Effect of Hydrosol Obtained from *Camellia japonica* Branch on α-MSH-Induced Melanin and Tyrosinase Activity in B16F10 Melanoma Cells

Si Young Ha,[a] Ji Young Jung,[a] Jung Myoung Lee,[b] and Jae-Kyung Yang [a,*]

Hydrosols that originate from various aromatic botanical sources in natural or organic settings contain a spectrum of fragrant compounds, which can be extracted from leaves, stems, peels, flowers, and roots. These compounds are known to exhibit diverse medicinal properties. However, there is limited research on hydrosols extracted from *Camellia japonica* branches, specifically in terms of their potential to inhibit tyrosinase. Hence, the aim of this study was to investigate the chemical composition of these hydrosols and their effects on inhibiting tyrosinase. Hexamethyl-cyclotrisiloxane (38.1%) and vanillin (25.3%) were identified as the primary constituents in the hydrosol through gas chromatography–mass spectrometry analysis. The inhibitory effects of the hydrosol, in comparison with the positive control arbutin, were evaluated against mushroom tyrosinase, revealing significant tyrosinase inhibitory properties for both the hydrosol and arbutin. Furthermore, in the presence of α-melanocyte-stimulating hormone, the hydrosol notably diminished melanogenesis, resulting in a substantial reduction in melanin production. Genetic and protein analyses were conducted to uncover the mechanisms behind the hydrosol's inhibition of tyrosinase and reduction of melanin. The results suggest that the hydrosol may effectively shield melanocytes from detrimental factors associated with tyrosinase-related proteins. The anti-tyrosinase activity of the hydrosol indicates its potential for promoting skin lightening.

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Keywords: Anti-tyrosinase activity; Hexamethyl-cyclotrisiloxane; Skin whitening; Volatile compound

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INTRODUCTION

Camellia japonica, commonly known as “tsubaki” in Japanese, holds a historical significance as a favored tree for ornamental gardening, oil extraction, and traditional medicine in Japan. In the Korean context, *C. japonica*, a perennial tree within the Theaceae family and *Camellia* genus, thrives as an evergreen presence. Particularly abundant in regions like Jeju Island and Jeollanam-do, it constitutes a significant proportion, encompassing 67% of the nation’s total planted area.

C. japonica has a rich heritage of employment as a cosmetic safeguard to nurture skin and hair vitality and as a calming agent. Literature reveals a diverse spectrum of biological activities attributed to *C. japonica*, including documented instances of its

antibacterial efficacy (Huang *et al.* 2012), inhibition of human immunodeficiency virus type 1 protease (Onodera *et al.* 2006), suppression of Epstein-Barr virus (Salinero *et al.* 2012), mitigation of metastatic progression (Lee *et al.* 2017), antioxidative prowess (Kim *et al.* 2001; Choi *et al.* 2013), control over human type I pro-collagen production (Kim *et al.* 2012), and modulation of allergic responses (Jung *et al.* 2007).

There is little previous research concerning the effects of *C. japonica* on tyrosinase activity inhibition. The multifaceted nature of botanical volatile compounds is well-established, encompassing robust antibacterial properties, potential anti-aging effects, and facilitation of skin revitalization (Schwab *et al.* 2008). As a result, continuous efforts are being earnestly channeled into the identification of a natural agent for skin lightening, with concern for both effectiveness and safety (Tayarani-Najaran *et al.* 2016).

Skin complexion is influenced by various pigments present in distinct skin layers, such as melanin within the epidermis, hemoglobin in dermal blood vessels, and carotene in the subcutaneous tissue. Notably, the external appearance of skin hinges on the abundance and dispersion of melanin pigment (Niu and Asia 2017). The enzymes central to melanin synthesis include tyrosinase, tyrosinase-related protein-1 (TRP-1), and dopachrome tautomerase (DCT, TRP-2) (Zolghadri *et al.* 2019). Among these, tyrosinase holds a pivotal role, initiating the primary reaction that dictates the pace of melanin synthesis by converting tyrosine to DOPA-quinone (Kim *et al.* 2023). Consequently, substances capable of inhibiting tyrosinase, TRP-1, and TRP-2 have the potential to hinder melanin formation, thereby manifesting skin lightening properties (Chou *et al.* 2013).

Melanin serves a critical function in shielding the skin against UV radiation and external hazards. However, excessive melanin production, coupled with its accumulation on the skin, can lead to undesirable outcomes such as melasma, freckles, and pigmented lesions. Moreover, this surplus melanin can pose toxicity concerns due to precursor substances, potentially leading to cellular damage and even conditions such as skin cancer (Choi *et al.* 2017). Hence, the primary objective of this investigation was to delve into the chemical constitution of the *C. japonica* branch using gas chromatography-mass spectrometry (GC-MS) and concurrently to appraise its potential to inhibit tyrosinase activity. To comprehensively evaluate this inhibitory potential, the study entailed a meticulous analysis of the impact of the *C. japonica* branch on α-melanocyte-stimulating hormone (α-MSH)-induced melanogenesis and its ability to hinder tyrosinase activity within B16F10 melanoma cells.

EXPERIMENTAL

Chemicals and Materials

C. japonica (cultivar: 'Tricolor'; plant age: about 30 years) branch were purchased in March 2022 from the Seohyeon Herbal Medicine Farming Association, Nonsan, South Korea. Plant materials were identified by Hee-Gon Kang, a representative from the experimental forest of Gyeongsang National University. The samples were washed with running tap water before being chopped into pieces (about 2 cm).

Hydrosol Preparation

A quantity of 500 g of *C. japonica* branches was introduced into a container and subjected to a 30-min distillation process using 2,000 mL of water. The vapor thus

produced underwent a controlled cooling procedure *via* an enclosed cooling mechanism, leading to condensation and collection of the resultant liquid in a receptacle. During this phase, the oil component gravitated towards the upper segment of the distilled liquid, while the aqueous portion settled in the lower layer. As a consequence, the upper fraction of the liquid was extracted to obtain the hydrosol, which was subsequently preserved at a temperature of -20 ℃ until required for analysis.

GC–MS Analysis

The volatile constituents of the hydrosol were subjected to analysis utilizing GC–MS instrumentation (Clarus 600 GC-MS, Perkin Elmer, Shelton, CT, USA). For this purpose, the PerkinElmer Elite-5ms column (30 mm × 0.3 mm × 0.25 μm) was employed. Helium gas (1.0 mL/min) was employed as the mobile phase. The temperature within the oven commenced at 40 ℃ and was gradually escalated at a rate of 10 ℃/min, holding at this temperature for 1.0 min. Subsequently, a temperature increase to 230 °C was executed at a rate of 10 ℃/min, where it was maintained for 5 min. The injector temperature was fixed at 200 ℃, while the detector temperature was set at 250 ℃. The interpretation of the analysis findings was accomplished employing the NIST Mass Spectral Search Program (Version 2.0 g, National Institute of Standards and Technology, Gaithersburg, MD, USA).

Cell Culture

B16F10 murine melanoma cells were procured from the Korea Cell Line Bank. Cell cultivation was executed employing DMEM medium (WELGENE, Daegu, Korea), supplemented with 10% FBS (fetal bovine serum, WELGENE, Daegu, Korea), and 1% penicillin-streptomycin (Gibco BRL). The culturing environment was established at a temperature of 37 °C under 5% CO_2 conditions. To mitigate the issue of cellular overpopulation stemming from proliferation, the cultivated B16F10 cells were vigilantly maintained at a suitable density *via* the application of trypsin (HyClone, USA).

Cell Viability Assay

B16F10 melanoma cells were introduced into a 6-well plate at a concentration of 3×10^4/mL for the purpose of cell culture. The hydrosol treatment was meticulously administered across each well, employing varying concentrations ranging from 31.25 ppm to 500 ppm. Following a 72-h incubation period, the medium was extracted, and a 200 μL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (Promega, Madison, WI, USA) was introduced. Following a 2-h incubation within a CO_2 incubator at 37 °C, the MTT reagent was meticulously eliminated. Subsequently, for cell staining, 2 mL of DMSO was added to facilitate dissolution of the formazan generated within the wells. The assessment of cell viability was undertaken through absorbance measurements at 540 nm utilizing an ELISA reader (SpectraMax 190, Molecular Devices LLC, San Jose, CA, USA).

Tyrosinase Activity Assay

Tyrosinase activity was assessed through the determination of the L-DOPA oxidation rate. B16F10 cells, numbering 2×10^5 cells per well, were subjected to treatment involving α-melanocyte stimulating hormone (α-MSH) and hydrosol, and were cultured over a span of 3 days. Following this incubation period, cells were meticulously collected, and subsequent to the addition of lysis buffer, they were subjected to lysis at a temperature

of 4 ℃ for a duration of 1 h. Subsequent to centrifugation at 12,000 × g for 15 min, the supernatant was meticulously gathered. Upon quantifying the protein content within the gathered supernatant, L-DOPA at a concentration of 10 mmol/mL was introduced. The cells were then subjected to incubation within a CO_2 incubator set at 37 °C for a duration of 30 min, following which absorbance readings at 490 nm were captured employing an absorbance microplate reader (SpectraMax 190, Molecular Devices LLC, San Jose, CA, USA). The obtained data were subjected to analysis using the ensuing equation: tyrosinase activity (%) = (OD 490 of sample/OD 490 of control) × 100.

Melanin Content Assay

B16F10 cells were introduced into a six-well plate (2×10^5 cells/well), and following a 12-h period of initial adherence, the cell culture medium was renewed for those cells previously exposed to different hydrosol concentrations and α-MSH over a span of 48 h. Following this, the cells were thoroughly washed twice with phosphate-buffered saline (PBS). B16F10 cells were then subjected to a combined treatment involving α-MSH and hydrosol, with a subsequent 3-day incubation period. Post-incubation, the cells were carefully harvested, and a double wash was performed using PBS. Subsequently, these cells were subjected to treatment with 1 N NaOH containing 10% DMSO, and this mixture was subjected to reaction at a temperature of 80 ℃ for a duration of 1 h. In order to gauge melanin content, absorbance readings were captured at 475 nm using an absorbance microplate reader (SpectraMax 190, Molecular Devices LLC, San Jose, CA, USA). The percentage value corresponding to the cells treated with hydrosol was calculated relative to the negative control. Specifically, the BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, US) was deployed to ascertain the protein concentration in each sample. The quantified melanin content was then normalized to the cellular protein concentration (absorbance melanin/μg protein) (Ha *et al*. 2021).

Investigation of Genes

B16F10 melanoma cells were introduced into a 100 mm culture dish, with an initial seeding density of 1×10^6 cells, and they were nurtured in DMEM medium (GIBCO, USA) supplemented with 10% bovine serum and 1% antibiotics. These cells were then placed within a 5% CO_2 environment at 37 °C for incubation. Following a medium exchange to fresh 10% DMEM, the addition of hydrosol to the culture plate was executed, with a 3-day cultivation duration. Additionally, 1% Amisoft was introduced as a surfactant, in a quantity corresponding to 1/1,000 of the medium volume. Subsequent to this 3-day period, the cells were treated with 1 mL of TRIzol (Invitrogen, USA) for the purpose of assessing the mRNA expression level of genes associated with skin whitening. The ensuing RNA isolation was conducted utilizing Invitrogen's RNA isolation method. After quantifying the RNA quantity at 260 nm employing an ultraviolet detector, reverse transcription-polymerase chain reaction (RT-PCR) was carried out. This RT-PCR procedure utilized an all-in-one RT-PCR kit (Super Bio, Korea), following the manufacturer's guidelines, with primer sequences and reaction conditions detailed in Table 1. Ultimately, a total of 72 PCR reactions were undertaken, spanning 25 cycles over 1 min each.

Table 1. The Primers and Reaction Conditions

Primer	Reaction Conditions
Beta-actine	5'-GAG ACC TTC AAC ACC CCA GCC-3'; anti-sense, 5'-GGC CAT CTC TTG CTC GAA GTC-3'; reverse transcription at 50 °C for 30 min; reverse transcriptase inactivated at 96 °C for 3 min, 94 °C for 30 s, 62 °C for 1 min, followed by 25 cycles at 72 °C for 1 min.
Tyrosinase	5'-GGC CAG CTT TCA GGC AGA GGT-3'; anti-sense, 5'-TGG TGC TTC ATG GGC AAA ATC-3'; denatured at 90 °C for 30 s; reverse transcription at 60 °C for 30 min; reverse transcriptase inactivated at 94 °C for 1 min. Thereafter, a PCR was performed for 30 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min
Tyrosinase-related protein 1 (TRP-1)	5'-GCT GCA GGA GCC TTC TTT CTC-3'; anti-sense, 5'-AAG ACG CTG CAC TGC TGG TCT-3'; denatured at 90 °C for 30 s; reverse transcription at 60 °C for 30 min; reverse transcriptase inactivated at 94 °C for 1 min. Thereafter, a PCR was performed for 30 cycles at 94 °C for 30 s, 56 °C for 30 s, and 72 °C for 1 min.
Tyrosinase-related protein 2 (TRP-2)	5'-TGA CCG TGA GCA ATG GCC-3'; anti-sense, 5'-CGG TTG TGA CCA ATG GGT GCC-3'; reverse transcription at 50 °C for 30 min; inactivation of reverse transcriptase at 96 °C for 3 min, 94 °C for 1 min, and 60 °C for 1 min.

Note: the PCR was described previously in Ha *et al*. (2021)

Investigation of Protein Expression

Murine B16F10 melanoma cells were introduced into a 100 mm culture dish at a seeding density of 5×10^5 cells, utilizing DMEM medium enriched with 10% FBS and 1% antibiotics. The cells were then cultivated within a 5% CO_2 atmosphere at a temperature of 37 °C for a duration of 1 day. Following this initial period, a medium replacement was executed, and hydrosol was introduced at varying concentrations, with the cells undergoing a subsequent 3-day incubation. A 1% aqueous solution of Amisoft, functioning as a surfactant, was added to the medium in an amount corresponding to 1/1000 of the medium volume.

The cultured cells were subjected to PBS washing before being transferred into a 1.5 mL microtube. These cells were subsequently introduced into a cell disruption buffer (comprising 40 mM Tris-Cl [pH 7.4], 10 mM EDTA, 120 mM NaCl, 0.1% NP-40, 1 mM PMSF, and a protease inhibitor cocktail). Following the addition and disruption of cells, centrifugation was executed at 15,000 rpm and at a temperature of 4 °C for 10 min, with the ensuing supernatant being isolated for protein separation. The quantification of isolated proteins was accomplished through Sigma's BCA method, with the subsequent implementation of SDS-PAGE. Following the transfer of SDS-PAGE gel onto a PVDF membrane, the protein content was labeled using a secondary antibody conjugated with a primary antibody and peroxidase. This treated membrane was then exposed to an X-ray film, employing a western blot detection kit (Intron, Korea). Subsequently, an assessment of expression levels was undertaken.

Statistical Analysis

Statistical analyses of the results were performed at a 5% significance level. Differences between the means of individual groups were assessed using the Student's t-test (language R, R Development Core Team 2020, Vienna, Austria) and Duncan's multiple range test (SAS, SAS Institute, Inc., North Carolina, USA).

RESULTS AND DISCUSSION

The Chemical Composition of Hydrosol

The hydrosol was acquired with a 17% w/w yield. Employing GC–MS analysis (Fig. 1), the hydrosol's chemical composition was scrutinized. GC-MS analysis of *C. japonica* branches showed 6 major peaks indicating the presence of various phytochemical constituents. On comparison of the mass spectra of constituents with the main library, all these compounds were characterized and probably identified (Table 2). Notably, the principal constituents within the hydrosol encompassed hexamethyl-cyclotrisiloxane (38.1%), octamethyl-cyclotetra-siloxane (22.4%), and vanillin (25.3%). Chemical derivatives of siloxane in *C. japonica* branches were detected using GC-MS rather than calibration standards. Siloxanes, a subgroup of silicones, consist of Si–O bonds with aliphatic chains (mostly methyl groups) attached to Si atoms. Due to their low surface tension, high thermal stability, and lubricating properties, siloxanes have been widely used for decades in industrial processes and consumer products such as cosmetics and health care products. Prior investigations have indeed identified hexamethyl-cyclotrisiloxane in leaf and stem extracts of *Bauhinia acuminata* Linn (Anjukrishna *et al.* 2015). In the context of *Moringa oleifera*, the volatile components were primarily constituted by esters, acids, and aldehydes with hexamethyl-cyclotrisiloxane (Dandan *et al.* 2018). Likewise, the present findings validate hexamethyl-cyclotrisiloxane's prominence within the hydrosol. Noteworthy among the hydrosol's core constituents, vanillin, has been discerned within the essential oil of *Eugenia caryophyllata*, *Ocimum basilicum* (Khanafari *et al.* 2011), *Hyssopus officinalis* L. (Lamiaceae) (Schulz and Stahl-Biskup 1991), and *Calea clematidea* (Flach *et al.* 2002). Prior research has indeed attested to vanillin's potential to impede monophenolase and diphenolase activities intrinsic to tyrosinase (Gou *et al.* 2017). It is notable that certain low molecular weight cyclic volatile methylsiloxane compounds, including hexamethyl-cyclotrisiloxane, have found application in cosmetics, personal care products, and diverse consumer items (Wang *et al.* 2009). In future endeavors, the compound responsible for the inhibition of melanin synthesis and tyrosinase function could be extracted for potential application in natural cosmetics or medicinal contexts.

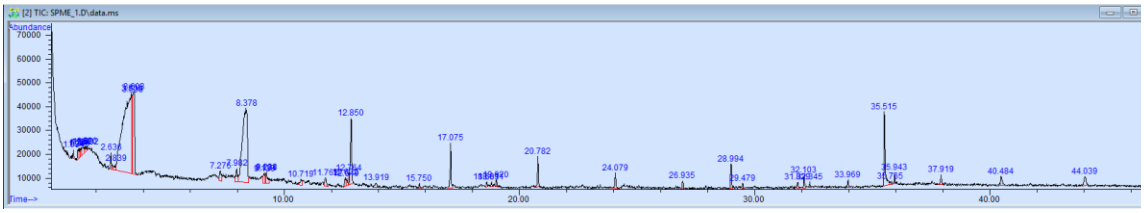


Fig. 1. Chromatograms of a hydrosol obtained by HS-SPME. Peaks numbers are identified according to Table 2

Table 2. Gas Chromatography-Mass Spectrometry (GC/MS) Analysis of *C. japonica* Branch

Compounds	Retention Time (min)	Area (%)
Hexamethyl-cyclotrisiloxane	3.50	38.1
Octamethyl-cyclotetrasiloxane	8.38	22.4
Vanillin	17.1	25.3
n-Hexadecanoic acid	29.0	5.42
Hexanedioic acid	35.5	3.56
Formic acid	40.5	1.12
Total		**95.9**

Cell Viability of Melanoma Cells Induced by Hydrosol

The findings indicate that exposure of murine melanoma B16F10 cells to hydrosol concentrations ranging from 31.2 to 500 ppm for a 24-h interval did not precipitate any discernible alterations in cell viability. Especially, the cell viability at these concentrations was within the range of 80% to 100%, which means that hydrosol did not show cytotoxicity (Fig. 2). Consequently, the entire spectrum of concentrations (31.25 to 500 ppm) was deemed suitable for subsequent exploration of hydrosol's impact on both tyrosinase activity and melanin synthesis within the B16F10 cell context.

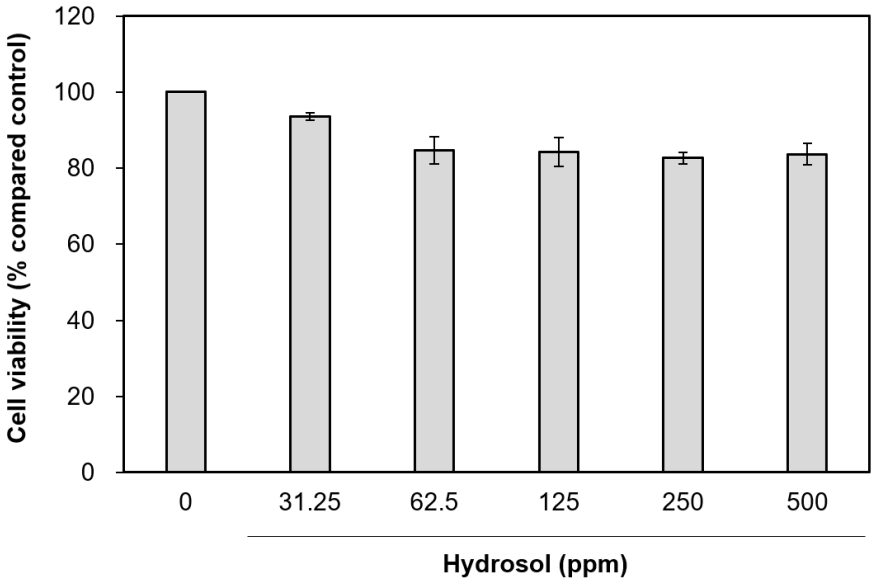


Fig. 2. The effects of hydrosol obtained from *C. japonica* branch on the cell viability of α-MSH-treated B16 cells. *: $p < 0.05$ compared non-treated (0 ppm); but there not significant.

Inhibition of Intracellular Tyrosinase Activity of Hydrosol

The impact of hydrosol, alongside that of arbutin, a widely recognized tyrosinase inhibitor, on the L-DOPA oxidation catalyzed by tyrosinase was meticulously examined. As illustrated in Fig. 3, both hydrosol and arbutin demonstrated substantial dose-dependent inhibitory influences on L-DOPA oxidase activity. The outcomes corroborate that hydrosol and arbutin evinced comparable tyrosinase inhibitory propensities. Statistical analysis showed that arbutin displayed significantly greater tyrosinase inhibitory efficacy in

comparison to hydrosol (125, 250, and 500 ppm). However, at concentrations of 31.2 ppm, and 62.5 ppm, similar inhibitory effects (not statistically significant) were evident in relation to tyrosinase, when contrasted against the well-established tyrosinase inhibitory potential of hydrosol.

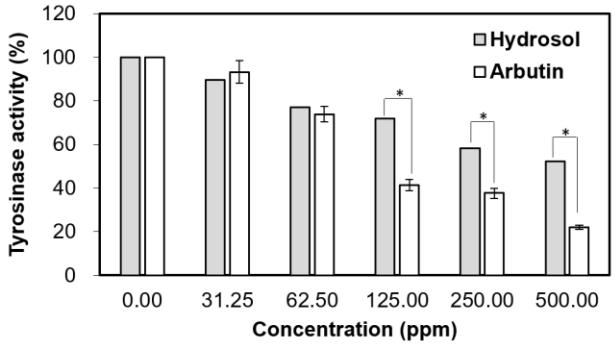


Fig. 3. Tyrosinase inhibitory activities of hydrosol obtained from *C. japonica* branch. The data are representative of three independent experiments and expressed as the mean ± standard error of the mean. *: $p < 0.05$ compared arbutin each at the same concentration.

Effect of Hydrosol on Melanin Content in B16F10 Cells

To substantiate the potential contribution of hydrosol in impeding melanin production, alterations were investigated concerning melanin secretion following treatment with distinct hydrosol concentrations within a melanin-inducing environment facilitated by α-MSH stimulation. The hydrosol was administered at concentrations of 31.25, 62.5, 125, 250, and 500 ppm, employing a methodology akin to that utilized for tyrosinase activity analysis. Notably, the hydrosol exhibited a pronounced capacity to hinder melanogenesis within the α-MSH-treated group, resulting in a marked reduction in melanin levels (Fig. 4). This observation paralleled the outcomes observed in the positive control group treated with arbutin (not shown). Remarkably, even at the lowest concentration of 31.2 ppm within the treated group, a statistically significant decrease in melanin content was discerned when contrasted against the untreated group (0 ppm).

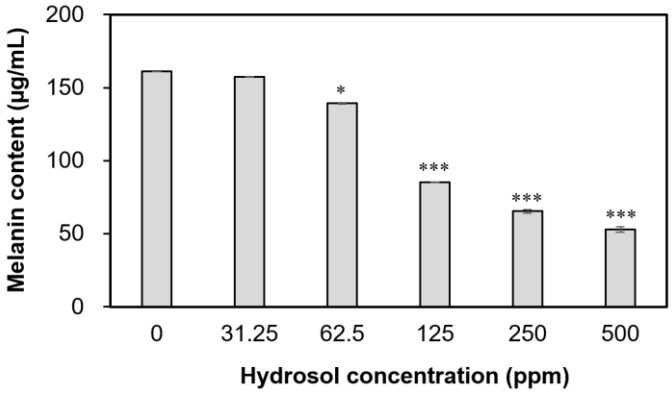


Fig. 4. Effects of hydrosol obtained from *C. japonica* branch on melanin content in α-MSH-treated B16 cells. The data are representative of 3 independent experiments and expressed as the mean ± standard error of the mean. *: $p < 0.05$, ***<0.001 compared arbutin each at the same concentration.

Therefore, it can be deduced that hydrosol adeptly curtails the synthesis or discharge of melanin within B16F10 cells. Within the 31.25, 62.5, 125, 250, and 500 ppm hydrosol-treated groups, melanin content demonstrated reductions of 1.1, 1.5, 2.6, 2.7, and 4.3 times, correspondingly, relative to the untreated group. This unequivocally underscores the discernible capacity of hydrosol to impede melanin secretion. The outcomes spanning cytotoxicity assessment, tyrosinase inhibition, and melanin content analysis collectively accentuate the extraordinary potential of hydrosol as an invaluable constituent in the realm of skin-whitening cosmetics.

Evidently, volatile compounds have been reported to manifest robust antioxidant properties (Yee 2019). Among these, the described hydrosol stands out, characterized by its distinct pungent aroma. Synthesized by plants as secondary metabolites, the hydrosol's multifaceted benefits encompass bactericidal, virucidal, fungicidal, anticancer, antioxidant, and antidiabetic attributes (Kong *et al.* 2021). In this investigation, the hydrosol prominently featured hexamethyl-cyclotrisiloxane, contributing to 42.4% of the total chemical makeup. In a parallel vein, *Toxicodendron vernicifluum*, encompassing hexamethyl-cyclotrisiloxane, has been shown to display diverse biological and pharmacological facets, spanning central, antimicrobial, and antitumor activities (Saravanakumar *et al.* 2019). Notably, elevated concentrations of hydrosol scarcely induced cytotoxic impacts on melanocytes in this study. Consequently, the inhibitory effect of hydrosol on α-MSH-induced B16F10 cell proliferation came into focus. The results lay bare that pretreatment with hydrosol elicited a dose-dependent decrease in α-MSH-induced cell propagation (Fig. 3), closely mirroring the outcomes observed with pure arbutin utilized as a positive control. Notably, the majority of natural essential oils in traditional medicine entail complex compound mixtures, veering away from pure compounds.

Genetic Investigation

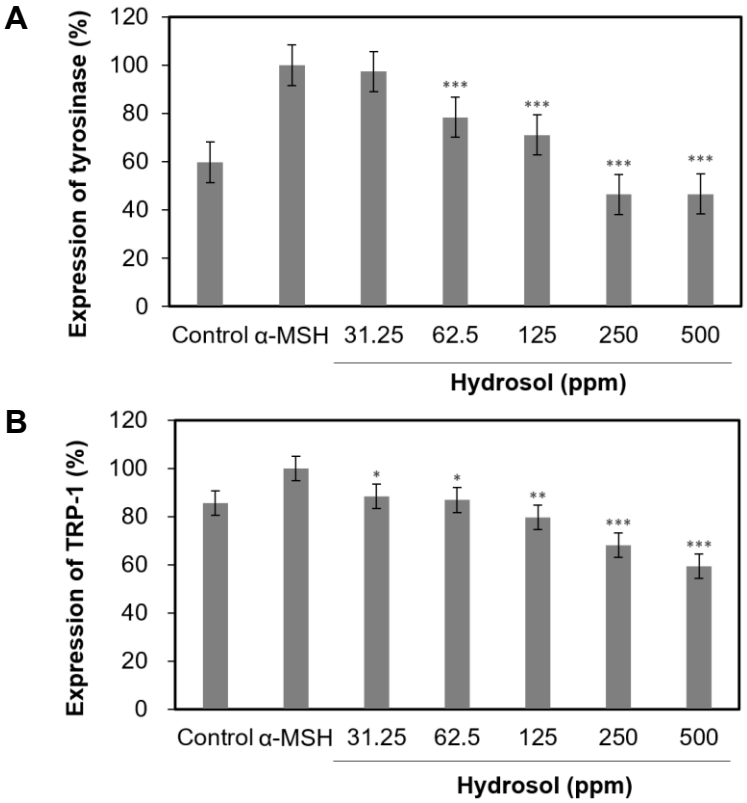
In pursuit of discerning the gene within hydrosol that thwarts melanin biosynthesis by influencing genes participating in the melanin biosynthesis pathway, an investigative endeavor employing the RT-PCR technique was conducted, with the outcomes unveiled in Fig. 5. Serving as the negative control, α-MSH was juxtaposed against hydrosol, which exhibited pronounced inhibitory effects on the expression of tyrosinase, TRP-1, and TRP-2 across all concentrations. Notably, at 125 ppm or above, hydrosol elicited a reduction of more than 50% in tyrosinase expression. Intriguingly, the inhibitory efficacy on tyrosinase, TRP-1, and TRP-2 expression demonstrated a diminishing trend as the concentration of hydrosol escalated. These outcomes intriguingly underscore the proposition that hydrosol's melanogenesis inhibitory potential correlates with its ability to suppress the expression of tyrosinase, TRP-1, and TRP-2.

Tyrosinase, a pivotal enzyme implicated in melanin production through an enzymatic oxidative pathway, governs the coloration of skin, hair, eyes, and even the browning of certain foods (Pillaiyar *et al.* 2018). Chemical agents showcasing anti-tyrosinase activity have found clinical utility in managing dermatologic conditions linked with melanin hyperpigmentation (Rodboon *et al.* 2020). Moreover, melanin production holds implications for specific histopathological attributes unique to malignant cancers (Sarna *et al.* 2019), reinforcing the potential therapeutic relevance of anti-tyrosinase agents in skin cancer treatment.

The contemporary trend leans towards embracing natural products *in lieu* of chemical or synthetic compounds, which can be economic viability, environmental

friendliness, and safety (Gullón *et al.* 2020). Simultaneously, the research and development of sustainable technology and cost-effective raw materials bear significance for both the industry and the optimization of plant resource utilization. Recent literature has spotlighted the anti-tyrosinase activity of select plant species (Cheraif *et al.* 2020), yet information regarding essential oils sourced from natural plants remains limited. Consequently, the quest for identifying plant essential oils harboring robust anti-tyrosinase potential has garnered substantial interest.

The etiology of pigmentation remains relatively enigmatic. The cascade of melanin biosynthesis hinges on melanocyte-specific enzymes such as TRP-1 and TRP-2 (Ryu *et al.* 2021). Hence, tyrosinase emerges as a pivotal gauge in addressing pigmentation concerns. Within the present investigation, hydrosol wielded a suppressive influence on melanin synthesis and concurrently impacted anti-tyrosinase activity. As a corollary, it is surmised that the anti-melanogenic potential exhibited by hydrosol might be intertwined with other enzymes, notably TRP-1 and TRP-2. Moving forward, it becomes imperative to segregate the volatile constituents inherent to hydrosol and delve into the anti-melanogenic impact attributed to each isolated individual component. In line with the present findings, it is tentatively deduced that hydrosol potentially mitigates the impact of detrimental factors on melanocytes, including TRP-1 (as exemplified in Figs. 5 and 6).



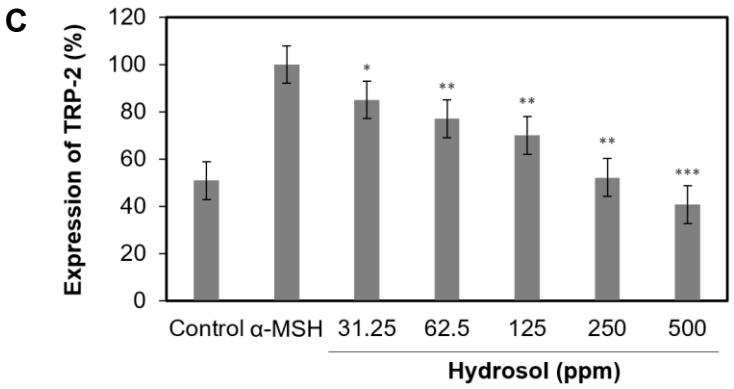
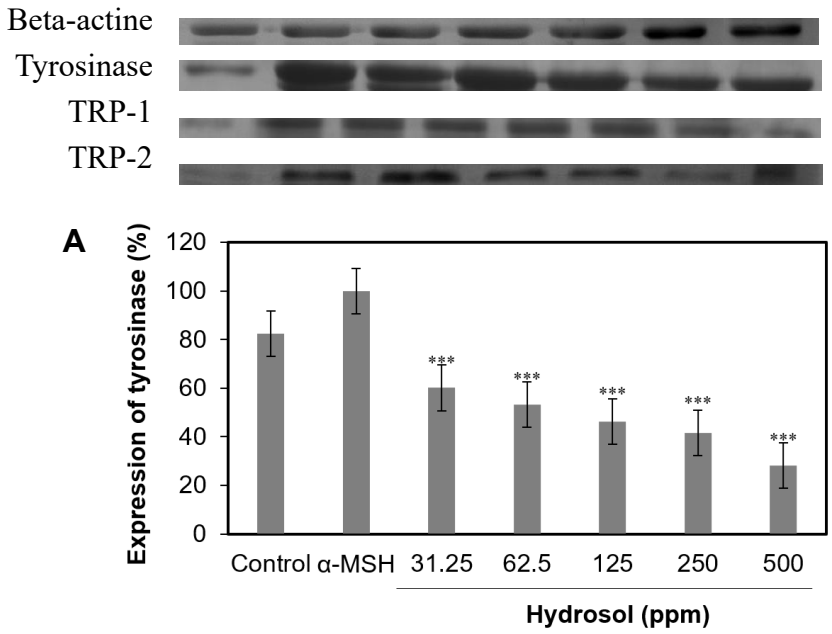


Fig. 5. The effects of hydrosol obtained from *C. japonica* branch on gene expression in α-MSH-treated B16 cells. The data are representative of three independent experiments and expressed as the mean ± standard error of the mean. *: $p < 0.05$, **: $p < 0.01$, ***<0.001 compared α-MSH.

Evidence suggested that hydrosol obtained from the *C. japonica* branch was effective in inhibiting melanin. This finding is supported by the fact that the functional components contained in hydrosol obtained from the *C. japonica* branch stimulated melanin. A total of six components were detected by GC-MS, and studies on whether each component influenced melanin stimulation are needed in the future.



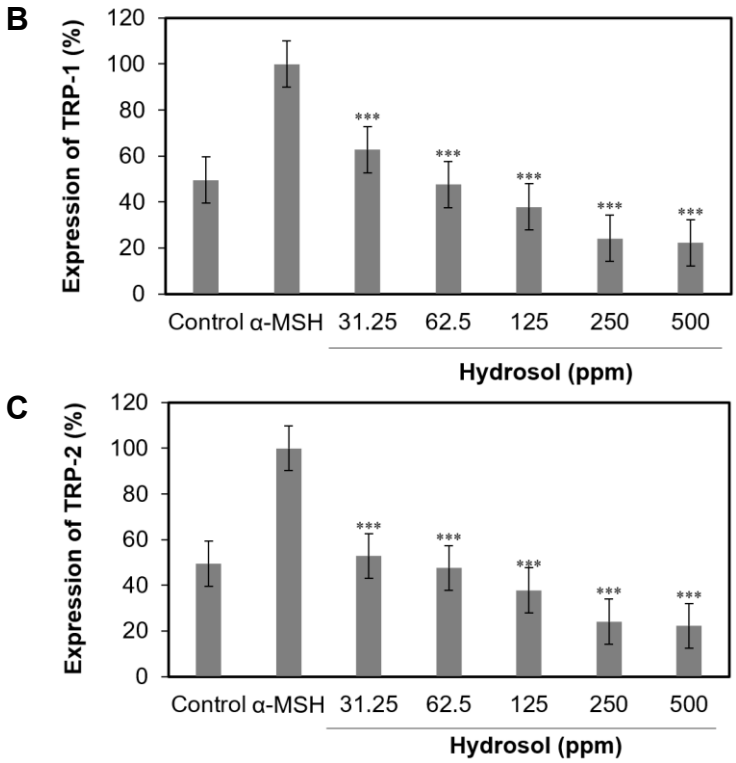


Fig. 6. The effects of hydrosol obtained from *C. japonica* branch on protein expression in α-MSH-treated B16 cells. The data are representative of three independent experiments and expressed as the mean ± standard error of the mean. ***<0.001 compared α-MSH.

Protein Expression Investigation

The impact of hydrosol on the expression of proteins integral to melanin biosynthesis was verified through western blotting, as illustrated in Fig. 5. When incubated against the negative control group comprising α-MSH-treated cells, it became evident that hydrosol exerted an inhibitory influence. Remarkably, hydrosol showcased its most pronounced inhibitory efficacy towards tyrosinase and TRP-2. Specifically, at 500 ppm concentration, the inhibition reached 46.6% for tyrosinase and 40.7% for TRP-2 in comparison to the α-MSH-treated group. While TRP-1 demonstrated comparatively lesser inhibitory potential, it still exhibited a significant decline in contrast to the α-MSH-treated group. As such, the protein analysis mirrored the trends observed in the gene analysis, thereby affirming that hydrosol incites a reduction in melanin *via* the stimulation of tyrosinase, TRP-1, and TRP-2. Although the present study was an *in vitro* experiment, it can be expected that similar effects may be shown for *in vivo* experiments in animals or volunteers. The inhibition of tyrosinase enzyme would need to be confirmed in the skin of animals or volunteers. However, for clear results, this should be studied in the future.

CONCLUSIONS

1. To the best of our knowledge, this marks the inaugural study unveiling the potential of a hydrosol from *Camellia japonica* to impede melanin synthesis within B16F10 melanoma cells.

2. The present findings underscore that the hydrosol intervenes in α-MSH-triggered melanogenesis by not only deactivating tyrosinase but also concurrently repressing the expression of proteins integral to melanin biosynthesis in B16F10 melanoma cells.
3. With its established safety profile, the hydrosol's non-cytotoxic nature was verified in this investigation. As such, the prospect of hydrosol's application as an efficacious skin-whitening agent gains prominence, arguing well for future advances in alternative medicine-based aromatherapy.

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Data Availability

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Si Young Ha, Ji Young Jung, Jung Myoung Lee and Jae-Kyung Yang contributed equally to this work.

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