



Journal of Herbal Medicine Research (ISSN: 2474-106X)



Effects of *Myrica esculenta* Bark Extracts on Melanin Biosynthesis

Kenji Ohguchi^{1*}, Rie Ozaki¹

¹ Department of Food and Nutrition, Sugiyama Jogakuen University, Aichi, Japan

ABSTRACT

In this study, we investigated the effect of crude extracts, derived from stem bark of *Myrica esculenta* on melanin production in mouse B16 melanoma cells. In response to α -melanocyte-stimulating hormone (α -MSH), B16 melanoma cells underwent differentiation characterized by increased melanin biosynthesis. Treatment of *Myrica esculenta* bark extracts significantly blocked α -MSH-induced melanogenesis in a dose-dependent manner. α -MSH stimulated the activity of tyrosinase, a key melanogenic enzyme in melanin biosynthesis, which was significantly reduced by *Myrica esculenta* bark extracts. In addition, treatment of B16 melanoma cells with *Myrica esculenta* bark extracts decreased the protein expression level of tyrosinase. These results demonstrated that the inhibitory effects of *Myrica esculenta* bark extracts on melanogenesis may be due to the suppression of tyrosinase protein levels.

Keywords:

Myrica esculenta bark extracts, Myricaceae, Melanin, Melanogenesis, Tyrosinase

*Correspondence to Author:

Dr. Kenji Ohguchi. Department of Food and Nutrition, Sugiyama Jogakuen University, Aichi, Japan.
E-mail: kohguchi@sugiyama-u.ac.jp

How to cite this article:

Kenji Ohguchi and Rie Ozaki. Effects of *Myrica esculenta* Bark Extracts on Melanin Biosynthesis. Journal of Herbal Medicine Research, 2017, 2: 15.

eSciencePublisher®

eSciPub LLC, Houston, TX USA.

Website: <http://escipub.com/>

INTRODUCTION

Melanin production is principally responsible for skin color and plays an important role in prevention of sun-induced skin injury.^[1] Melanin is synthesized in melanocytes that are located in the basal layer of epidermis.^[2] Biosynthesis of melanin starts from the conversion of L-tyrosine to dopaquinone by tyrosinase, the enzyme playing a critical regulatory role in the melanin biosynthesis pathway.^[3] In melanocytes or melanoma cells, melanogenesis, one of the differentiation parameters is induced by melanogen, such as α -melanocyte-stimulating hormone (α -MSH).^[4] α -MSH binds to its specific receptor (MC1R), resulting in the activation of stimulatory GTP-binding protein (Gs), which in turn stimulates adenylate cyclase to produce cyclic-AMP.^[5] Cyclic-AMP undergoes melanogenesis mainly via activation of microphthalmia-associated transcription factor (MITF), a melanocyte-specific transcription factor, thereby leading to induction of tyrosinase expression.^[5] This tyrosinase process is involved in overproduction of melanin pigments such as hyperpigmentation. Therefore, many report have described pharmacologic and cosmetic agents that inhibit tyrosinase activity or that block melanogenic pathways, leading to skin lightening.^[6, 7]

The extracts of the bark of *Myrica esculenta* (Myricaceae) have been traditionally used in Ayurveda.^[8] It has been also reported that this extract shows various biological and pharmacological functions such as anti-oxidant^[9], anti-helminthic^[10], anti-allergic^[11], anti-inflammatory^[12], anti-microbial effects^[12]. However, there are no previous reports of the effects of *Myrica esculenta* bark extracts on the mechanisms involved in melanogenesis. In the present study, we describe the antimelanogenesis activity of *Myrica esculenta* bark extracts in B16 melanoma cells.

MATERIALS AND METHODS

Materials: *Myrica esculenta* bark extracts were prepared by Ichimaru Pharcos Co. Ltd. (Japan). The powder of stem bark of *Myrica esculenta* Buch.-Ham. was successively extracted with 50 % ethanol-water (24 h, at room temperature). The extract was filtered to remove any precipitate and further concentrated by evaporation. The resultant residue obtained was

dissolved in 50% ethanol-water. [Nle⁴, D-Phe⁷]- α -MSH, 3,4-dihydroxyphenylalanine (L-DOPA) and the antibody to β -actin were purchased from Sigma-Aldrich. Complete™, a protease inhibitor cocktail, was from Roche. Antibody against tyrosinase was from Santa Cruz Biotechnology. Anti-rabbit and -mouse antibodies conjugated with horseradish peroxidase (HRP) and the ECL Western blotting detection kit were obtained from GE Healthcare. All other reagents and chemicals were of the highest quality available.

Cell culture: Mouse B16 melanoma cells were purchased from RIKEN BioResource Center (Tsukuba, Japan). The B16 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum at 37°C in an atmosphere containing 5% CO₂.

Determination of melanin content: B16 cells were seeded onto 24-well cell culture plates at a density of 4 x 10⁴ cells. After 24 h, the cells were stimulated with 1 μ M α -MSH and treated with *Myrica esculenta* bark extracts. To determine the melanin content, the B16 cells were washed twice with phosphate-buffered saline (PBS) and lysed in 2 M NaOH for 1h at 60 °C. The melanin content was determined by measuring the absorbance at 470 nm.

Measurement of cell viability: The cell viability was determined using the trypan blue exclusion test.

Measurement of tyrosinase activity: To determine the tyrosinase activity, the B16 cells were lysed in lysis buffer (10 mM Tris-HCl, pH7.5, 150 mM NaCl, 1% NP-40, 0.1% sodium deoxycholate, 0.1% SDS, 1 mM EDTA) containing protease inhibitor cocktail. After clarifying the lysates by centrifuge at 14,000 x g for 30 min. The resulting supernatant and 0.05% L-DOPA were mixed in a 96 well plate. After incubation at 37 °C for 20 min, the absorbance at wavelength 470 nm was measured.

Protein assay: Protein concentrations were assayed using the DC protein assay reagent (Bio-Rad) with BSA as a standard.

Western blot analysis: B16 cells were lysed in the lysis buffer containing protease inhibitor

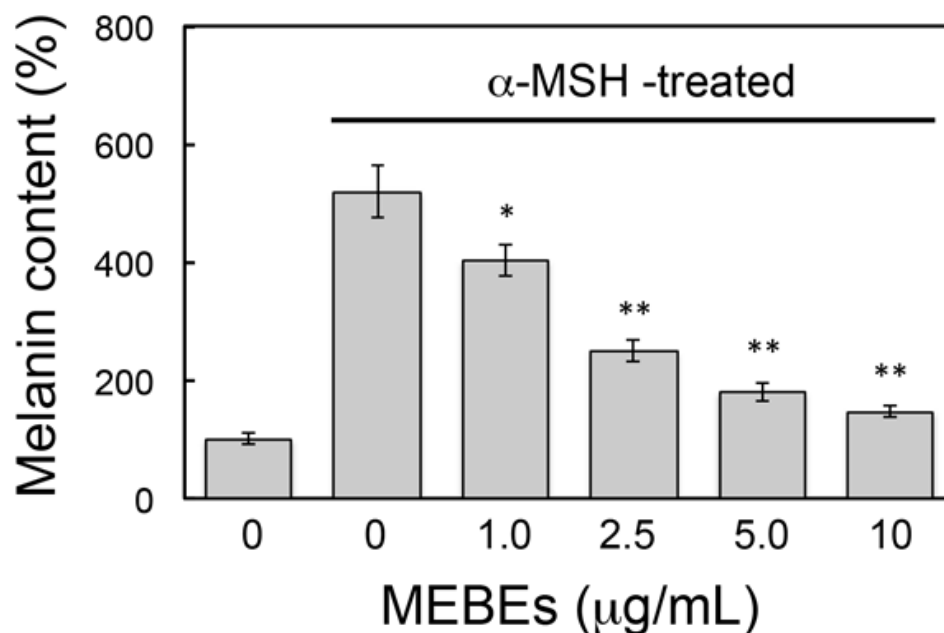


Figure 1: Effects of *Myrica esculenta* bark extracts on melanin content in B16 melanoma cells. The cells were treated with 1 µM α-MSH and various concentrations of *Myrica esculenta* bark extracts (MEBEs) for 72 h. The melanin content was determined as described under MATERIALS AND METHODS. Melanin content was expressed as the percentage of the values obtained in the control cells. Data represent the mean ± S.D. of three different experiments each carried out in duplicate. Asterisks indicate statistical significance as determined by Dunnett's test (* $p < 0.05$, ** $p < 0.01$ vs. IBMX-treated control).

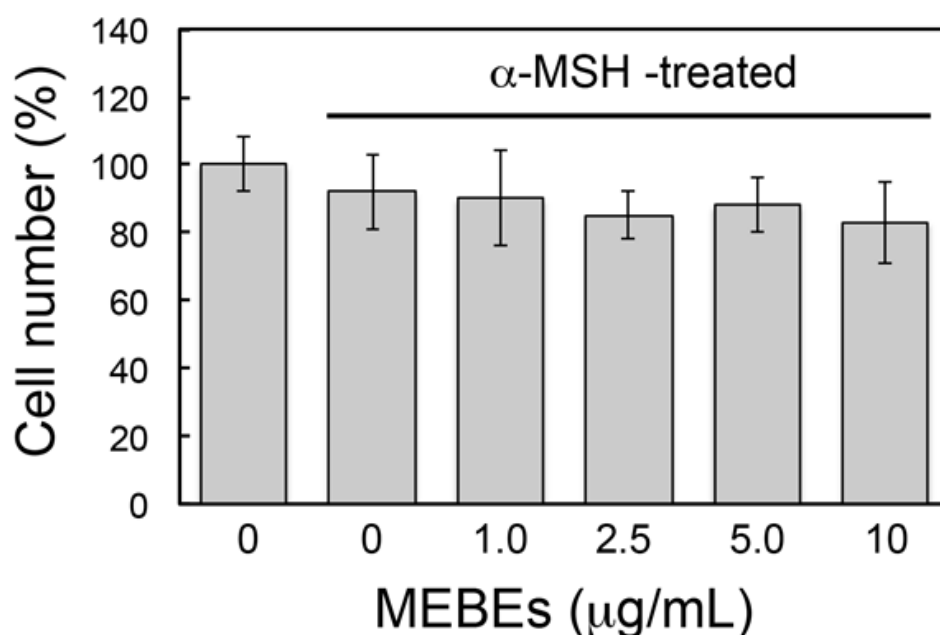


Figure 2: Effects of *Myrica esculenta* bark extracts on cell number in B16 melanoma cells. The cells were treated with 1 µM α-MSH and various concentrations of *Myrica esculenta* bark extracts (MEBEs) for 72 h. The cell viability was determined under MATERIALS AND METHODS. Cell number was expressed as the percentage of the values obtained in the control cells. Data represent the mean ± S.D. of three different experiments each carried out in duplicate.

cocktail described above. The resultant lysate (10 ug of protein per lane) was electrophoresed in a 7.5 % sodium dodecyl sulfate-polyacrylamide gel. Separated proteins were transferred electrophoretically onto a PVDF membrane. The membrane was blocked using Tris-buffered saline containing 5 % nonfat milk and 0.05 % Tween 20. Blots were incubated with each primary antibody, and then further incubated with HRP-conjugated secondary antibody. After washing, proteins were detected by the ECL detection kit.

RESULTS AND DISCUSSION

To investigate the effects of *Myrica esculenta* bark extracts on melanin production, B16 melanoma cells were cultured in the presence of *Myrica esculenta* bark extracts. In response to α -MSH or cyclic-AMP-elevating agents, mouse B16 melanoma cells underwent differentiation characterized by increased melanin biosynthesis. In this study, melanogenesis in B16 melanoma cells was started by the addition of 1uM α -MSH and was assessed by determining the intracellular melanin content. As shown in Fig. 1, when B16 melanoma cells were treated with α -MSH in the presence of *Myrica esculenta* bark extracts, significant decreases in α -MSH-induced melanin production was observed, compared with that in the absence of *Myrica esculenta* bark extracts. The amounts of melanin in cells decreased in a dose-dependent manner by *Myrica esculenta* bark extracts, with the maximal level at 10 ug/ml (Fig. 1). To exclude the possibility that the above inhibitory effects of *Myrica esculenta* bark extracts on melanogenesis might be caused by the cytotoxic effect, we examined the number of cells grown in the presence of *Myrica esculenta* bark extracts. Cell number was not significantly changed at 10 ug/ml *Myrica esculenta* bark extracts (Fig. 2).

Tyrosinase is a rate-limiting enzyme for melanogenesis [3], and we examined the effect of *Myrica esculenta* bark extracts on α -MSH-mediated increase in the activity of tyrosinase. Although α -MSH greatly stimulated the tyrosinase activity, which was strongly suppressed by *Myrica esculenta* bark extracts (Fig. 3).

To further investigate the mechanism underlying *Myrica esculenta* bark extracts-induced inhibition

of melanin production, we examined the effect of *Myrica esculenta* bark extracts on the protein expression level of tyrosinase proteins in the cells by Western blot analysis. As shown in Fig. 4, *Myrica esculenta* bark extracts exhibited a significant decrease in the protein levels of tyrosinase. The inhibition of melanin production by *Myrica esculenta* bark extracts was well correlated with the suppression in the activity of tyrosinase, which deserves its protein expression level.

CONCLUSION

In summary, the present study has demonstrated that *Myrica esculenta* bark extracts suppresses melanin production by modulating the protein amounts of tyrosinase in B16 melanoma cells. Therefore, *Myrica esculenta* bark extracts will be useful for application in skin care field. More extensive studies including identify the effective compounds in *Myrica esculenta* bark extracts, which are under current progress in our laboratory.

ACKNOWLEDGEMENT

We thank Ichimaru Pharcos Co. Ltd. for providing us with *Myrica esculenta* bark extracts.

REFERENCES

1. Kondo T, Hearing VJ (2011). Update on the regulation of mammalian melanocyte function and skin pigmentation. *Expert Rev Dermatol.* 6: 97-108.
2. Costin GE, Hearing VJ (2007). Human skin pigmentation: melanocytes modulate skin color in response to stress. *FASEB J.* 21: 976-994.
3. Land EJ, Ito S, Wakamatsu K, Riley PA (2003). Rate constants for the first two chemical steps of eumelanogenesis. *Pigment Cell Res.* 16: 487-493.
4. Thody AJ, Graham A (1998). Does alpha-MSH have a role in regulating skin pigmentation in humans? *Pigment. Cell Res.* 11: 265-274.
5. Busca R, Ballotti R (2000). Cyclic AMP a key messenger in the regulation of skin pigmentation. *Pigment. Cell Res.* 13: 60-69.
6. Solano F, Briganti S, Picardo M, Ghanem G (2006). Hypopigmenting agents: an updated review on biological, chemical and clinical aspects. *Pigment Cell Res.* 19: 550-571.

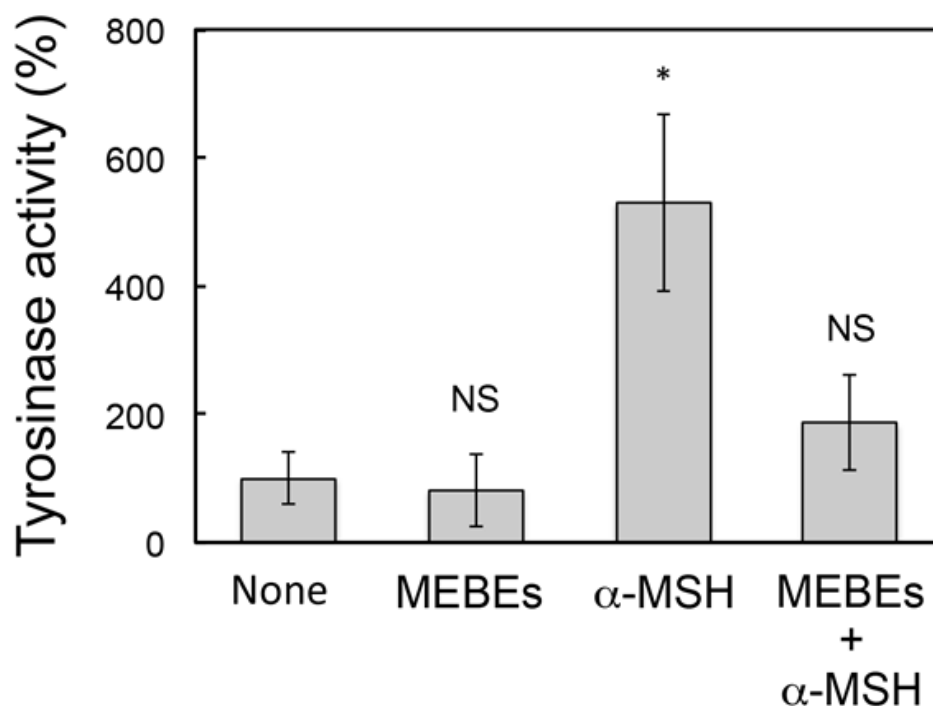


Figure 3: Effects of *Myrica esculenta* bark extracts on the activity of tyrosinase in B16 melanoma cells. The cells were treated with 10 ug/ml *Myrica esculenta* bark extracts (MEBEs) in the presence of 1 μ M α -MSH for 72 h, and cellular tyrosinase activity was determined by measuring the formation of dopachrome as described under MATERIALS AND METHODS. Data represent the mean \pm S.D. of three different experiments each carried out in duplicate. Asterisks indicate statistical significance as determined by Dunnett's test (* $p < 0.05$ vs. untreated control).

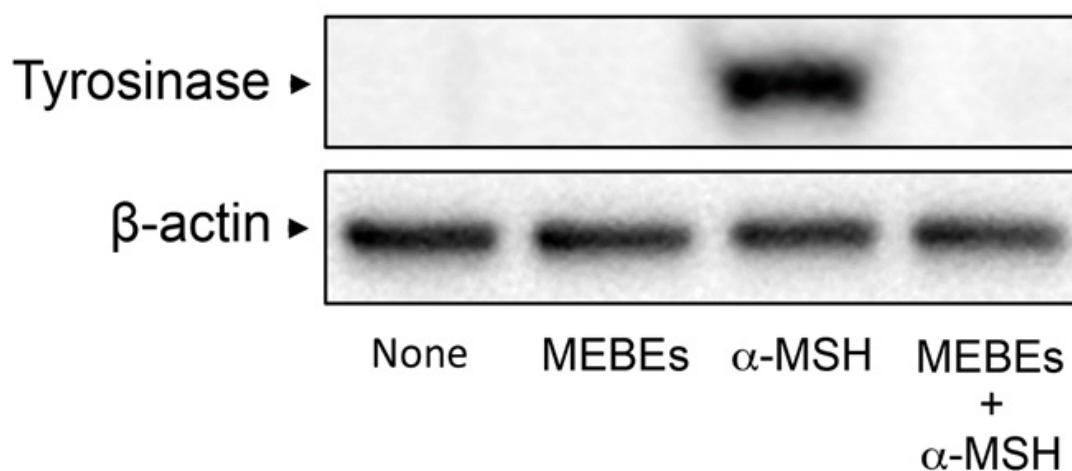


Figure 4: Effects of *Myrica esculenta* bark extracts on the protein expression of tyrosinase in B16 melanoma cells. The cells were treated with 10 ug/ml *Myrica esculenta* bark extracts (MEBEs) in the presence of 1 μ M α -MSH for 72 h, and the levels of tyrosinase and β -actin as an internal loading control in total cell lysates were analyzed by Western blot analysis as described under MATERIALS AND METHODS. A representative blot of three independent experiments is shown.

7. Gillbro JM, Olsson MJ (2011). The melanogenesis and mechanisms of skin-lightening agents-existing and new approaches. *Int J Cosmet Sci.* 33: 210-221.
8. Srivastava B, Sharma VC, Pant P, Pandey NK, Jadhav AD (2016). Evaluation for substitution of stem bark with small branches of *Myrica esculenta* for medicinal use - A comparative phytochemical study. *J Ayurveda Integr Med.* 7: 218-223.
9. Rana RK, Patel RK (2014). Antioxidant activity of bark of *Myrica nagi*. *Int J Pharm Sci Rev Res,* 28, 99–101.
10. Jain VK, Jain B. (2010) Antihelmintic activity of ethanolic extract of bark of *Myrica esculenta*. *J Pharm Sci.* 1: 129–131.
11. Patel KG, Rao NJ, Gajera V, Bhatt P, Patel K, Gandhi T (2010). Anti-allergic activity of stem bark of *Myrica esculenta* Buch.-Ham. (Myricaceae). *J Young Pharm.* 2: 74–78.
12. Agnihotri S, Wakode S, Ali M (2012). Essential oil of *Myrica esculenta* Buch. Ham.: composition, antimicrobial and topical anti-inflammatory activities. *Nat Prod Res.* 26: 2266–2269.

