition effect (whitening effect) of *Nelumbo nucifera's* leaf, seed and flower extract was 59%, 57% and 50%, respectively. *Nelumbo nucifera's* leaf, seed and flower extract showed 56%, 49%, and 54% elastase inhibition (anti-wrinkle effect) at 200 μg/ml, while adenosine indicated 26% inhibition. Water cream including *Nelumbo nucifera's* root, leaf, flower, stem extract did not cause significant skin irritation. Water cream including 4% *Nelumbo nucifera* extract was stable for 30 days under various temperature conditions. From the study, *Nelumbo nucifera's* leaf, flower and seed extracts showed strong possibility for whitening and anti-wrinkle functional cosmetic agent.

Key words: *Nelumbo nucifera*, Cosmetics, Whitening, Anti-wrinkle, Safety

INTRODUCTION

Melanin produced at melanocyte in human epidermis is a major defensive chemical against ultraviolet of sunlight [1]. However, excessive ultraviolet increases the production of melanin pigment in human skin such as sunburn, liver spots, and freckles [2]. Melanin synthesis starts from tyrosine (amino acid) with tyrosinase as an enzyme and tyrosine is converted to DOPA, DOPA quinine, and eventually melanin by complex reactions [3]. During the process, several enzymes such as tyrosinase, tyrosinase-relative protein 1 (TRP1) and TRP2 are known to play important roles.

In dermis of human skin, collagens and elastines are well organized to give elasticity. When an outer stimulus such as UV and reactive oxygen species (ROS) is given in fibroblast cells, cells begin to generate collagenase and elastase, which degrades collagen and elastin, respectively [4]. The degradation is responsible for wrinkle formation and skin aging.

Functional cosmetics are defined in Korean Cosmetics Law as cosmetics including whitening, anti-wrinkle, and UV protection effects. Market size of functional cosmetics in Korea is 774 billion Won ($673 million) and has increased rapidly to become the second category among cosmetics [5].

According to the Korea Food and Drug Administration (KFDA), arbutin, ethyl ascorbyl ether, *Broussonetia* extract powder, and oil soluble *licorice* (Glycyrrhiza) extract are registered as whitening agents [6]. Among whitening agents, arbutin and ethyl ascorbyl ether have been widely used in whitening functional cosmetics. However, it was reported that arbutin caused minor skin irritation and that vitamin C derivative (ethyl ascorbyl ether) was easily degraded during storage [7].

As anti-wrinkle agents, retinol, retinyl palmitate, adenosine, and polyethoxylated retinamide are registered in KFDA [6]. However, retinol and vitamin A derivatives such as retinoic acids and retinaldehydes are known to cause skin irritation [7]. Many studies have been done to find new materials for whitening and anti-wrinkle agents. Typical whitening agents include Johimbe bark, Kazinoki Papermul-Berry, Runamoon, *Schizophyllum commune*, *Acanthopeltis japonica*, *Pimpinella brachycarpa*, citrus fruits, and Forsythia suspense [8,9]. As anti-wrinkle agents, Oat, *Geranium nepalense*, Spirulina, *Equisetum arvense*, *Zostera marina* have been studied by cosmetic companies [10,11].

Nowdays in Korea, cosmetics based on traditional Korean medicine are very popular and increasing in market share. In Dongeuibogam (Korean traditional medical book), *Nelumbo nucifera* flower is reported to stabilize the mind, prevent facial aging, and not to have poisonous properties [12]. Dongeuibogam also mentions *Nelumbo nucifera's* leaf to stop thirst and to treat fungi poisoning, and *Nelumbo nucifera's* root water extract to contain sweetness, to strengthen internal organs, to prevent parasites and not to have poisonous properties.

Nelumbo nucifera has several advantages compared with traditional Korean herbal medicine for cosmetic ingredients. First, it has been consumed for hundreds of years in Korean cuisine and there are no reports about significant malfunction, which suggests that *Nelumbo nucifera* is safe for food. Generally, ingredients safe for food are also safe for cosmetics. Secondly, Korea produces a large volume of *Nelumbo nucifera* and the price is cheap compared with traditional herbal medicines.

In the study, *Nelumbo nucifera's* root, leaf, flower, stem and seed extracts were collected, and cosmetic assays (efficacy, safety and stability test) were done to see possible application for functional cosmetic agent.

EXPERIMENTAL

1. *Nelumbo nucifera* Extraction

Nelumbo nucifera was purchased at Chilseo-myun Haman-gun,

†To whom correspondence should be addressed.
E-mail: pedkim@inje.ac.kr

Gyongnam. *Nelumbo nucifera's* root, flower, leaf, seed, and stem were ground and dried with freeze dryer. 2,000 ml of distilled water including 50 g of *Nelumbo nucifera* was heated at 100 °C until solution volume was reduced to 50% (1st extraction). 2,000 ml of fresh water was added in the solution and heated again until total solution volume became 50% (2nd extraction). Third extraction was done at the same condition. Final solution was cooled to room temperature and treated with preservatives including 20% propylene glycol (Sigma, USA) and 0.5% Phenonip (Sigma, USA). Whole solution was then filtered with 200 mesh (0.75 μm) filter (Millipore, USA), refrigerated for 24 hours and filtered again with mixed cellulose ester filter (0.45 μm) (Millipore, USA).

2. Cosmetic Assays

As efficacy test, anti-oxidation effect, whitening effect and anti-wrinkle effect were measured based on standard methods. Anti-oxidation effect was measured by DPPH free radical-scavenging assay [13]. 100 μl of DPPH (α,α-diphenyl-β-picrylhydrazyl, Sigma, USA) and 200 μl of the extract was reacted at room temperature for 20 min and absorbance was measured at 517 nm. DPPH inhibition (%) was calculated as follows:

$$\text{DPPH inhibition (\%)} = [1 - (\text{Exp.} - \text{Blank})/\text{Control}] \times 100 \quad (1)$$

Whitening effect was measured by yrosinase inhibition assay and DOPA-oxidase inhibition assay [14]. When L-tyrosine (Sigma, USA) was used as a substrate, the reaction mixture included 220 μl of 0.1 M potassium phosphate buffer (pH 6.8), 40 μl of 1.5 mM of L-tyrosine, 20 μl of mushroom tyrosinase (2,000 U/ml, Sigma, USA) and 20 μl of the extract. Reaction mixtures were incubated at 37 °C for 10 min and absorbance was measured at 490 nm using an ELISA reader (Synergy HT, BIOTEK, USA).

When DOPA was used as a substrate, the reaction mixture contained 200 μl of 0.1 M potassium phosphate buffer (pH 7.0), 20 μl of mushroom tyrosinase (2,000 U/ml) and 40 μl of the extract. Reaction mixtures were incubated at 37 °C for 6 min. 40 μl of 0.06 mM of DOPA was added in solution and incubated at 37 °C for 1 min. The absorbance was measured at 475 nm using an ELISA reader.

The anti-wrinkle effect was measured by elastase inhibition assay [15]. 200 μl of 0.12 M Tris-Cl buffer (pH 8.0, Sigma, USA) was mixed with N-succinyl-$(Ala)_3$-p-nitroanilide (Sigma, USA) to make 1.0 mM concentration. 20 μl sample and 20 μl of 2.5 U/ml porcine pancreas elastase (PPE, Sigma, USA) were mixed with solution. After incubating for 10 min at 25 °C, absorbance was measured at 410 nm by ELISA reader.

As *in vivo* safety test, a human patch test was done for 20 applicants from Amaranth Cosmetics. Patch including *Nelumbo nucifera* extract was put on a forearm using Haye's test chamber (Epitest ltd Oy, Finland) for 24 hours [6]. Skin condition in the tested area was compared with a blank to see the difference between samples.

As stability test, *Nelumbo nucifera's* root extract was mixed with water and left up to 30 days under room temperature, 40 °C, 50 °C and refrigeration. During the storage, physical appearance such as phase separation, color and fragrance was checked.

RESULTS AND DISCUSSION

1. Physical Property

Physical properties of *Nelumbo nucifera's* root, leaf, flower, stem and seed extracts were measured for appearance, pH, specific gravity, heavy metals and microbes. Table 1 shows the physical properties of *Nelumbo nucifera's* flower extract. *Nelumbo nucifera's* flower extract satisfied all cosmetic specifications [6]. Other parts of *Nelumbo nucifera* (root, leaf, seed and stem) showed the same results. From the experiment, *Nelumbo nucifera's* extracts seemed to have suitable physical properties for a cosmetic agent.

Table 1. Physical properties of *Nelumbo nucifera's* flower extract

Testing item	Specification	Results
Appearance	Dark Yellowish liquid	Confirm
pH	4.0-7.0	5.30
Confirm test 1	Light red brown	Confirm
Confirm test 2	Light red brown	Confirm
Specific gravity	0.98-1.04	1.022
Heavy metal	10 ppm	Confirm
Arsenic	2 ppm	Confirm
Microbes	100 cfu/ml	Confirm

2. Efficacy Test

Antioxidation effect of *Nelumbo nucifera's* leaf, root, flower, stem and seed extracts was measured by DPPH free radical scavenging assay as shown in Fig. 1. As concentration of extracts was increased, DPPH free radical scavenging activity was generally increased. At 100 μg/ml extract concentration, *Nelumbo nucifera's* leaf, flower and seed extract showed 78%, 78% and 80% anti-oxidation effect, respectively. IC_{50} (50% inhibition concentration) of *Nelumbo nucifera's* extracts is summarized in Table 2 with vitamin C (typical anti-oxi-

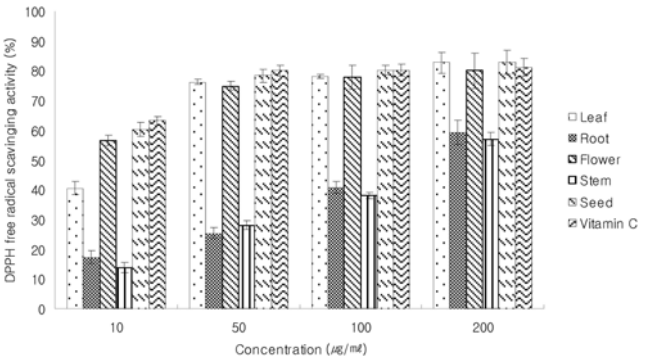

Fig. 1. Anti-oxidation effect of *Nelumbo nucifera's* leaf, root, flower, stem and seed extracts measured by DPPH free radical scavenging assay.

Table 2. Anti-oxidation effect of *nucifera's* extracts as IC_{50} (50% inhibition concentration) value

Extracts/reference	IC_{50} μg/ml
Flower	5
Seed	5
Leaf	29
Root	150
Stem	162
Vitamin C	5

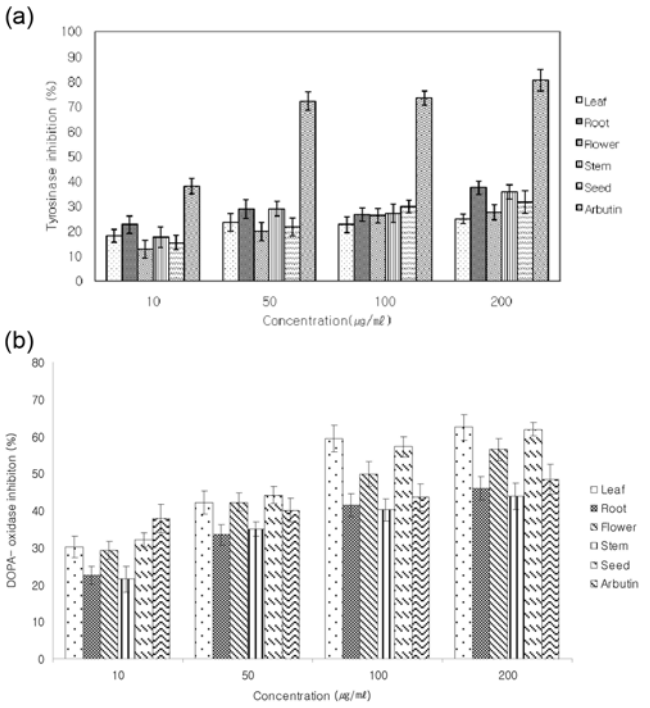

Fig. 2. Whitening effect of *Nelumbo nucifera's* leaf, root, flower, stem and seed extracts measured by (a) tyrosinase inhibition assay and (b) DOPA oxidase inhibition assay.

dation reference ingredient). The anti-oxidation effect of *Nelumbo nucifera's* flower and seed extract was outstanding and was as good as vitamin C. However, the antioxidation effect of *Nelumbo nucifera's* leaf, root and stem extracts was relatively lower compared with other parts.

Whitening effect of *Nelumbo nucifera's* leaf, root, flower, stem and seed extracts was measured by tyrosinase inhibition assay (Fig. 2(a)) and DOPA-oxidase inhibition assay (2(b)). In Fig. 2, arbutin was registered as a whitening agent by KFDA. Tyrosinase inhibition effect of all *Nelumbo nucifera's* extracts was significantly lower than that of arbutin. At 100 μg/ml extract concentration, *Nelumbo nucifera's* seed extract showed 30% tyrosinase inhibition and the other parts indicated 22-29% inhibition.

DOPA-oxidase inhibition effect of *Nelumbo nucifera's* extracts was much better than tyrosinase inhibition. At 100 μg/ml extract concentration, DOPA-oxidase inhibition effect of *Nelumbo nucifera's* leaf, seed and flower extract was 59%, 57% and 50%, respectively, whereas that of arbutin was 44%. From Fig. 2, *Nelumbo nucifera's* leaf, seed and flower extract did not have meaningful tyrosinase inhibition; however, they possessed strong DOPA-oxidase inhibition effect. Whitening effect did not necessarily require both pathway inhibition and DOPA-oxidase inhibition was strong enough to block melanin synthesis.

Anti-wrinkle effect of *Nelumbo nucifera's* leaf, root, flower, stem and seed extracts was measured by elastase inhibition assay as shown in Fig. 3. Adenosine registered anti-wrinkle agent by KFDA was used as a reference material. All parts of *Nelumbo nucifera's* extracts showed higher elastase inhibition effect than adenosine at concentration range tested. Among extracts, leaf, flower and seed extracts showed 56%, 54% and 49% elastase inhibition at 200 μg/ml, while

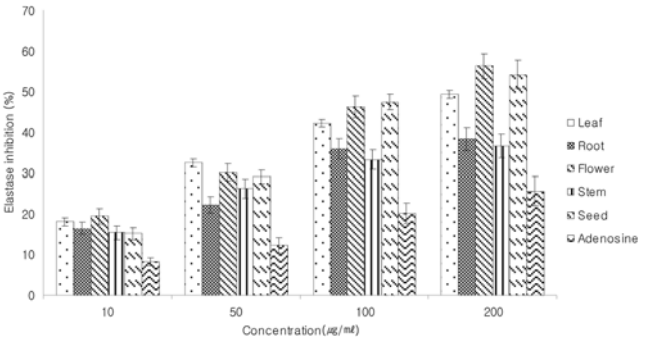

Fig. 3. Anti-wrinkle effect of *Nelumbo nucifera's* leaf, root, flower, stem and seed extracts measured by elastase inhibition assay.

adenosine indicated 26% inhibition. Therefore, *Nelumbo nucifera's* leaf, flower and seed extracts had strong potential for anti-wrinkle cosmetic agent. From efficacy test, *Nelumbo nucifera's* flower and seed extracts were shown to possess powerful anti-oxidation, whitening and anti-wrinkle capacity.

Nelumbo nucifera leaves were known to contain N-nornuciferine, O-nornuciferine, nuciferine, and roemerine as active compounds [16]. Nuciferine is an alkaloid found within the plants *Nymphaea caerulea* and *Nelumbo nucifera*. It has a profile of action associated with dopamine receptor blockade. It has been reported to induce catalepsy, inhibit spontaneous motor activity, conditioned avoidance response, amphetamine toxicity and stereotypy [17]. From the literature, nuciferine in Nelumbo nucifera may be biologically active material behind anti-oxidation, whitening and anti-wrinkle effect.

3. Safety Test

Cosmetic products are used for longer time compared with drugs, and safety is a very important factor in cosmetic tests. As *in vivo* test, a human patch test was done for 20 applicants from Amaranth Cosmetics. Patches including 1% extract solution were put on a forearm using Haye's test Chamber for 24 hours. Skin condition in tested area was compared with a blank to see any irritation after removal of the patch. For 20 volunteers, there was not any skin irritation for all test subjects when skin conditions were checked 30 min, 1day, 2 day and 3 day after patch removal. Same experiments were repeated for *Nelumbo nucifera's* root, leaf, seed and stem extracts and skin irritation was not observed.

To check safety for cosmetics including *Nelumbo nucifera* extracts, water cream containing 1% root, leaf, flower, stem extract and 4% combined extract was tested for skin irritation in 20 applicants. *Nelumbo nucifera's* root, leaf, flower and stem extracts did not cause any skin irritation within concentration range. From the result, *Nelumbo nucifera's* extracts were safe enough to be used in cosmetic agent.

4. Stability Test

To test the stability of extracts, 4% *Nelumbo nucifera* root extract was left for 30 days under room temperature, 40 °C, 50 °C and refrigeration. During the period, physical appearance was checked for phase separation, color change and fragrance. *Nelumbo nucifera's* root extract solution did not show significant variation for phase separation, color change and fragrance in 30 days regardless of temperature condition. Same experiments were repeated for leaf, flower, stem and seed extracts, and each extract solution showed stable phys-

ical appearance.

Stability test for cosmetics was performed using water cream including 1% *Nelumbo nucifera's* root extract, 1% leaf extract, 1% flower extract and 1% stem extract. Experimental condition applied was the same and the water cream maintained stable condition for 30 days under various temperature condition. From the result, *Nelumbo nucifera* extracts showed excellent stability for cosmetic agent.

CONCLUSIONS

From *Nelumbo nucifera*, water extracts of leaf, root, flower, stem and seed were obtained and functional cosmetic effect was studied. All of *Nelumbo nucifera* extracts satisfied cosmetic specifications. Anti-oxidation effect of *Nelumbo nucifera's* flower and seed extract was excellent such that 50% inhibition concentration for *Nelumbo nucifera* flower and seed extracts were 5 μg/ml each. DOPA-oxidase inhibition effect (whitening effect) of *Nelumbo nucifera's* leaf, seed and flower extract was 59%, 57% and 50%, respectively, whereas that of arbutin was 44%. *Nelumbo nucifera's* leaf, flower and seed extracts showed 56%, 54% and 49% elastase inhibition at 200 μg/ml while adenosine indicates 26% inhibition. Water cream including *Nelumbo nucifera* 1% root, leaf, flower, stem extract and 4% combined extracts was tested for skin irritation in 20 applicants and did not cause any skin irritation. Water cream including 4% *Nelumbo nucifera* extracts was stable for 30 days under room temperature, refrigeration, 40 °C and 50 °C. From the study, *Nelumbo nucifera's* flower and seed extracts showed strong potential for whitening and anti-wrinkle functional cosmetic agent.

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