J. Microbiol. Biotechnol. (2015), 25(4), 448–451
http://dx.doi.org/10.4014/jmb.1409.09033

jmb

# Whitening Effect of Octaphlorethol A Isolated from *Ishige foliacea* in an *In Vivo* Zebrafish Model S

**Kil-Nam Kim[1†], Hye-Mi Yang[2†], Sung-Myung Kang[2], Ginnae Ahn[3], Seong Woon Roh[1], WonWoo Lee[2], Daekyung Kim[1*], and You-Jin Jeon[2*]**

[1]*Jeju Center, Korea Basic Science Institute (KBSI), Jeju 690-140, Republic of Korea*
[2]*Department of Marine Life Science, Jeju National University, Jeju 690-756, Republic of Korea*
[3]*Department of Marine Bio-Food Sciences, Chonnam National University, Yeosu 550-749, Republic of Korea*

*Received: September 12, 2014*
*Revised: October 20, 2014*
*Accepted: October 22, 2014*

First published online
October 23, 2014

*Corresponding authors
D.K.
Phone: +82-64-754-3475;
Fax: +82-64-756-3493;
E-mail: dkim@kbsi.re.kr
Y.-J.J.
Phone: +82-64-754-3475;
Fax: +82-64-756-3493;
E-mail: youjinj@jejunu.ac.kr

†These authors contributed equally to this work.

Supplementary data for this paper are available on-line only at http://jmb.or.kr.

pISSN 1017-7825, eISSN 1738-8872



In a previous study, we isolated octaphlorethol A (OPA) from *Ishige foliacea* and evaluated its anti-melanogenesis activity in a murine melanoma cell line. However, the whitening effect and toxicity of OPA have not yet been examined *in vivo*. Therefore, in this study, we investigated the inhibitory effect of OPA on melanin synthesis and tyrosinase activity in an *in vivo* zebrafish model. More than 90% of subject embryos survived upon exposure to OPA concentrations below 25 μM, which was not significantly different from the finding in the control group. OPA markedly inhibited melanin synthesis and tyrosinase activity in a concentration-dependent manner.

**Keywords:** Octaphlorethol A, melanin synthesis, tyrosinase, whitening, zebrafish

Skin pigmentation results from the production and distribution of melanin in the epidermis, and is the major physiological defense against solar irradiation. However, increased melanin synthesis can darken the skin and induce a number of hyperpigmentary skin conditions, such as freckles, chloasma, age spots, and actinic keratosis [7, 10]. Therefore, there is a need to develop safer and more effective skin-whitening agents.

The zebrafish (*Danio rerio*) is a small tropical freshwater fish that has emerged as a useful vertebrate model organism because of its small size, large clutches, transparent body, and physiological similarity to mammals, and low cost of breeding [5, 6, 16]. The administration of drugs and other small molecules to zebrafish is particularly easy, because the early-stage embryo rapidly absorbs small molecular compounds in the bathing medium through the skin and gills. In addition, zebrafish have melanin pigments on the body surface, which allow observation of pigmentation without the need for complicated experimental procedures [4]. Therefore, the zebrafish is an ideal model for the study of melanogenesis in the context of skin whitening [3, 4].

Phlorotannins are a class of polyphenolic compounds that are one of the most common classes of secondary metabolites in marine algae. Phlorotannins have been shown to have a variety of biological functions, including anticoagulant, anti-melanogenesis, antifungal, anti-inflammatory,

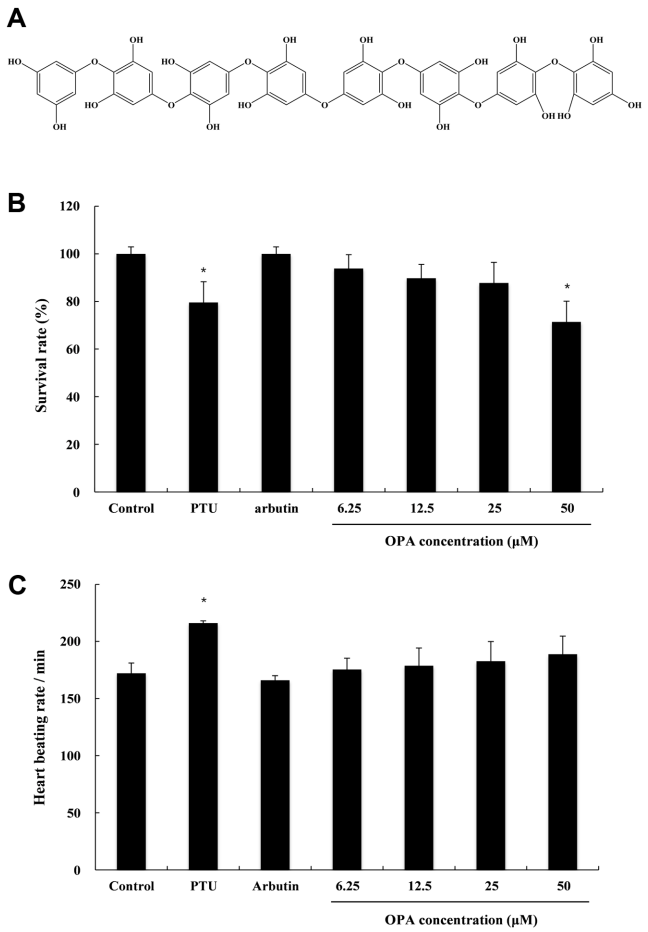


**Fig. 1.** (**A**) Chemical structure of OPA and its effect on (**B**) survival and (**C**) heart rate.

Methods are described in the supplementary information. 1-Phenyl-2-thiourea (PTU; 50 μM) and 500 μM arbutin were used as the positive control treatments. The experiments were conducted in triplicate and the data are expressed as the mean ± standard error. Statistical tests were performed to compare the experimental groups with the control groups. *$p < 0.05$.

and anticancer activities [2]. Octaphlorethol A (OPA, purity: ≥95.0%; Fig. 1A) is a phlorotannin isolated from the marine algae *Ishige foliacea*, and has been reported to possess several biological activities, including anti-inflammatory [13] and antidiabetic effects, which it is believed to exert by mediating changes in glucose uptake and reducing oxidative stress [11, 12]. Furthermore, we previously demonstrated that OPA inhibits melanogenesis induced by alpha-melanocyte-stimulating hormone (α-MSH) in B16F10 melanoma cells [9]. However, the whitening effect and toxicity of OPA have not been examined *in vivo*. Therefore, in this study, we examined the inhibitory effects of OPA on melanin synthesis and tyrosinase activity in zebrafish.

Several compounds and molecules that are known to inhibit melanin biosynthesis are used in cosmetic products. Many skin-lightening products such as linoleic acid, hinokitiol, kojic acid, and naturally occurring hydroquinone and catechol have been reported to inhibit melanogenesis [17]; however, these compounds show low clinical activity, and produce side effects and toxicity. These shortcomings of available melanin synthesis inhibitors have led to the search for new agents, such as molecules derived from plant-extract-based skin-lightening compounds, in order to develop more effective cosmetic products with fewer side effects [15]. Among plant-derived molecules, polyphenols have attracted considerable interest, and have been examined in the context of several biological functions, including anticancer, antioxidant, anti-inflammatory, and anti-melanogenesis activities. The purpose of this study was to determine the capability of the algae-derived polyphenol OPA to inhibit tyrosinase and melanin synthesis *in vivo*, and to evaluate its toxicity.

First, experiments were performed to determine the toxicity of the melanogenic inhibitors 1-phenyl-2-thiourea (PTU), arbutin, and OPA by measuring the survival rate and heart rate of the exposed zebrafish embryos. The survival rates of zebrafish embryos exposed to these melanogenic inhibitors are shown in Fig. 1. More than 90% of the embryos survived treatments with arbutin and lower concentrations of OPA (6.25, 12.5, and 25 μM), and the survival rates of these groups were not significantly different from that of the control group. The survival rate of the embryos exposed to 50 μM PTU and 50 μM OPA was significantly decreased compared with that of the control group (Fig. 1B). In the heart rate test, PTU exposure resulted in a significant increase in heart rate compared with the heart rate in the control group, whereas OPA and arbutin did not (Fig. 1C). OPA doses below 25 μM were chosen for studies to determine the effects of OPA on tyrosinase activity and melanin synthesis, because these doses did not alter heart rates or survival rates.

Because melanin synthesis is ultimately regulated by tyrosinase [1], we determined whether OPA inhibits melanin synthesis and tyrosinase activity. The effects of the melanogenic inhibitors used in these studies on pigmentation are shown in Fig. 2. Arbutin and 6.25 μM OPA showed little effect on pigmentation of the zebrafish embryo body (Figs. 2B and 2D). In contrast, 50 μM PTU and 25 μM OPA remarkably decreased pigmentation (Figs. 2C and 2F). Others have similarly reported that phlorotannins from *Ecklonia cava* inhibited increased pigmentation caused by UV-B radiation in zebrafish [3].

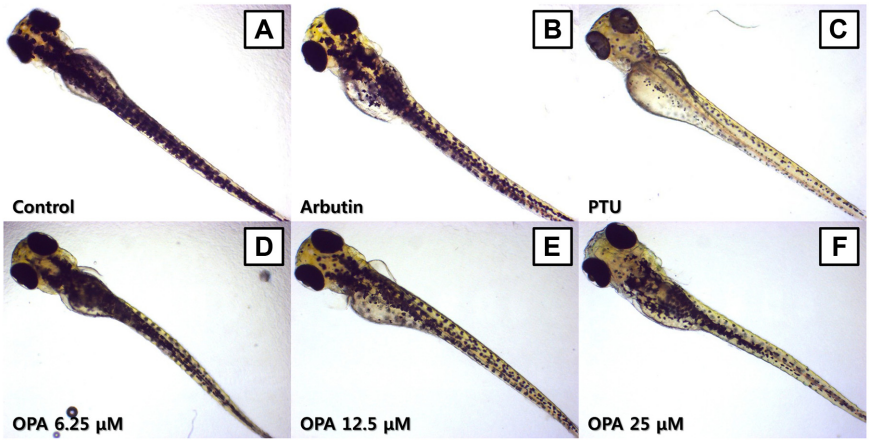


**Fig. 2.** Effect of melanogenic inhibitors on pigmentation in zebrafish embryos.
Methods are described in the supplementary information. Zebrafish pigmentation was assessed using a microscope. (**A**) Untreated zebrafish embryos were used as a control group. (**B**, **C**) 1-Phenyl-2-thiourea (PTU; 50 μM) and 500 μM arbutin were used as positive control treatments. (**D**, **E**, **F**) 6.25–25 μM OPA.

To quantify the inhibitory activity of the melanogenic inhibitors used in this study, we measured the melanin synthesis and tyrosinase activity using whole zebrafish extracts. PTU (50 μM) and 500 μM arbutin served as positive controls, and the embryos exposed to them showed significantly reduced melanin synthesis compared with that in untreated embryos (44.5% and 15%, respectively). OPA was found to inhibit melanin synthesis in a dose-dependent manner (5.2%, 15.4%, 25.6%, and 27.8% at 3.125, 6.25, 12.5, and 25 μM, respectively; Fig. 3A). Interestingly, OPA doses higher than 12.5 μM were significantly more effective than 500 μM arbutin at inhibiting melanin synthesis. PTU treatment (40.2%) and treatment with OPA doses higher than 12.5 μM (25.3% and 32.1% at 12.5 and 25 μM, respectively) significantly inhibited tyrosinase activity, whereas arbutin (17.3%) and OPA doses less than 12.5 μM produced little tyrosinase inhibition (12.0% and 15.7% at 3.125 and 6.25 μM, respectively; Fig. 3B). These results suggest that OPA downregulated tyrosinase activity in exposed zebrafish embryos, and that this inhibitory effect led to decreased melatonin synthesis.

The results reported herein contribute to the growing body of knowledge regarding melanogenic inhibitors isolated from algae, which have yielded information on relative efficacy and molecular mechanisms that serve to guide future studies. Kim *et al.* [8] reported that phlorotannins

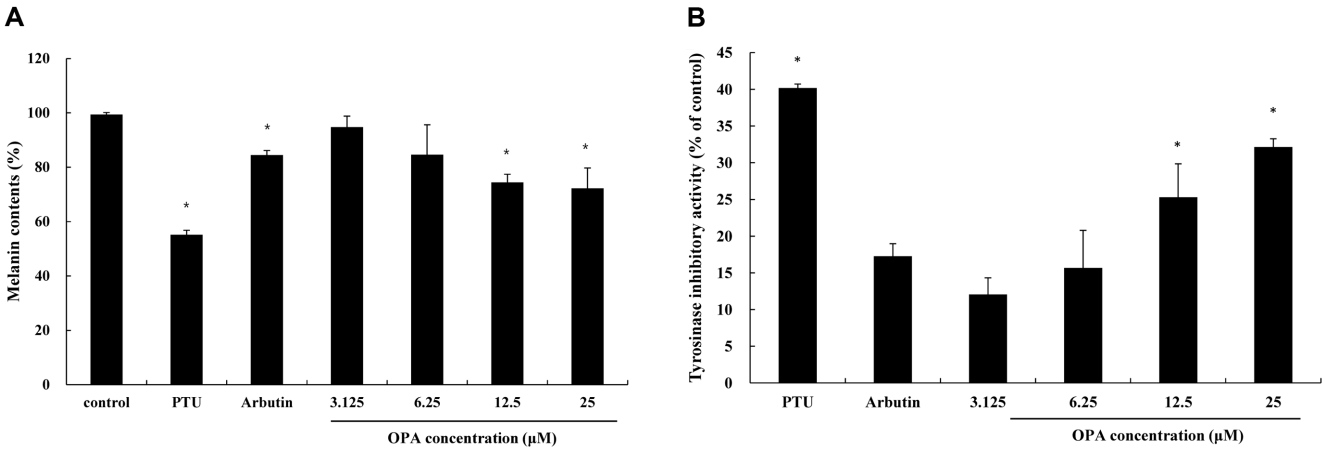


**Fig. 3.** Effects of melanogenic inhibitors on (**A**) melanin synthesis and (**B**) tyrosinase activity in zebrafish embryos.
Methods are described in the supplementary information. 1-Phenyl-2-thiourea (PTU; 50 μM) and 500 μM arbutin were used as positive control treatments. The concentration of OPA was 6.25–50 μM. The experiments were conducted in triplicate and the data are expressed as the mean ± standard error. Statistical tests were performed to compare the experimental groups with the control groups. *$p < 0.05$.

isolated from *Ecklonia cava* showed more pronounced tyrosinase inhibitory activity than arbutin and mulberry root powder. Park *et al.* [14] also reported that tyrosinase inhibitory compounds isolated from the brown alga *Ecklonia stolonifera* were phenolic compounds with reducing ability, and further suggested that these compounds inhibited tyrosinase activity noncompetitively, by directly binding to a site other than the active site of tyrosinase.

The present study explored the potential of OPA derived from a marine alga, *I. foliacea*, as a skin-whitening agent for use in cosmetic products. Our results clearly show that OPA produces a skin-whitening effect *via* tyrosinase inhibition, and therefore might be used as a skin-whitening agent. However, prior to this use, studies are necessary to further elucidate the precise molecular mechanism underlying OPA's whitening effect, and to characterize this effect using human skin.

## Acknowledgments

This research was supported by Ministry of Agriculture, Food and Rural Affairs (MAFRA), Ministry of Oceans and Fisheries (MOF), Rural Development Administration (RDA) and Korea Forest Service (KFS) (213004-04-2-SB930). This research was financially supported by the Ministry of Education (MOE) and National Research Foundation of Korea (NRF) through the Human Resource Training Project for Regional Innovation (NRF-2012H1B8A2025863).

## References

1. Ando H, Kondoh H, Ichihashi M, Hearing VJ. 2007. Approaches to identify inhibitors of melanin biosynthesis *via* the quality control of tyrosinase. *J. Invest. Dermatol.* **127:** 751-761.
2. Balboa EM, Conde E, Moure A, Falque E, Dominguez H. 2013. *In vitro* antioxidant properties of crude extracts and compounds from brown algae. *Food Chem.* **138:** 1764-1785.
3. Cha SH, Ko SC, Kim D, Jeon YJ. 2011. Screening of marine algae for potential tyrosinase inhibitor: those inhibitors reduced tyrosinase activity and melanin synthesis in zebrafish. *J. Dermatol.* **38:** 354-363.
4. Choi TY, Kim JH, Ko DH, Kim CH, Hwang JS, Ahn S, *et al.* 2007. Zebrafish as a new model for phenotype-based screening of melanogenic regulatory compounds. *Pigment Cell Res.* **20:** 120-127.
5. Eisen JS. 1996. Zebrafish make a big splash. *Cell* **87:** 969-977.
6. Fishman MC. 1999. Zebrafish genetics: the enigma of arrival. *Proc. Natl. Acad. Sci. USA* **96:** 10554-10556.
7. Jones K, Hughes J, Hong M, Jia Q, Orndorff S. 2002. Modulation of melanogenesis by aloesin: a competitive inhibitor of tyrosinase. *Pigment Cell Res.* **15:** 335-340.
8. Kim JA, Lee JM, Shin DB, Lee NH. 2004. The antioxidant activity and tyrosinase inhibitory activity of phlorotannins in *Ecklonia cava*. *Food Sci. Biotechnol.* **13:** 476-480.
9. Kim KN, Yang HM, Kang SM, Kim D, Ahn G, Jeon YJ. 2013. Octaphlorethol A isolated from *Ishige foliacea* inhibits alpha-MSH-stimulated induced melanogenesis *via* ERK pathway in B16F10 melanoma cells. *Food Chem. Toxicol.* **59:** 521-526.
10. Kim YJ, Kang KS, Yokozawa T. 2008. The anti-melanogenic effect of pycnogenol by its anti-oxidative actions. *Food Chem. Toxicol.* **46:** 2466-2471.
11. Lee SH, Kang SM, Ko SC, Kang MC, Jeon YJ. 2013. Octaphlorethol A, a novel phenolic compound isolated from *Ishige foliacea*, protects against streptozotocin-induced pancreatic beta cell damage by reducing oxidative stress and apoptosis. *Food Chem. Toxicol.* **59:** 643-649.
12. Lee SH, Kang SM, Ko SC, Lee DH, Jeon YJ. 2012. Octaphlorethol A, a novel phenolic compound isolated from a brown alga, *Ishige foliacea*, increases glucose transporter 4-mediated glucose uptake in skeletal muscle cells. *Biochem. Biophys. Res. Commun.* **420:** 576-581.
13. Manzoor Z, Mathema VB, Chae D, Kang HK, Yoo ES, Jeon YJ, Koh YS. 2013. Octaphlorethol A inhibits the CpG-induced inflammatory response by attenuating the mitogen-activated protein kinase and NF-kappaB pathways. *Biosci. Biotechnol. Biochem.* **77:** 1970-1972.
14. Park DC, Ji CI, Kim SH, Jung KJ, Lee TG, Kim IS, *et al.* 2000. Characteristics of tyrosinase inhibitory extract from *Ecklonia stolonifera*. *J. Fish Sci. Tech.* **3:** 195-199.
15. Roh JS, Han JY, Kim JH, Hwang JK. 2004. Inhibitory effects of active compounds isolated from safflower (*Carthamus tinctorius* L.) seeds for melanogenesis. *Biol. Pharm. Bull.* **27:** 1976-1978.
16. Yang HM, Ham YM, Yoon WJ, Roh SW, Jeon YJ, Oda T, *et al.* 2012. Quercitrin protects against ultraviolet B-induced cell death *in vitro* and in an *in vivo* zebrafish model. *J. Photochem. Photobiol. B* **114:** 126-131.
17. Zawar VP, Mhaskar ST. 2004. Exogenous ochronosis following hydroquinone for melasma. *J. Cosmet. Dermatol.* **3:** 234-236.