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Skin Lightening and Wrinkle Improving Efficacy of Organic *Portulaca oleracea* Extract in Skin Care Cosmetic

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Abstract

To find the new raw material having skin care activity, P. oleracea of organic native plant was extracted and concentrated with solvent extracting method. As a cosmetic active material, it is to develop an active raw molecule for discovering new skin pathway such as anti-aging, whitening and wrinkle skin improvement. The skin irritating evaluations (in-vitro) were performed to confirm the skin safety of its extract. Both free radical scavenging activity and collagen biosynthesis activity for skin anti-aging activity were evaluated with control sample (pure ascorbic acid). Melanin inhibition activity (in-vitro) as a skin lightning effect, to find the skin whitening was tested to use its material. As an application study, two high functional emulsion lotion having almost natural sources containing P. oleracea extract were formulated without any preservatives and totally evaluated the skin improvement effect (whitening, anti-aging activity and fine wrinkle diminish effect, human volunteer test, in-vivo). Therefore, the organic P. oleracea extract can contribute to the development of new natural active ingredients for advanced skin care cosmetic.

Keywords: *plant extract, anti-aging, whitening, skin care, cosmetic*

1. Introduction

It has been observed that the increase of anti-aging and melanin synthesis or uneven distribution can cause local hyper-pigmentation or spot. The pigmentary disorders are caused by various factors, including inflammation, imbalance of hormones, and genetic disorders [1]. Recently, a variety of oriental cosmetic products using bio- or natural botanical ingredients has been developed since 2004, which are focused on moisturizing, whitening, fine wrinkle improvement, and anti-aging. Especially, various additives which come from oriental medical plants have been discovered in term of their physiological properties as well as validate their effectiveness on the skin epidermis. Therefore, they have been subjected to several processes such as extraction, isolation and concentration, and popularly introduced to cosmetic industry [2-5]. On the other hand, *P. oleracea* extract is well-known as an only anti-inflammatory additive, which calms from the side-effect of chemical materials or outside irritations. But its material has not become clear for their functional pathway yet [6].

In this study, in order to find the new effectiveness, *P. oleracea* of organic native plant was extracted and high concentrated with solvent extracting method [7]. As a cosmetic material, it is to develop an active molecule for discovering new pathway such as anti-aging, whitening, and wrinkle improvement. At first, to confirm the skin irritation or safety of its extract, skin cytotoxicity (*in-vitro*) evaluations were performed. Second, free radical scavenging activity

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and collagen biosynthesis activity for skin anti-aging effectiveness were evaluated. Third, melanin inhibition activity to find the whitening was tested to use its material. Finally, as an application study, development of anti-aging emulsion cosmetic was formulated and totally evaluated the skin improvement effect (whitening & fine wrinkle diminish effect, human volunteer test, *in-vivo*) to apply high concentrated its extract.

Figure 1. Pictures of *Portulaca Oleracea* Plant from Origin to Extraction (a); Leaves and Flowers, (b); Dried Material, (c) Extracted Sample

2. Materials and Methods

2.1. Materials

Kojic acid, arbutin, and ascorbic acid were purchased from Sigma-Aldrich chemicals. All other reagents were used directly without further purification. Mushroom tyrosinase was also purchased from Sigma chemicals (EC 1.14, 18.1).

2.1. Equipment

Evaluation of whitening and diminishing fine wrinkle improvement were measured by Skin Analyzer (Model: Aramo TS, Aram Hubis Co. Ltd, Korea).

2.2. Preparation of Extract

A *P. oleracea* stems and leaves **(Figure 1)** were only used that has grown in Korea and Japan. 100g of hashed sample was soaked in 300mL of 50 wt% ethanolic aqueous solution at room temperature for 7 days. After filtration, the ethanol and water were evaporated to dryness under vacuum at 70-80°C. These extracts were used for the further biological study including anti-oxidative and free radical scavenging activities as well as anti-aging and tyrosinase inhibitive activity.

2.3. Free Radical Scavenging Effect

Scavenging effect against free radical generation was measured following the procedure of Fugita *et al.*, [8]. Reaction mixtures containing 1wt% of test samples (dissolved in ethanol) and 0.004wt% of 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) ethanolic solution were incubated at 25°C for 30 minutes. Absorbance of the resulting solutions were measured at 520nm using a Spectra Max Plus (Molecular Devices).

2.4. Determination of Collagen Biosynthesis Effect

Fibroblast was placed in 96-well microplates (2×10^4 cells/well) for 24 hrs. The medium was changed for serum free medium containing *P. oleracea* extract for 48 hrs of incubation. During the final 24 hrs, test sample (50μg/mL) was added to wells to promote collagen

synthesis. At the end of incubation, the wells were washed, supernatants were collected to determine the amounts of pro-collagen type I C-peptide with and enzyme-linked immunosorbent assay kit [9].

2.5. Tyrosinase Inhibitive Activity

Skin whitening test was performed by tyrosinase inhibitive activity from founded cell based general method. Tyrosinase activity is generally determined by spectrophotometer. The procedure followed that described by Vanny *et al.*, [9, 10]. For the assay, the test reaction mixture was prepared by adding 0.5mL of a solution of the ramulus mori compound, to which 200 units of mushroom tyrosinase had been added, to 0.5mL of 0.1 mg/mL L-tyrosine and 0.5mL of 50mM sodium phosphate buffer (pH 6.8) of the test mixture. After incubation for 10 minutes at 37 °C, we measured tyrosinase inhibitive activity by absorbance at 475 nm. We determined the effect of the test compound on tyrosinase inhibition by IC_{50}, the concentration of the compound at which half the original tyrosinase activity is inhibited. We calculated the percent the inhibition of tyrosinase activity as follows.

$$\% \text{ inhibitive activity} = [(A-B)/A] \times 100$$

Where A = absorbance at 475 nm without test sample, and B = absorbance at 475 nm with test sample. Our ramulus mori compound is more potent tyrosinase inhibitor than kojic acid and arbutin. The compound shows a strong inhibitive effect on tyrosinase at very low concentration.

2.6. Measurement of Melanin Contents in B16 Melanoma

B16 melanoma cells were purchased from Korean Cells line bank and cultured in DMEM (Dubecco's modified eagle's medium, sigma, D-2902, St. Louis, MO 63178 USA) supplemented with penicillin(100 U/mL), and 10% fetal bovine serum (Fetal bovine serum, gibico, 26140-079, Invitrogen) at 37°C in an incubator flushed continuously with 5% CO_2. B16 melanoma cells cultured above conditions were seeded at 2 x 10^5 cell/mL in 12-multiwell plates (Nunc). After one day, media were changed and 0.34 g/mL of a-MSH (α-Melanocyte stimulating hormone, Sigma, M-4135) was treated. The desired concentrations of samples were added to each well. The cells were incubated at 37°C in a humidified incubator for four days and media of each well was transferred into 15mL tube. After washing with PBS (phosphate buffered saline, 136.89 mM NaCl, 2.68 mM KCl, 8.06 mM Na_2HPO_4 pH7.2), 1.47 mM KH_2PO_4, pH7.4), 100 mL of Typsin-EDTA (Gibco, 25300-054) was treated in each well in order to collect remained cells of well, Cells and media were centrifuged at 3000rpm for 30 min and supernatants were discarded. 20 min Freed melanin was transferred 96 well plates (Nunc) and measured by ELISA reader (Tecan A-5082, Austria) at 470 nm [11].

2.7. Human Volunteer Evaluation (*in-vivo*)

Skin improving test of anti-aging and whitening lotion was applied twice a day on eye zone of the face using 7 male volunteers (25-57 years old) respectively for 42 days. Skin clearing activity and fine wrinkle improvement were measured respectively interval one week after application. Wrinkle and whitening activity were evaluated using the Aramo TS (Aram Hubis, Korea) in incubation room at 25°C and 50% relative humidity (RH) [12, 13]

3. Result and Discussions

3.1. High Concentrated Extract of P. oleracea and Determination of Key Ingredients:

The high concentrated extract was extracted from dried plant with 50wt% ethanol aqueous solution. Then, it was analyzed by HPLC, TLC, and spectrophotometer. Yield was 17.9 wt% of powder. Appearance was pale yellowish color with special odor. The major ingredients into its extract using HPLC was contained in polysaccharide, starch, omega-3 fatty acid (linoleic acid), vitamin C, vitamin E, vitamin B_1, carotene, tannin and a few minerals such as Ca, Zn, Fe, *etc* (Figure 2).

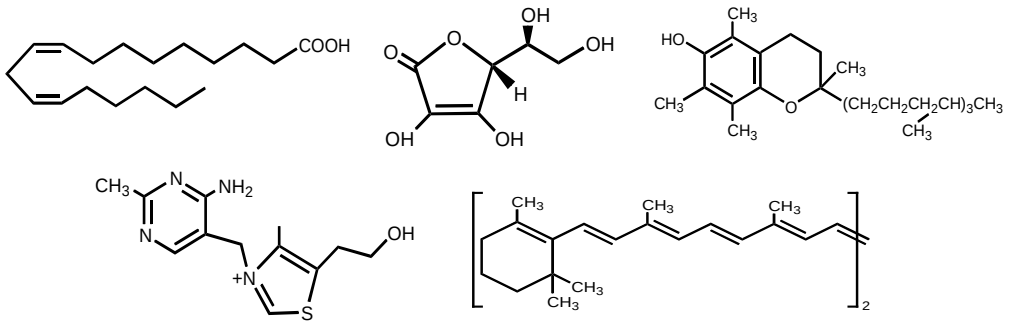

Figure 2. Major Active Ingredients in *P. oeracea* Extract; (a) Omega-3 Fatty Acid (Linolein Acid), (b) Vitamin C, (c) Vitamin E, (d) Vitamin B_1, (e) Carotene

3.2. Free Radical Scavenging Effect

To test free-radical scavenging activity, we applied 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as a free radical source. Free radical scavenging activity of *P. oleracea* extract was very high compared with ascorbic acid, arbutin and kojic acid (Figure 3). Free-radical strong scavenging activity found that was as follows *P. oleracea* extract > ascorbic acid ≥ kojic acid > arbutin.

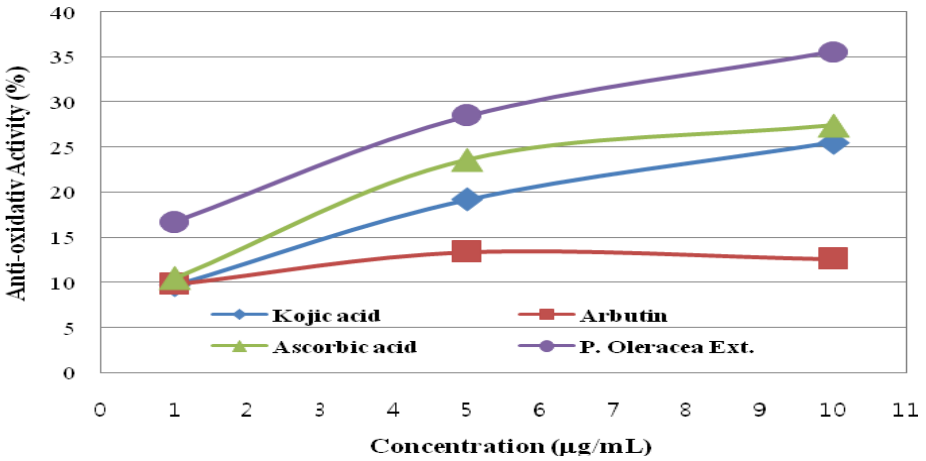

Figure 3. Anti-oxidative Activity of *P. oleracea* Extract by Measuring the DPPH Method

3.3. Collagen Synthesis Activity (*in-vitro*)

In collagen synthesis through fibroblast culture, *P. oleracea* extract was significantly increased with protein synthesis compared with other three ingredients (Figure 4). Collagen synthesis assay is summarized in Figure 4. Collagen synthesis increased 23.5% by *P. oleracea* extract, -12.7% by kojic acid, -8.5% by arbutin, and 9.6% by ascorbic acid at 5x 10^{-7} M respectively. Therefore, we suggest that the protein induced by *P. oleracea* extract may be a one of a number of cell adhesion proteins.

3.4. Melanin Inhibitive Test *(in-vitro)*

Inhibitive activity of *P. oleracea* extract for tyrosinase, compared with ascorbic acid, kojic acid and arbutin was show in Figure 5. *P. oleracea* extract showed higher tyrosinase inhibitive activity than arbutin and kojic acid depend on increasing concentration, but it was same activity when comparison *P. oleracea* extract with ascorbic acid was measured (Figure 5; tyrosinase inhibitive activity: *P. oleracea* extract = ascorbic acid > kojic acid > arbutin). This result can be recognized to decrease tyrosinase inhibition activity due to the several vitamin complexes and contained several minerals such as Ca, Zn, and Fe.

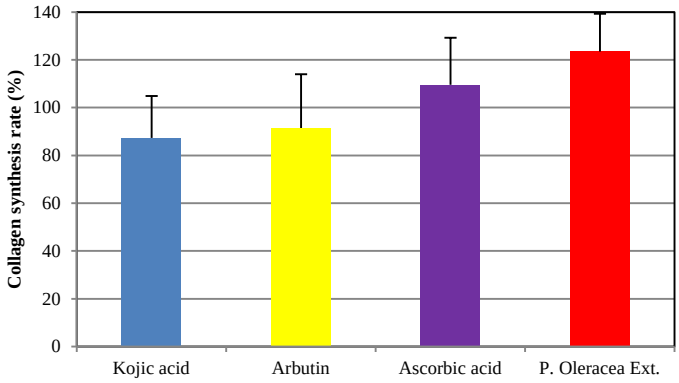

Figure 4. Collagen Synthesis Activity of *P. oleracea* Extract with Fibroblast Culture

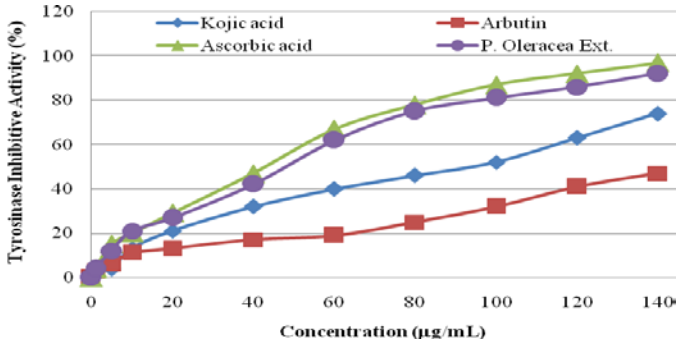

Figure 5. Tyrosinase Inhibitive Activity of *P. oleracea* Extract Depend on Increasing Concentration Compared with other Ingredients

3.5. Inhibition of Melanin Formation in B16 Melanoma Cell (*in-vitro*)

Inhibitive activity of *P. oleracea* extract for melanin formation in B16 melanoma was measured compared with kojic acid, arbutin, and ascorbic acid. Inhibitive activity of *P. oleracea* extract was not found within ranges of test concentrations (Figure 6). Because *P. oleracea* extract molecule was contained in high concentrating actives within vitamin complexes, could not easily permeate cell membrane, inhibitive activity of *P. oleracea* extract against melanin formation in B16 melanoma might be low, even though *P. oleracea* extract showed high inhibitive activity for tyrosinase. We will carry out further study with other evaluation systems to verify exact action mechanism of *P. oleracea* extract.

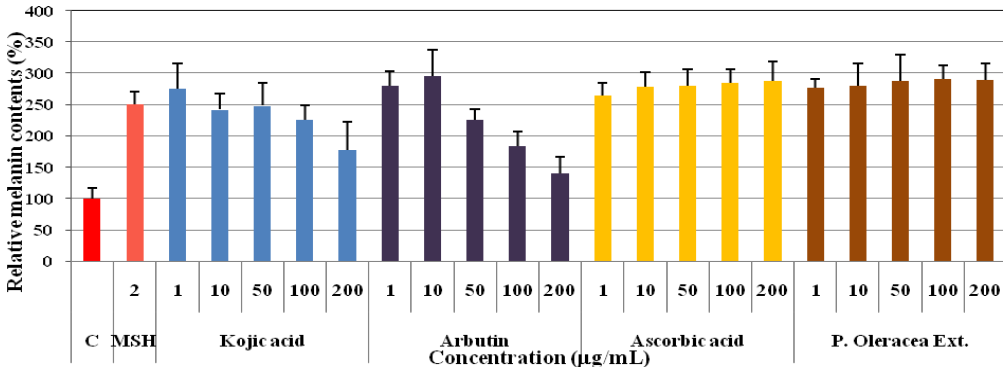

Figure 6. Inhibitive Activity of Melanin Formation in B16 Melanoma of *P. oleracea* Extract with Fibroblast Culture compared with Kojic Acid, Arbutin, and Ascorbic Acid

3.6. Skin Whitening and Fine Wrinkle Diminishing Activity (in-vivo)

3.6.1. Whitening Effect

Skin whitening activity of final formulation was evaluated and compared with before treatment (Figure 7) by measuring volunteer skin with 3D-images with Aramo TS (Aram Hubis Co., Ltd, Korea). Figure 7 shows the changes of skin clearing activity. As shown in Figure 7 (placebo application, left), the skin clearance was no changed prior to the application. After 6 weeks of applying final formulation (Figure **7,** right), the skin epidermis tended to be a change of skin clearance. It was diminished and removed pigmentation. However, there was no change on skin whitening improvement in four weeks following placebo application.

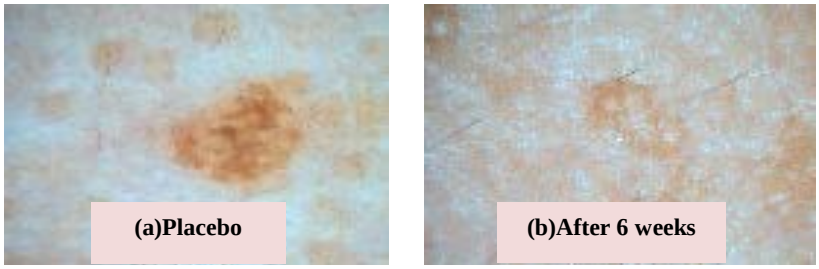

Figure 7. Skin Whitening Activity of Whitening Lotion with *P. oleracea* Extract using Human Volunteers; (a) Placebo Application, (b) Application after 6 Weeks (n=6, Application Twice a Day for 6 Weeks)

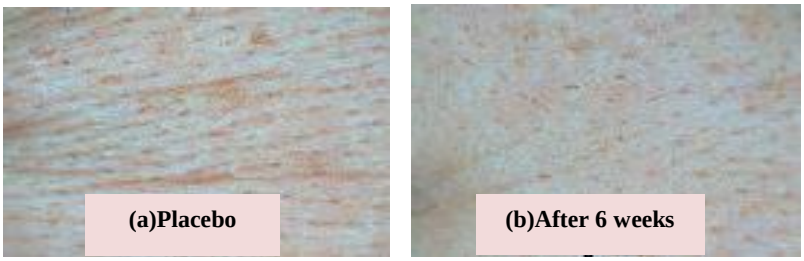

Figure 8. Fine Wrinkle Diminish Evaluation of Anti-aging Essence with *P. oleracea* Extract using Human Volunteers; (a) Before Application, (b) Application after 6 weeks (n=6, Application Twice a Day for 6 Weeks)

3.6.2. Anti-wrinkle Effect

Fine wrinkle diminishing effect was carried out same samples with final formulations. Skin conditions after application were observed by measuring volunteer skin with 3D-images with Aramo TS. As shown in Figure 8 (placebo formulation, left), the skin clearance was no changed prior to the application. After 6 weeks of applying final formulation, the skin epidermis tended to be a change of skin clearance. It was recognized to reduce fine wrinkles (Figure 8, final formulation, right).

3.7. Development of Formulation Containing Plant Origin

As an application, whitening lotion and anti-aging lotion of almost 100wt% natural sources containing *P. oleracea* extract were developed except for preservatives. Not only Figure 9 (a) shows a whitening essence but also Figure 9 (b) shows anti-aging lotion.

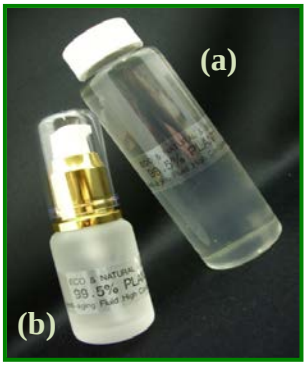

Figure 9. Two Formulations of 99.5 % Natural Origin using *P. oleracea* Extract; (a) Whitening Lotion, (b) Anti-aging Essence

4. Conclusions

This study was conducted to find new sources of additional performances to enhance the protective functions of cosmetic formulations. We demonstrated that *P. oleracea* extract have potent anti-oxidative effect and/or free radical scavenging activity. Additionally, this extract could know that collagen synthesis was increased by protein synthesis. In terms of tyrosinase inhibitive activity were significantly increased compared with kojic acid and arbutin. This extract has potent diminished fine wrinkle and clearing activity of the skin through human

volunteers. Therefore, this study can contribute to the development of new organic natural source for formulating advanced skin care cosmetics.

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References

[1] M. Mizuno and T. Tanaka, Fragrance Journal, vol. 30, **(1986)**, pp. 2-9.
[2] K. H. Kim, K. I. Ko, E. J. Kang, E. K. Yang and S. N. Park, J. Soc. Cosmet Scientists Korea, vol. 30, no. 3, pp. 329-343. **(2004)**.
[3] S. B. Shim, S. G. Oh and Y. J. Chun, Journal of the Korea Academia-Industrial cooperation Society, vol. 12, no. 5, **(2011)**, pp. 2434-2438.
[4] S. B. Shim, S. G. Oh and Y. J. Chun, Journal of the Korea Academia-Industrial cooperation Society, vol. 12, no. 9, **(2011)**, pp. 4257-4260.
[5] S. B. Shim and Y. J. Chun, Journal of the Korea Academia-Industrial cooperation Society, vol. 13, no. 11, **(2012)**, pp. 5623-5627.
[6] P. Kim and K. A. Hyeon, J. Soc. Cosmet Scientists Korea, vol. 31, no. 1, **(2005)**, pp. 51-58.
[7] A. N. Rashed, F. U. Afifi, M. Shaedah and M. O. Taha, Jouranl of Pharmaceutical Sciences, vol. 17, no. 1, **(2004)**, pp. 37-45.
[8] M. Cuendet, K. Hostettmann, O. Potterat and W. Dyatmiko, Irioid, Heiv Chim Acta., vol. 80, **(1997)**, pp. 1144-1152.
[9] S. Ando, O. Ando, Y. Suemoto and Y. Mishima, J. Invest Dermatol, vol. 100, **(1993)**, pp. 150-155.
[10] G. Imokawa and Y. Mishima, J. Invest Dermatol, vol. 83, **(1984)**, pp. 196-201.
[11] K. Maeda and M. Jukuda, J. Soc Cosmet Chem., vol. 42, **(1991)**, pp. 361-368.
[12] T. Mosmann, J Immunol Methods, vol. 65, **(1983)**, pp. 55-63.
[13] R. F. Diegelmann and B. Peterkofsky, Dev Biol., vol. 28, **(1972)**, pp. 443-453.

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I am working at Soongsil University as a professor. Also, I am working at Korea Kolmar Co., Ltd. as well as Cosmecca Korea Co., Ltd. as a technical advisor. And I have variously experienced in field of cosmetic industry; Hankook cosmetic Co., Ltd. R&D Center for 5 years, CJ group, cosmetic division skin science R&D Center for 8.5 years, NIKKO Chemicals Co., Ltd. Cosmos Technical Center in Tokyo Japan for 5 years, LOREAL KSP R&D Center in PARIS FRANCE for 2 years. My major job is develop the new raw materials for cosmetic fields such as moisturizing actives, whitening and anti-wrinkle actives, texture modifiers, uv light absorbers, plant extract, etc.

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