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Bioassay-guided Fractionation of Clove Buds Extract Identifies Eugenol as Potent Melanogenic Inducer in Melanoma Cells

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Abstract: Clove, a dried flower buds of *Syzygium aromaticum*, is used in traditional medicine, for culinary purposes, and in essential oil production. In our preliminary screening of crude drugs used in Japanese Kampo formulas, a methanol (MeOH) extract of clove buds was found to exhibit a melanin induction. To date, the effects of clove buds or their constituents on the activation of melanogenesis remain unclear. Thus, this study aimed to isolate active compounds from the MeOH extract of clove buds associated with melanin synthesis in melanoma cells and to investigate the molecular mechanism involved. The MeOH extract of clove buds increased melanin content in murine B16-F1 melanoma cells. To identify the active compounds responsible for melanin induction, the MeOH extract was suspended in water and successively partitioned using hexane, ethyl acetate (EtOAc), and *n*-butanol (*n*-BuOH). Comparative analysis revealed that the EtOAc fraction induced melanin synthesis. Bioassay-guided separation of the EtOAc fraction isolated three compounds including eugenol. The analysis of structure-activity relationships of eugenol and structurally related compounds indicated that eugenol was the most potent melanin inducer among the 11 compounds, and that a hydroxyl group at C-1 and a methoxy group at C-2 may contribute to melanin induction. Eugenol induced melanin synthesis in human HMV-II melanoma cells as well as in B16-F1 cells. Further analysis indicated that eugenol may invoke intracellular tyrosinase activity and expression of tyrosinase, tyrosinase-related protein (TRP)-1, TRP-2, and microphthalmia-associated transcription factor (MITF). These results suggest that eugenol enhances melanin synthesis by upregulating the expression of MITF and subsequent expression of melanogenic enzymes, and that it may be a potent therapeutic agent for hypopigmentation.

Key words: clove, eugenol, *Syzygium aromaticum*, melanin synthesis, melanoma cells

1 Introduction

Melanin is an important factor determining the color of human skin, hair, and eyes[1]. Melanin protects the skin from damage caused by ultraviolet light, toxic drugs, and chemicals[2, 3]. In addition, hypopigmentation, which is the abnormal loss of melanin in the skin, causes vitiligo, albinism, and hair problems[4]. Melanogenesis is a physiological process that results in the synthesis of melanin pigments. In melanocytes and melanoma cells, melanogenesis is catalyzed by three types of melanogenic enzymes, such as tyrosinase, tyrosinase-related protein (TRP)-1, and TRP-2[5]. Tyrosinase catalyzes two rate-limiting reactions during melanin biosynthesis, including hydroxylation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA). TRP-2 catalyzes the tautomerization of DOPA-chrome to produce 5,6-dihydroxyindole-2-carboxylic acid (DHICA)[5]. TRP-1 also oxidizes DHICA to produce carboxylate indole-quinone[6]. TRP-1 and TRP-2 function downstream from tyrosinase in the melanin biosynthesis pathway[6]. At the transcriptional level, microphthalmia-associated transcription factor (MITF) has a crucial role in the transcription of melanogenic genes, binding to the M-box motif in their promoter regions[7, 8]. Therefore, MITF plays an important role in activating melanogenesis. Isolation and identification of melanogenesis inhibitors from natural products have contributed to cosmetic whitening product development and hyperpigmentation disease understanding; however, the number of studies on melanogenesis stimulators is limited.

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The discovery and development of melanin synthesis stimulators may help treat hypopigmentary disorders.

Essential oils are used in cosmetic products or perfumes because of their chemical complexity, strong fragrance, and natural marketing image[9]. Essential oils and their components are gaining popularity because of their many applications and consumer acceptance. Accumulating evidence suggests that essential oils have anti-acne, anti-aging, skin lightening, and sun protection properties, among others, making them highly valuable to cosmetic industries[10–15]. Several essential oils or their components have been reported to suppress melanin synthesis, such as the essential oils of *Melaleuca quinquenervia* leaves and stems[16], *Alpinia zerumbet* leaves[17], *Eucalyptus camaldulensis* flowers[18], *Vitex negundo* leaves[19], and *Cinnamomum cassia* stem bark[20]. In addition, recent studies have reported that the essential oil extracted from *Calocedrus formosana* wood reduced melanin synthesis in α-melanocyte-stimulating hormone (α-MSH) and forskolin-induced in B16-F10 cells; thymol is the active ingredient responsible for this anti-melanogenic activity[21]. The essential oil of *Camellia japonica* seeds also exhibits anti-melanogenesis activity in α-MSH- treated B16-F10 cells[22]. Furthermore, the essential oils of *Alpinia nantoensis* reduced forskolin-induced melanin production followed by the down-regulation of tyrosinase and TRP-1 expression via the degradation of MITF[23]. There are many reports on essential oils suppressing melanogenesis; however, few studies have reported on essential oils or their constituents activating melanogenesis.

Syzygium aromaticum (L.) Merr. & L.M Perry, is an evergreen tree and a member of the Myrtaceae family. The flower buds of *S. aromaticum*, known as cloves, are a spice used in folk medicine to treat many disorders. Clove is known to be an effective and beneficial herb with anti-inflammatory, antimicrobial, antithrombotic, antioxidant, antimutagenic, and anti-ulcerogenic properties[24, 25]. The essential oil obtained from clove has a wide range of pharmacological properties including antiangiogenic, anticancer, antioxidant, anti-inflammatory, and antimutagenic activities[26–31]. Phytochemical analysis revealed that concentrations up to 20% of essential oil can be found in flower buds, and roughly, 70–90% of the clove essential oil is eugenol[32, 33]. The other main phytochemicals isolated from the essential oil include acetyl eugenol, β-caryophyllene, and several sesquiterpenes[26, 34]. Among different active ingredients, eugenol properties include antioxidant, anticarcinogenic, antibacterial, antifungal, and insecticidal activities[35]. However, few studies have evaluated the effects of clove or its constituents on melanin synthesis.

Ongoing research aims at identifying stimulators of melanin synthesis from natural products. In a preliminary study, we screened approximately 130 crude drugs used in the Kampo formulas and 250 natural compounds to identify stimulators of melanogenesis. This screening revealed that liquiritin and liquiritigenin, which are major flavonoids in licorice root, enhanced melanin synthesis by upregulating the expression of melanogenic enzymes[36]. In addition, we found that (+)-magnolin, which is one of the furofuran lignans contained in the flower buds of *Magnolia biondii*, induced melanin production in both melanoma cells and a 3D human skin equivalent[37]. We recently reported that silibinin, which is a flavonolignan isolated from milk thistle (*Silybum marianum*), induced melanin synthesis in melanoma cells[38]. These findings may contribute to the development of a potentially potent therapeutic agent for use in the treatment of hypopigmentation, and further research is required to identify more effective stimulators of melanin synthesis. The screening results indicated that the MeOH extract of clove buds was associated with melanin induction in melanoma cells. In this study, we conducted a bioassay-guided fractionation of the MeOH extract of clove buds to isolate the stimulators of melanin synthesis. We then explored the structure-activity relationships of the isolated and structurally related compounds in melanin induction. Finally, we aimed to elucidate the underlying mechanisms. To the best of our knowledge, this is the first study to describe the properties of compounds in clove buds and the molecular mechanisms associated with the activation of melanogenesis.

2 Experimental Procedures

2.1 Materials

Dulbecco's Modified Eagle's Medium (DMEM) and RPMI1640 were obtained from Nissui Pharmaceutical (Tokyo, Japan). Fetal bovine serum was obtained from Gibco (Gaithersburg, MD, USA). Antibody against MITF was purchased from Cell Signaling Technology (Beverly, MA, USA). Other antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). α-MSH was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methyl eugenol, acetyl isoeugenol, isoeugenol, and 2-methoxy-4-ethylphenol were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Chavicol was were purchased from Nacalai tesque Inc. (Kyoto, Japan). All other chemicals were obtained from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Before use, eugenol and structurally related compounds were dissolved in ethanol and stored at −20℃.

2.2 Cell cultures

B16-F1 and HMV-II cells were obtained from the European Collection of Authenticated Cell Cultures (ECACC) through DS Pharma Biomedical (Osaka, Japan). B16-F1 and HMV-II cells were grown in DMEM and RPMI1640 medium supplemented with 10% FBS and 1% penicillin–

streptomycin, respectively. Both cells were incubated at 37℃ in a 5% CO_2 atmosphere. During the cell treatments, the concentration of dimethyl sulfoxide or ethanol in the cell culture medium did not exceed 0.2% (v/v), and the controls were treated with the same amount of dimethyl sulfoxide or ethanol as was used in the corresponding experiments.

2.3 Plant material, extraction, fractionation, and isolation of compounds

Dried clove buds were obtained from Kojima Kampo (Osaka, Japan). Clove buds (2.5 kg) were extracted with MeOH (5.0 L × 4 times) at 40℃ under sonication for 12 h. Evaporation of the solvent under reduced pressure provided the MeOH extract (723.76 g). A part of the MeOH extract (527.99 g) was suspended in 1.0 L of water and partitioned with hexane, EtOAc, and *n*-BuOH (1.0 mL × 3 times) to obtain soluble fractions of hexane (273.22 g), EtOAc (100.31 g), *n*-BuOH (87.93 g), and H_2O (55.97 g), respectively. A part of the EtOAc fraction (32.84 g) was subjected to normal-phase silica gel column chromatography [hexane-EtOAc (10:1→5:1→2:1→1:1→1:2→1:5→1:10, v/v)→EtOAc→EtOAc-MeOH (2:1, v/v)→MeOH] to give 12 fractions (fractions 1～12). Fraction 3 (5.97 g) was further separated using reversed-phase silica gel column chromatography [MeOH-H_2O (5:5→6:4→7:3→8:2→1:0, v/v)] to give 27 fractions (fractions 3-1～3-27). Fraction 3-9 (0.16 g) was subjected to purified reversed-phase silica gel column chromatography [MeOH-H_2O (5:5→6:4→7:3→8:2, v/v)] to yield eugenol (**1**, 38.9 mg), acetyl eugenol (**2**, 19.4 mg), and vanillin (1.7 mg). The isolated compounds were identified by comparing their physical characteristics (^{1}H-NMR, ^{13}C-NMR, and MS) with those of the reference compounds.

2.4 Measurement of cellular melanin content

B16-F1 cells (2×10^4 cells/well) or HMV-II cells (5×10^4 cells/well) were seeded into 24-well plates. After 24 h of incubation, the cells were treated with each agent at various concentrations for 72 h. After treatment, the medium was removed, and the cells were dissolved in 120 μL of 1M NaOH at 80℃ for 20 min. Then, the absorbance at 415 nm was measured using a microplate reader (iMark, BioRad, Tokyo, Japan). Melanin content was expressed as the ratio of the control cultures.

2.5 Cell viability assay

B16-F1 cells (0.3×10^4 cells/well) or HMV-II cells (0.8×10^4 cells/well) were seeded into 96-well plates. After incubation for 24 h, the cells were treated with each agent at various concentrations for 72 h. After treatment, 10 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazol-3-ium bromide (MTT) solution was added to each well, and the cells were incubated for another 4 h. The precipitated MTT formazan was dissolved with 100 μL of 0.04 N HCl-isopropanol, and the amount of formazan was measured at 595 nm using a microplate reader (iMark, BioRad, Tokyo, Japan). Cell viability was expressed as a percentage of the control culture.

2.6 Measurement of intracellular tyrosinase activity

B16-F1 cells (1.2×10^5 cells/dish) were seeded into 6-well plates and then incubated for 24 h. The cells were treated with each agent at various concentrations for 72 h. After treatment, the cells were washed twice with ice-cold phosphate buffered saline (PBS) and lysed in 500 μL of 0.1 M sodium phosphate buffer (pH 6.8) containing 1% Triton X-100 and proteinase inhibitor cocktail. The lysate was centrifuged at 12,000 × *g* for 15 min at 4℃ and the supernatants were collected. The protein concentration was determined using a dye-binding protein assay kit (Bio-Rad, Tokyo, Japan). A reaction mixture containing 90 μL of equal amounts of protein lysate and 10 μL of 5 mM L-DOPA was placed into a 96-well plate. After 30–60 min of incubation (according to the content of dopachrome formation) at 37℃ in the dark, the absorbance was measured at 415 nm using a microplate reader (iMark, BioRad, Tokyo, Japan). The intracellular tyrosinase activity was calculated from the absorbance ratio relative to that of the control culture.

2.7 Western blot analysis

B16-F1 cells (3×10^5 cells/dish) were seeded in 6-cm dishes and incubated for 24 h. The cells were then treated with each agent at various concentrations. At the end of the incubation period, the cells were washed twice with ice-cold PBS and lysed in RIPA lysis buffer containing protease and phosphatase inhibitors. The protein concentration was determined using a dye-binding protein assay kit (Bio-Rad, Tokyo, Japan). Equal amounts of protein lysate from each sample were added to sodium dodecyl sulfate sample buffer and proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrotransfer to polyvinylidene fluoride membranes, the membranes were blocked with 5% skim milk in Tris-buffered saline containing 0.1% Tween 20 (TBS-T) and incubated with specific primary antibodies at a dilution ratio of 1:1000. The membrane was washed with TBS-T and incubated with horseradish peroxidase-conjugated secondary antibodies at a dilution ratio of 1:2500 for 1 h. After washing with TBS-T, chemiluminescent ECL Prime kit (GE Healthcare Japan, Tokyo, Japan) was used for visualizing proteins expressions.

2.8 Statistical analysis

All statistical analyses were presented as mean ± SD of at least three independent experiments. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test using GraphPad Prism 6 software (Graph-

Pad Software Inc., San Diego, CA, USA). p value of less than 0.05 was considered to be statistically significance.

3 Results and Discussion

3.1 Effect of MeOH extract of clove buds on melanin content in B16-F1 cells

To investigate the effect of the MeOH extract of clove buds on melanin synthesis, B16-F1 cells were treated with the extract at various concentrations (10, 20, or 40 μg/mL) for 72 h. α-MSH was used as a positive control[36–38]. As indicated in Fig. 1A, melanin content was increased in response to treatment with the extract, as well as α-MSH. In addition, we measured the cytotoxicity of the extract in B16-F1 cells using the MTT assay. As shown in Fig. 1B, although 40 μg/mL of the extract attenuated cell growth by 11.5 ± 6.7%, as compared with untreated control, 10 and 20 μg/mL of the extract did not affect cell viability. These results indicate that the MeOH extract of clove buds promotes melanin synthesis in B16-F1 cells in a manner independent of cell growth up-regulation.

3.2 Comparison of melanin induction activities of fractions prepared from MeOH extract and the effect of the isolated compounds on melanin content

To identify the active compounds responsible for melanin induction, the MeOH extract was suspended in water and then fractionated successively with equal volumes of hexane, EtOAc, and n-BuOH, leaving residual aqueous fraction. The B16-F1 cells were treated with 10, 20, and 40 μg/mL of each fraction for 72 h, and melanin content and cell viability were measured. As shown in Fig. 2A, the fractions of hexane, EtOAc, and n-BuOH significantly increased melanin content, and the induction potency of the EtOAc fraction was strongest among the four fractions. The EtOAc fraction did not affect cell viability at concentrations that increased melanin content (Supplementary Fig. 1A). The EtOAc fraction was further separated into 12 fractions (Fr. 1–12) using silica gel normal phase chromatography. Figure 2B and Supplementary Fig. 1B show melanin content and cell viability of each fraction, respectively. Among 12 fractions, Fr. 3 exhibited the strongest melanin induction with low cytotoxicity. To confirm the presence of the major compounds, Fr. 3 was further isolated by combined chromatography techniques, and 3 compounds were isolated as follows: eugenol (1), acetyl eugenol (2), and vanillin; structures were determined based on extensive spectroscopic spectra and comparisons with reference compounds (Fig. 3A). The yield of 1, 2, and vanillin from fraction 3-9 (0.16 g) were 38.9 mg, 19.4 mg, and 1.7 mg, respectively. Compound 1 and 2 were major and specific compounds in clove buds. On the other hand, the yield of vanillin was very low compared with that of 1 and 2. In addition, it has been reported that vanillin had no effect on melanin induction in B16-F1 cells[39]. Thus, our analysis focused on 1, 2, and structurally related compounds.

Next, we examined the effects of 1, 2, and structurally related compounds (3–11) at 200 μM for 72 h on melanin synthesis and cell viability. As shown in Fig. 3B, 1 (3.72 ± 0.13-fold) strongly induced melanin content. Compounds 2

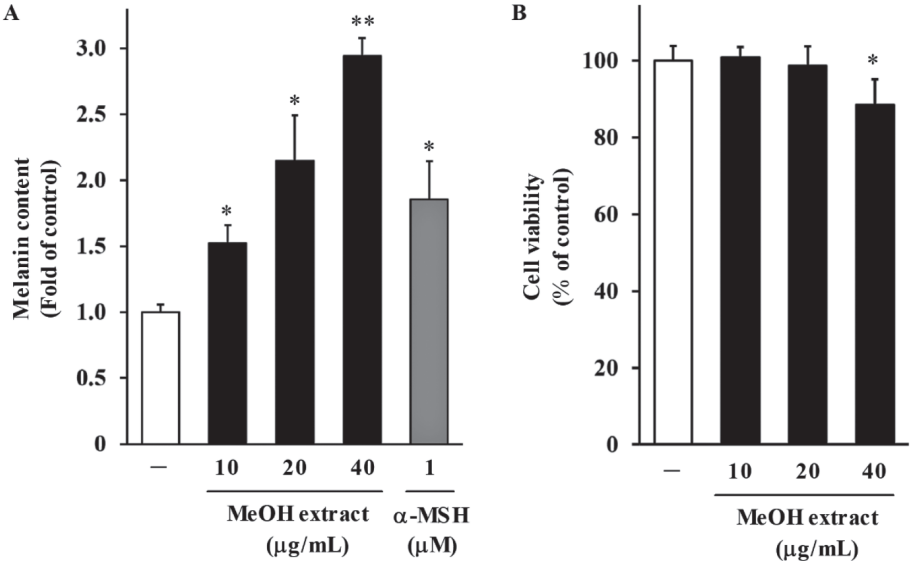

Fig. 1 Effects of clove buds MeOH extract on melanin synthesis (A) and cell viability (B) in B16-F1 cells. The cells were treated with the extract or α-MSH at the indicated concentrations for 72 h, and the melanin content and the cell viability were measured. Values are mean ± SD of three independent experiments. *$p < 0.05$ and **$p < 0.01$ for comparisons with controls.

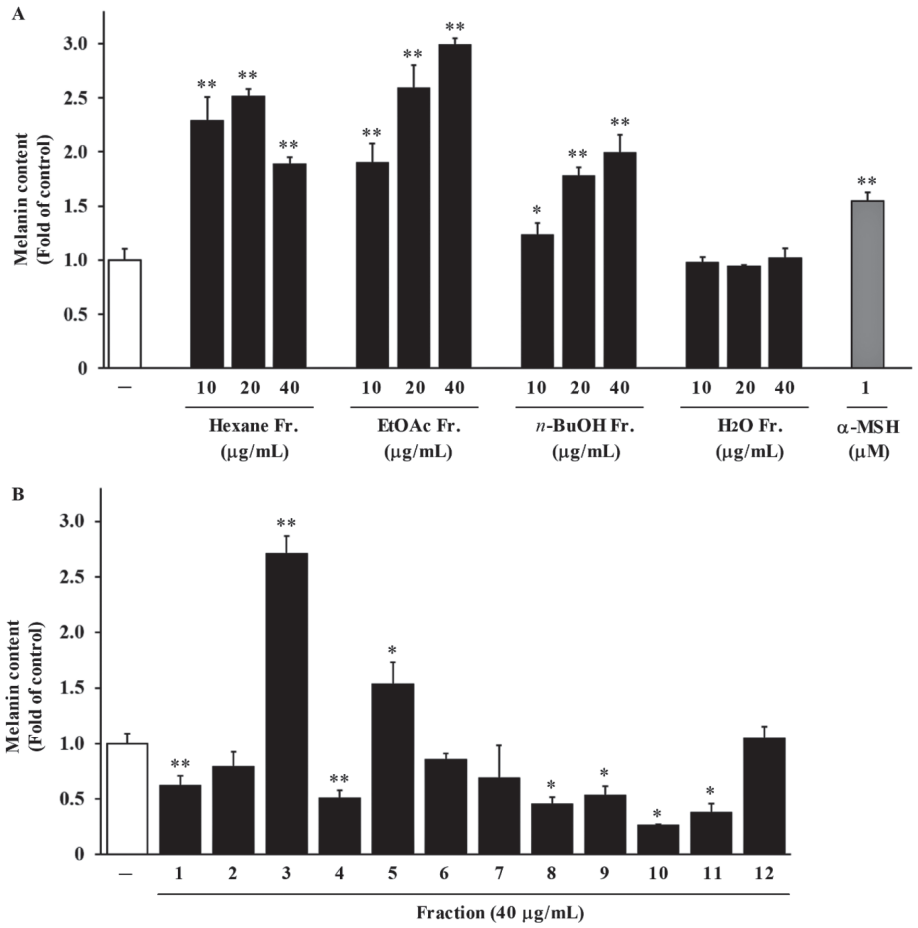

Fig. 2 Comparison of melanin induction values of fractions prepared from MeOH extract. The cells were treated with hexane, EtOAc, and *n*-BuOH, and aqueous fraction (**A**) or Fr. 1 – 12 separated from EtOAc fraction (**B**) at the indicated concentrations for 72 h, and melanin content was measured. Values are mean ± SD of three independent experiments. *$p<0.05$ and **$p<0.01$ for comparisons with controls.

(2.77 ± 0.07-fold), **5** (2.57 ± 0.20-fold), **6** (2.47 ± 0.07-fold), **7** (2.79 ± 0.23-fold), **8** (2.23 ± 0.15-fold), **9** (2.47 ± 0.08-fold), and **10** (2.77 ± 0.10-fold) exhibited melanin induction of between 2- and 3-fold, but their induction potencies were weaker than that of **1**. Meanwhile, **3** (1.64 ± 0.10-fold) and **4** (1.89 ± 0.09-fold) weakly induced melanin content, and **11** (0.96 ± 0.07-fold) showed no melanin induction. None of the compounds affected cell viability (Supplementary Fig. 2). Overall, the rank order of induction potencies of melanin content was **1**>**2**, **5**, **6**, **7**, **8**, **9**, **10**>**3**, **4**>**11**. These results suggest that **1** was the most potent inducer among the 11 compounds.

By comparing melanin induction potency of **1**–**11**, we investigated the structures required for the enhancement. The induction potencies of **1** and **2** were stronger than those of **6** and **5**, respectively, suggesting that the double bond position of the allyl side chain at C2'-C3' has a stronger induction than that at C1'-C2'. Compounds **6** and **7** containing three allyl carbons exhibited higher induction than **8** and **9** containing two allyl carbons, respectively. Focusing on the substituent at C-1 of **1**, the induction potency of **1** with a hydroxy group was stronger than that of **4** with a methoxy group or **2** with an acetyl group. Comparing the potency of **1** and **3** suggested that the position of a hydroxyl group also contributes to the induction potency. In addition, comparing the potency of **1**, **10**, and **11** suggested that the presence of a methoxy group at C-6 reduced the potency, while the presence of a methoxy group at C-2 enhanced the potency. Among these structure-activity relationships, the presence of a hydroxyl group at C-1 and a methoxy group at C-2 contributed particularly to melanin induction.

Eugenol is classified as a phenylpropanoid. It has been reported that several phenylpropanoids suppress the tyrosinase activity and melanin content. Cinnamic acid and its derivatives contained in *Cinnamomum cassia* bark were reported to have tyrosinase inhibitory activities[40, 41]. Nam *et al.* demonstrated that *Foeniculum vulgare* extract and its constituent, *trans*-anethole, inhibited UV-induced melanogenesis[42]. Furthermore, Morikawa *et al.*

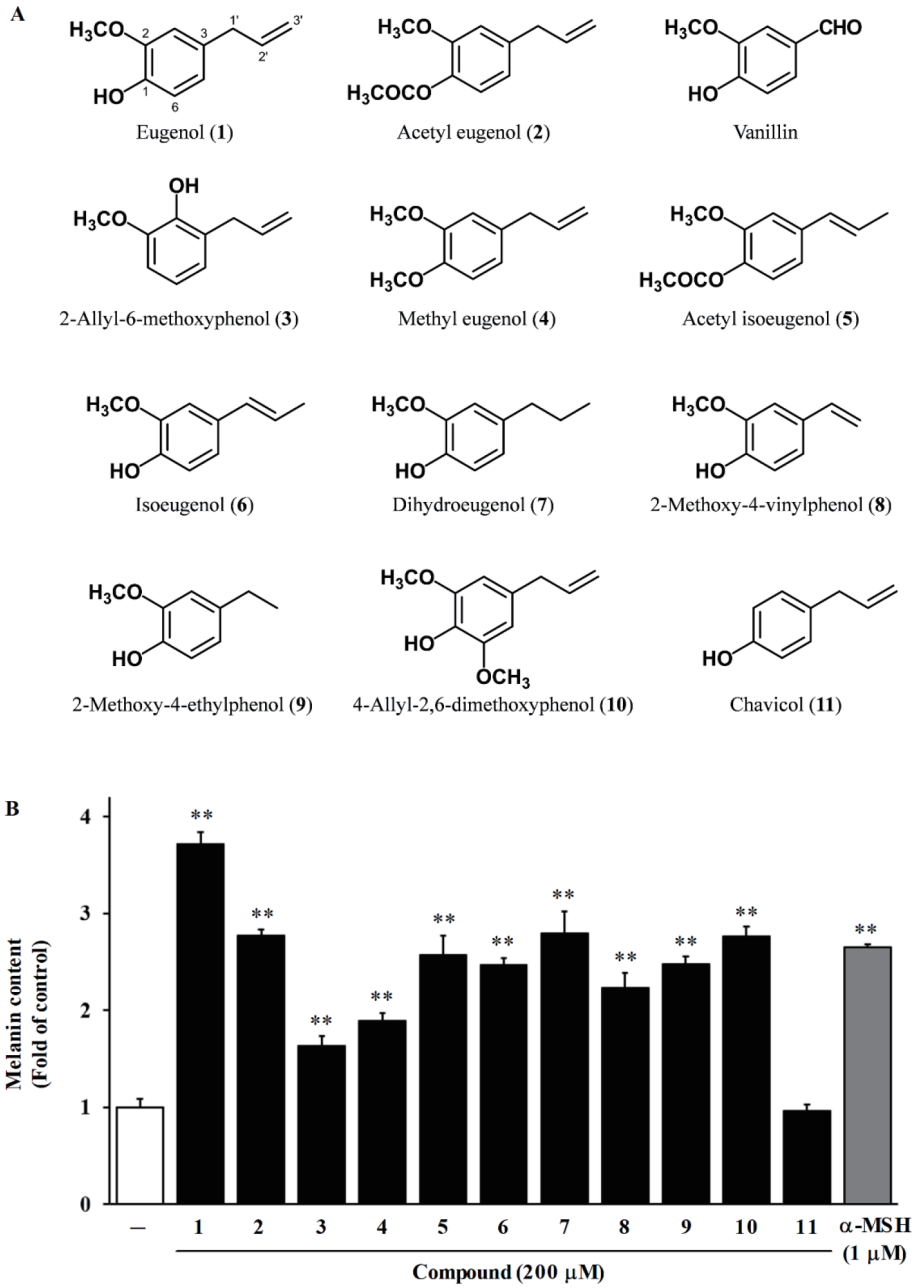

Fig. 3 (A) Chemical structures of 1 – 11. (B) Effect of 1 – 11 on melanin synthesis in B16-F1 cells. The cells were treated with 200 μM of 1 – 11 for 72 h, and melanin content was measured. Values are mean ± SD of three independent experiments. $^{**}p < 0.01$ for comparisons with controls.

found that the MeOH extract of flowers of *Narcissus tazetta* var. *chinensis* inhibited melanin synthesis in B16 melanoma 4A5 cells and several phenylethanoid and phenylpropanoid glycosides isolated from the extract showed anti-melanogenesis activity without notable cytotoxicity at the effective concentrations[43]. The difference of mechanisms of action between eugenol and these phenylpropanoids is not elucidated; thus, further studies focusing on their structure-activity relationships and the mechanisms of action are needed.

3.3 Effect of eugenol on melanin content in B16-F1 and HMV-II cells

To investigate the effect of eugenol (1) on melanin synthesis, B16-F1 and HMV-II cells were treated with 25, 50, 100, or 200 μM eugenol for 72 h. After treatment with eugenol, the B16-F1 cell pellets were significantly darker than the control cell pellets (Fig. 4A). Similarly, melanin content increased in a dose-dependent manner in response to eugenol treatment (Fig. 4B). In this concentration range, eugenol had no effect on cell viability (Supplementary Fig.

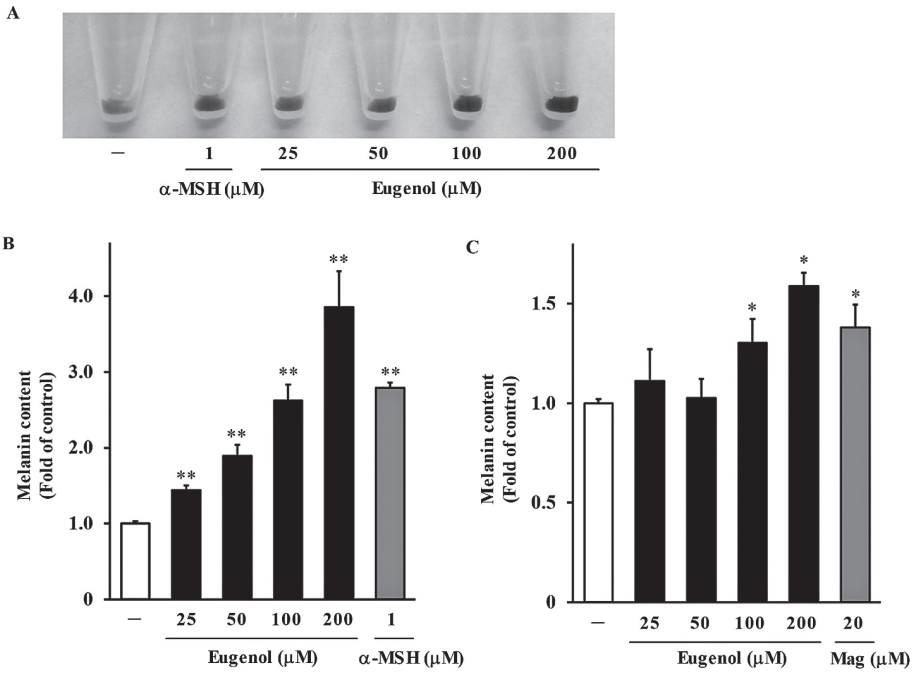

Fig. 4 Effect of eugenol on melanin synthesis in B16-F1 (A, B) and HMV-II (C) cells. Cells were treated with eugenol or α-MSH or Mag at the indicated concentrations for 72 h, and melanin content was determined. Values are mean ± SD of three independent experiments. *$p<0.05$ and **$p<0.01$ for comparisons with controls.

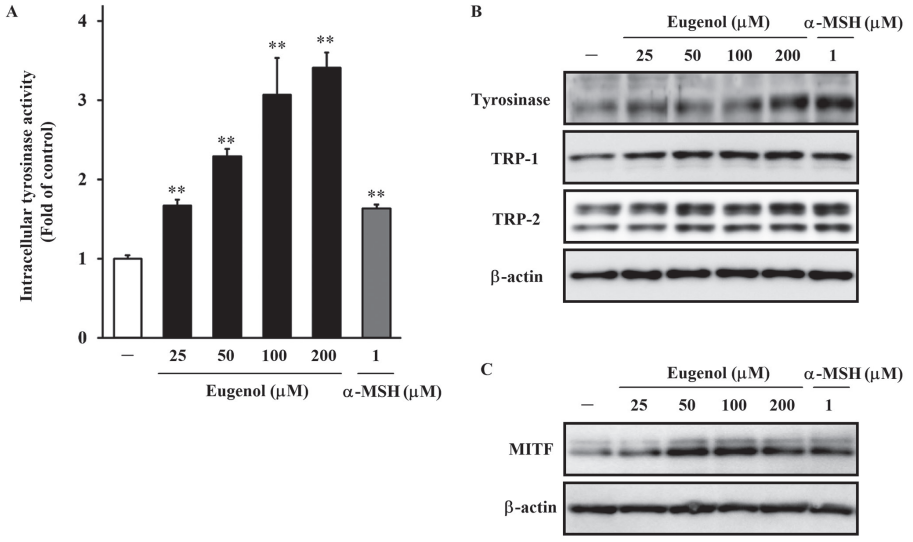

Fig. 5 (A) Effect of eugenol on intracellular tyrosinase activity. The cells were treated with eugenol or α-MSH at the indicated concentrations for 72 h and intracellular tyrosinase activity was determined. Values are mean ± SD of three independent experiments. **$p<0.01$ for comparisons with controls. Effect of eugenol on the protein levels of melanogenic enzymes (B) and MITF (C). B16-F1 cells were treated with eugenol or α-MSH at the indicated concentrations for 48 h, and the protein expression levels were determined by western blotting. The data shown represent at least three independent experiments.

3A). Furthermore, we observed the effect of eugenol on melanin synthesis in human melanoma HMV-II cells. (+)-magnolin (Mag) was used as a positive control for HMV-II cells[37]. Eugenol increased melanin content in a manner similar to that observed in B16-F1 cells without cytotoxicity (Fig. 4C and Supplementary Fig. 3B). These results indicate that eugenol promotes melanin synthesis in B16-F1 and HMV-II cells without regulating cell growth.

3.4 Effect of eugenol on melanogenesis-related proteins

We examined the effect of eugenol on the intracellular activity of tyrosinase in B16-F1 cells. As shown in Fig. 5A, eugenol induced intracellular tyrosinase activity in a dose-dependent manner, suggesting that eugenol regulates the expression of melanogenic enzymes. Furthermore, we examined the effect on melanogenic protein expression. Eugenol up-regulated the protein expression of tyrosinase, TRP-1, and TRP-2 (Fig. 5B). Moreover, MITF, which is a key transcription factor controlling the expression of melanogenic enzymes, was enhanced by treatment with eugenol (Fig. 5C). These results suggest that eugenol-induced melanin synthesis may be associated with the induction of melanogenesis-related proteins, such as tyrosinase, TRP-1, and TRP-2, through the activation of MITF expression.

4 Conclusion

Isolation studies of melanogenesis inhibitors from natural products have translated into cosmetic whitening applications and hyperpigmentation disease management, but studies on melanogenesis stimulators are limited. Thus, further research is required to identify the stimulators of melanin synthesis, which can be used to develop potent and safe therapeutic agents for the treatment of hypopigmentary disorders. In this study, we demonstrated that the MeOH extract of clove buds promoted melanin synthesis in B16-F1 cells. The bioassay-guided fractionation of the EtOAc fraction led to the isolation of 3 compounds including eugenol. The structure-activity relationship study of eugenol and structurally related compounds indicated that eugenol exhibited the strongest melanin induction properties among 11 compounds and that the presence of a hydroxyl group at C-1 and that of a methoxy group at C-2 in eugenol are important for the activation of melanogenesis. Furthermore, eugenol affects melanin induction in B16-F1 and in HMV-II cells. Our observations indicated that eugenol may upregulate the expression of melanogenic enzymes such as tyrosinase, TRP-1, and TRP-2, which may be associated with the activation of MITF expression that binds to the M-box motif in the promoter regions of melanogenic enzymes. This is the first study to demonstrate eugenol-related melanin induction properties, including the underlying mechanism. Our findings suggest that eugenol can be used as a potent and safe therapeutic agent for the treatment of hypopigmentary disorders.

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Supporting Information

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