

The Effect of Embelin's Physiological Activity as Cosmetics Ingredients

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ABSTRACT

Objectives: Embelin has been used in various treatments for skin diseases have excellent efficacy and have been widely used in folk remedies. Therefore, we want to observe the possibility of embelin as an additive to cosmetics.

Method: The cytotoxicity test was conducted to observe the safety of the component, and the antioxidant force of the component itself was observed using a DPPH solution. The effects on ROS generation using DCF-DA were observed. We observed the potential as a inhibitor of NO produced by inflammatory reactions and measured tyrosinase activity and melanin production to observe the possibility of utilization as skin whitening.

Findings: The results of observing the decimals using DPPH showed weak inhibition due to be weakly suppressed. Antioxidants were observed using Raw 264.7 macrophage. It inhibited ROS production in the cells induced by silica. In particular, production was inhibited on a concentration-dependent basis. As a result, the embelin has its own antioxidant function, has been shown to act as antioxidant in cells, and its concentration has inhibited NO production. Although the tyrosinase activity using L-dopa and L-tyrosine as substrates did not have any significant effect, melanin production in melanin cells resulted in concentration - dependent suppression.

Applications: Based on these results, it was found that embelin was highly antioxidant and was likely to be used as an anti-aging cosmetics ingredient.

Keywords: Embelin, Cosmetic, Whitening, ROS, anti-inflammation, anti-aging

Introduction

Embelin (2,5-dihydroxy-3-undecyl,1,4-benzoquinone) is a traditional medicinal herb effective ingredient that works against cancer¹ and various diseases, increasing the cytotoxic effects as well as the anti-bacterial activity and inhibiting the proliferation of various cancer cells². In addition, embelin is quinone with an alkyl-substituted hydrophilic acid, an important active ingredient contained in the seeds of *E. ribes*. Embelin's various pharmacological activities include

anti-inflammatory, fever, pain, anti-tumor, anti-cancer properties³. Recent interest in natural orientation or health has been increasing among modern people, and the preference for naturalism and well-being throughout life has led to numerous development of products using natural materials for both food and cosmetics, and the study of natural substances has emerged as a major challenge in the development of functional raw materials for cosmetics that impede aging. Among all the chemicals that are made from plant roots, leaves, flowers, and berries, the ingredients that exist in plants are called phytochemical⁴. Most cosmetics currently on the market are phytochemical, the main ingredients of vegetables and fruits that are actually available for consumption, and development of these products is on the rise. Therefore, in this study, we would like to explore the potential as a cosmetic material by measuring the antioxidant activity of Evelin, which is separated from natural products.

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Oxidative stress can be amplified by a continuous cycle of metabolic stress, tissue damage and apoptosis, leading to increased reactive oxygen production and destruction of free radical scavengers and scavenger systems, further exacerbating oxidative stress⁵. The skin is inherently very good antioxidant defense. However, it cannot protect damaged tissue from free radicals. Therefore, it is recommended that the body delay the rate of aging by minimizing oxidative stress through antioxidant supplements. ROS accelerates senescence by decreasing the DNA regeneration ability of cells and decreasing cell proliferation ability. ROS may also be produced during the process of making melanin pigments. Melanin is a pigment present in an organism that exists in the human skin and represents the color of the skin and acts as a defense function to protect the skin from ultraviolet rays. This melanin is formed by the melanin synthesis process that takes place in the melanocytes, which causes oxidative stress⁶.

Therefore, we monitor the possibility of natural antioxidants and whitening cosmetic ingredients using embelin belonging to polyphenol compounds, and confirm that they can be expected as a cosmetic material in terms of spices.

Material and Method

Reagent and Cell Culture: Embelin(2,5-Dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione, Embelic acid, Embetine), 3-(4,5-dimethyliazol-2-yl)-2, 5-diphenyl tetrazolium bromide(MTT), L-DOPA, Mushroom Tyrosinase, L-tyrosine purchased from Sigma-Aldrich, Inc.(St. Louis. Mo. USA). 2',7'-dichlorofluorescein diacetate (DCF-DA) was purchased from the Molecular Probe Co. (Eugene, OR, USA). Raw 264.7 macrophage and B16 F10 melanocyte were purchased from Seoul National University's Cellular Bank. Raw 264.7 macrophage and B16 F10 melanocyte were grown at a concentration of 37°C with 10% phthalate serum and a 5% concentration of phenicillin/streptomycin (100 IU/50 µg/mL).

Cytotoxicity Measurement using MTT: To confirm the cytotoxicity of embelin, the MTT method was applied. Raw 264.7 macrophage was used, and divided 1×10^4 cells per well in 96 well plates, cultivated for 24 hours, added sample by concentration, and cultivated at 37°C, CO₂ incubator for 72 hours. After 72 hours, the cultivation solution was removed, and 1 mL of 500 µg/

mL of MTT solution dissolved in Krebs solution (mM :NaCl 137, KCl 2.7, Na₂HPO₄ 0.4, MgCl₂ 0.5, HEPES [pH 7.4] 10, CaCl₂ 1.8, glucose 5) to each well and cultivated for 4 hours in dark. Then, the supernatant was removed, and 200 µL of DMSO was added to each well to dissolve MTT formazan. After completely dissolving MTT formazan for 10 minutes in room temperature, the absorbance was measured in 570nm.

DPPH Radical Scavenging Activity: 180 µL of 0.1 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH) solution dissolved in ethanol to 96 well plates embelin prepared in each concentration was added 20 µL each, cultivated for 30 minutes in 37°C in the dark were processed to absorbance measurement in 517 nm using FL 600 spectro fluorometer (BioTek, Winooski, VT, USA).

$$\text{DPPH radical scavenging activity (\%)} = 100 - \left\{ \frac{\text{Absorbance of added}}{\text{Absorbance of non-added}} \times 100 \right\}$$

Intracellular Oxidation Stress Measurement: This experiment statistical analysis was performed using SPSS Window Version 17.0 (SPSS Inc., Illinois, USA), and the significance was tested by Student's t-test. Was carried out three times or more independently under the same conditions noted in Mean ± standard deviation (Mean ± SD), The experiment determined that there was a statistically significant difference when the p value was less than 0.05.

In-vitro Tyrosinase Activity: L-DOPA was dissolved with 2 mg/mL of PBS(potassium phosphate buffer 0.1 M, pH 6.8), and the concentration of tyrosinase was 25units/mL. To 90 µL of tyrosinase, 10 µL of Embelin dissolved in different concentration was put into eppendorf tube, mixed, divided into 40 µL to 96 well plates, added 200 µL of L-DOPA (2 mg/mL), let it react for 1 hour in 37°C, and measured absorbance in 475 nm. 0.3 mg/mL of L-tyrosine was completely dissolved with PBS(potassium phosphate buffer 0.1 M, pH 6.8) and the tyrosinase concentration was 50 units/mL. To 90 µL of tyrosinase, 10 µL of Embelin dissolved in different concentration was put into eppendorf tube, mixed, divided into 40 µL to 96 well plates, added 200 µL of L-tyrosine (0.3 mg/mL), let it react for 1 hour in 37°C, and measured absorbance in 475 nm.

Melanin Product Inhibition: After dividing B16F10 melanin cell into 3 mL to 6 well plates, it was cultivated for 12 hours in phenol red-free DMEM solution include 10 % FBS. And the sample with different concentration were cultivated for 10 minutes in 37°C for preprocessing,

and processed 1 μM of $\alpha\text{-MSH}$ (melanocyte stimulating hormone) and cultivated for 72 hours in 37°C. After cultivation, 100 μL of 10mM PBS (sodium phosphate buffer pH 6.8) containing 1 % (w/v) Triton was added, shook for 5 minutes, moved to tube and centrifuged for 5 minutes in 10,000 rpm. Then, 100 μL of 1 N NaOH and 100 ml of purified water was added to cell pellet, and cultivated in 60°C for 1 hour to completely dissolve melanin, moved 200 μL to 96 well plates to measure absorbance of 405 nm. The experiment was conducted 4 times repeatedly in same condition, and obtained average to calculate melanin produced from each well using calibration curve from melanin standard.

Data Analysis and Statistical Verification: Result of experiment was displayed in average \pm S.D and the experiment outcome was verified by non-paired student's t-test.

Results and Discussion

Cell Viability Assay: The cell viability of embelin by MTT assay was 95% at 1 $\mu\text{g}/\text{mL}$, but the survival rate was 86% at 100 $\mu\text{g}/\text{mL}$ [Figure 1]. These results support research data demonstrating the efficacy of embelin to inhibit cancer cell proliferation⁷ and emelin, which is associated with IR inhibition, to induce cell growth to inhibit cell death and maintain cell viability⁸. Therefore, the cell death observed in this study is not caused by the cytotoxicity of embelin but rather by the cytotoxicity observed in the process of inhibiting cell proliferation. When the MTT assay time is reduced from 72 hours to 48 hours, The results can be guessed. Therefore, embelin is considered to be a safe ingredient when used as a cosmetic ingredient.

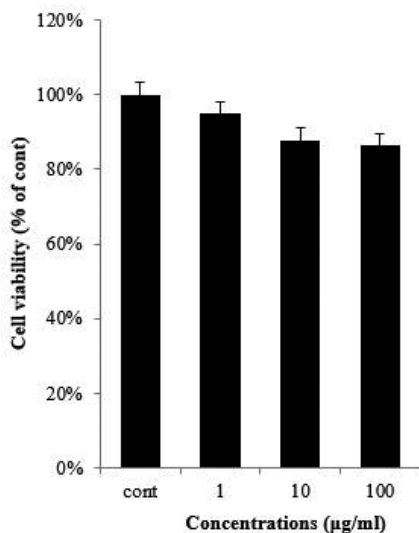


Figure 1: Cell toxicity of Embelin in Raw 264.7 macrophage. Results are means \pm SD from four separate experiments

Free Radical Scavenging Activity: Cold Ron⁹ observed radiation-induced ROS damage in DNA. In this study, the administration of embelin is regulated by reducing DNA damage due to UVB. Also, The skin is inherently very good antioxidant defense¹⁰. However, it cannot protect damaged tissue from free radicals. Therefore, it is desirable that the human body minimize oxidative stress through antioxidant supplements. ROS accelerates senescence by decreasing the DNA regeneration ability of cells and decreasing cell proliferation ability¹¹. Emelin is known to have antioxidant properties and was DPPH assay to observe antioxidants and activity on its own. At concentrations of 1 and 10 $\mu\text{g}/\text{mL}$, 1% and 5% radical scavenging ability was shown, respectively, and 14% scavenging activity was observed at 100 $\mu\text{g}/\text{mL}$. As a result, the antioxidant activity of the low concentration embelin was very small, but it was found that the thickening of the embelin caused the antioxidant activity [Figure 2].

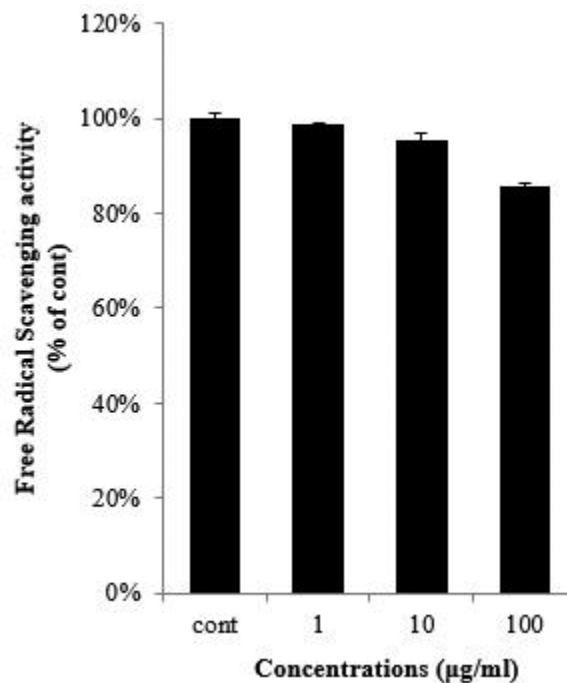


Figure 2: Antioxidant activity of Embelin in DPPH Assay. Results are means \pm SD from four separate experiments

In order to measure the antioxidative activity of embelin itself, the effect of inhibiting ROS formation was observed by using DCF-DA fluorescent material in Raw 264.7 macrophage induced at 1 mg/mL of Silica. Experimental results showed that the inhibitory effect on ROS production in cells was very strong and inhibited ROS production in a concentration dependent manner.

The inhibition of intracellular oxidation inhibited the production of 21% ROS at a low concentration of 1 µg/mL, while the inhibition of intracellular oxidation inhibited the production of 10 µg/mL 25% and 29% at 100 µg/mL [Figure 3], respectively. These results support the finding that embelin reduces lipid peroxidation levels in UVB-damaged cells and prevents DNA damage by UVB radiation¹².

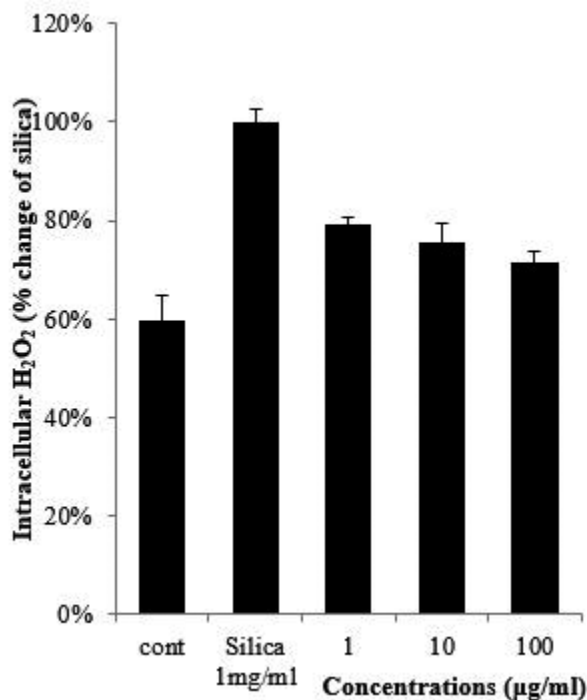


Figure 3: Antioxidant activity of Embelin 1 mg/mL silica-induced ROS production in Raw 264.7 macrophage. Results are means ± SD from 4 separate experiments

Whitening Action of Embelin: Tyrosinase, a polyphenol oxidizing enzyme, is deeply involved in the synthesis of melanin. In addition, tyrosinase inhibitors are used as skin whitening cosmetic materials, such as melanin, which is formed abnormally, as a pigment¹³. The effect of embelin on tyrosinase activity was measured using two substrates (L-Dopa, L-Tyrosine). Tyrosinase plays an important role in the early stages of melanin synthesis by oxidizing L-tyrosine (L-Tyr) to 3,4-dihydroxyphenylane (DOPA) and oxidizing DOPA to dopaquaone¹⁴. The effect of embelin on tyrosinase activity was measured using two substrates. As a result of measuring the activity of L-dopa by the substrate of mushroom tyrosinase 25unit, no enzyme inhibitory activity was observed [Figure 4], and the activity of L-tyrosine substrate was measured by using 50-unit tyrosinase enzyme. It did not appear [Figure 5]. Therefore, embelin did not inhibit the activity of

tyrosinase enzyme, and the possibility of using it as a whitening cosmetic ingredient due to inhibition of enzyme activity is small.

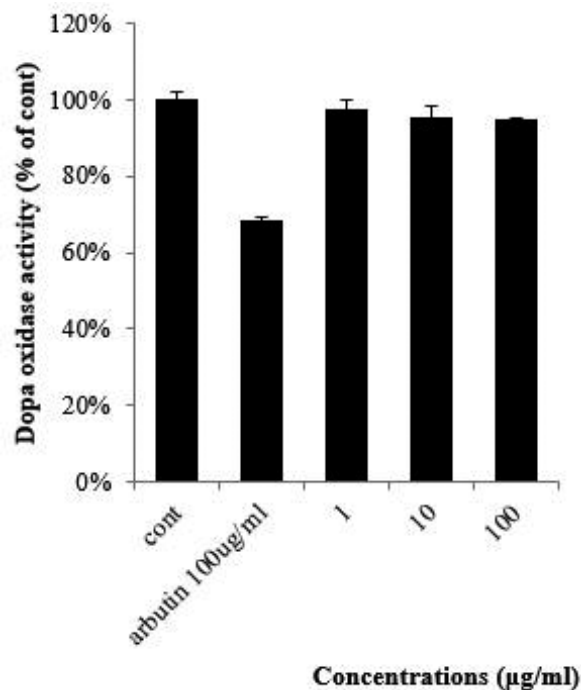


Figure 4: Effect of Embelin on L-dopa induced tyrosinase activity in Mushroom tyrosinase. Results are means ± SD from four separate experiments

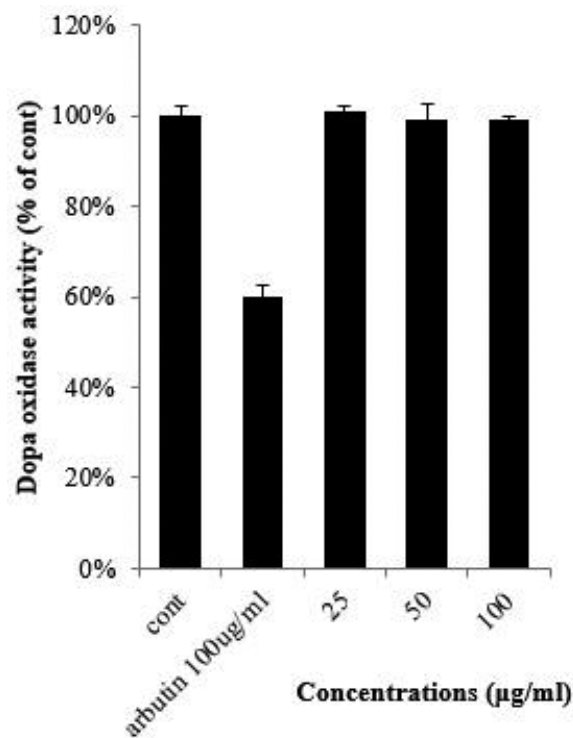


Figure 5: Effect of Embelin on L-tyrosine induced tyrosinase activity in Mushroom tyrosinase. Results are means ± SD from four separate experiments.

In order to investigate the effect of embelin on B16 F10 melanocyte, melanin production was measured by 1 μ M MSH-induced melanocyte-treated melanocytes, and melanin production was strongly inhibited. 33% Inhibition[Figure 6]. These results suggest that embelin can be used as a cosmetic ingredient for skin whitening function. It is not a mechanism to inhibit melanin production by inhibiting tyrosinase enzyme activity during the formation of melanin pigment, but it is directly involved in melanocyte cells to inhibit the formation of melanin pigment. Therefore, it is considered that embelin is useful value as a whitening cosmetic product.

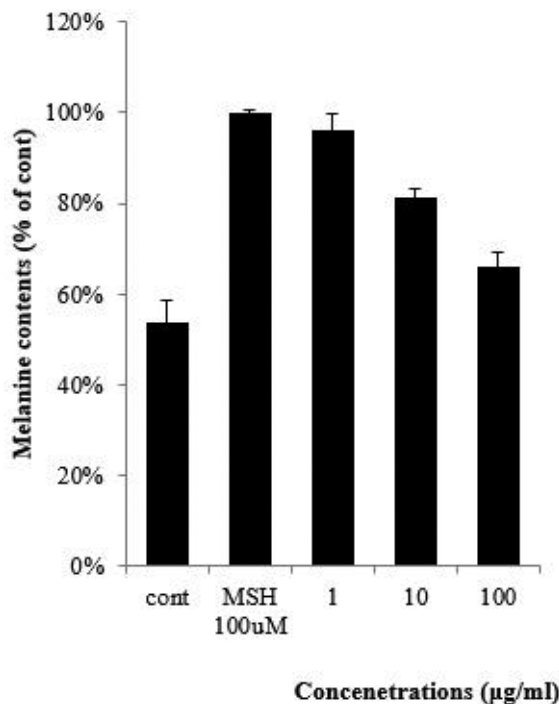


Figure 6: Effects of Embelin on melanin production by 1 μ M MSH in B16F10 melanocyte. Results are means \pm SD from four separate experiments.

Ethical Clearance: Not required

Source of Funding: Nil

Conflict of Interest: Nil

Conclusion

Embelin has been used in various treatments for skin diseases have excellent efficacy and have been widely used in folk remedies. Therefore, we want to observe the possibility of embelin as an additive to cosmetics.

The cytotoxicity test was conducted to observe the safety of the component, and the antioxidant force of the

component itself was observed using a DPPH solution. The effects on ROS generation using DCF-DA were observed. We observed the potential as a inhibitor of NO produced by inflammatory reactions and measured tyrosinase activity and melanin production to observe the possibility of utilization as skin whitening.

The results of observing the decimals using DPPH showed weak inhibition due to be weakly suppressed. Antioxidants were observed using Raw 264.7 macrophage. It inhibited ROS production in the cells induced by silica. In particular, production was inhibited on a concentration-dependent basis. NO production was observed in Raw 264.7 macrophage stimulated by LPS. It was strongly inhibited at all concentrations. As a result, the embelin has its own antioxidant function, has been shown to act as antioxidant in cells, and its concentration has inhibited NO production. Although the tyrosinase activity using L-dopa and L-tyrosine as substrates did not have any significant effect, melanin production in melanin cells resulted in concentration - dependent suppression. Based on these results, it was found that embelin was highly antioxidant and was likely to be used as an anti-aging cosmetics ingredient.

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