Lee *et al. Applied Biological Chemistry* (2025) 68:26
https://doi.org/10.1186/s13765-025-01004-x

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The Korean Society for Applied Biological Chemistry

ARTICLE Open Access

Cellular enzyme-modulating activity of *p*-acetoxycinnamyl alcohol from *Alpinia officinarum*

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Abstract
Alpinia officinarum, commonly referred to as lesser galangal, is a medicinal plant traditionally used in East Asian practices owing to its anti-inflammatory, antioxidant, and antimicrobial properties. This study aimed to isolate *p*-acetoxycinnamyl alcohol from *A. officinarum* and evaluate its effect on melanogenesis-related enzyme expression. *p*-Acetoxycinnamyl alcohol was successfully isolated and structurally characterized from *A. officinarum* using NMR spectroscopy. The compound's skin-whitening potential was investigated through a combination of molecular docking simulations and in vitro assays using B16F10 melanoma cells. The results demonstrated that *p*-acetoxycinnamyl alcohol showed potent binding affinity and inhibited the expression of microphthalmia-associated transcription factor, tyrosinase, tyrosinase-related protein (TRP)-1, and TRP-2 in melanocytes as shown by molecular docking simulations and in vitro assays. These findings indicate that *p*-acetoxycinnamyl alcohol has potential as a natural skin-whitening compound that controls melanogenic enzyme expression and functionality. The therapeutic potential of *A. officinarum* in functional cosmeceuticals and dermatological therapies emphasizes its pharmacognostical importance.

Keywords *Alpinia officinarum*, Melanogenesis, Molecular docking, *p*-acetoxycinnamyl alcohol, Skin-whitening, Tyrosinase

Introduction

Alpinia officinarum Hance, commonly referred to as lesser galangal, is a perennial herbaceous plant belonging to the Zingiberaceae family, traditionally used in East Asian medicine for its anti-inflammatory, antioxidant, and antimicrobial properties [1, 2]. Extensive pharmacological studies have demonstrated diverse biological activities including anti-inflammatory [3], antioxidant [4], anti-skin aging [5], antibacterial [6], and anticancer [7] properties. Moreover, its bioactive compounds exhibit anti-arthritic [8], analgesic [9], and immunomodulatory [6] effects.

The phytochemical composition of *A. officinarum* is characterized by the presence of flavonoids, glycosides, and diarylheptanoids, which contribute to its

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pharmacological properties [10]. Among these phytochemicals, flavonoids such as galangin and kaempferide, as well as diarylheptanoids, have been extensively studied for their biological activities [11, 12]. In particular, *p*-acetoxycinnamyl alcohol, a bioactive diarylheptanoid, has been identified as a key constituent of *A. officinarum* and is believed to play a crucial role in its therapeutic effects [13]. *p*-Acetoxycinnamyl alcohol was first reported to have been isolated in *A. officinarum* in a study in the year 2023 [13]. Although *p*-acetoxycinnamyl alcohol is commercially available, its isolation from *A. officinarum* is scientifically significant. As of today, though there have been a few studies on the compound in the context of the plant, there is still a need for more studies to uncover its pharmacologic potential.

Melanogenesis, the process of melanin production in melanocytes, is regulated by key enzymes such as tyrosinase (TYR), tyrosinase-related protein (TRP)-1, and TRP-2, along with microphthalmia-associated transcription factor (MITF), which acts as a critical transcriptional regulator [14–17]. Excessive melanin accumulation leads to hyperpigmentation disorders, increasing the demand for effective skin-whitening agents in dermatology and cosmetics [18–20].

Natural compounds are being explored as safer skin-whitening alternatives to synthetic agents such as hydroquinone and arbutin [21–23]. Notably, plant-derived polyphenols and diarylheptanoids exhibit depigmentation effects by downregulating MITF and TYR activities [24–26]. Molecular docking studies have shown that *p*-acetoxycinnamyl alcohol primarily interacts with MITF and TYR through the formation of hydrogen bonds. Similarly, the molecular interactions of other natural compounds with MITF and TYR suggest that hydrogen bonding plays a crucial role in their inhibitory mechanisms. Given its antioxidant and anti-inflammatory properties, *p*-acetoxycinnamyl alcohol from *A. officinarum* has been hypothesized to regulate melanin biosynthesis by modulating key pigmentation pathways [25]. Natural compounds such as resveratrol and glabridin have shown biphasic effects on melanogenesis, stimulating melanin production at low concentrations and inhibiting it at high doses through mechanisms involving redox balance and MITF modulation [27, 28]. This study proposed that *p*-acetoxycinnamyl alcohol may regulate melanogenesis via dual mechanisms, including tyrosinase inhibition and modulation of MITF-dependent transcription.

This study aimed to isolate and characterize *p*-acetoxycinnamyl alcohol from *A. officinarum* Hance using nuclear magnetic resonance (NMR) spectroscopy for structural elucidation. Additionally, the melanogenesis-inhibitory effects of *p*-acetoxycinnamyl alcohol were investigated in 3-isobutyl-1-methylxanthine (IBMX)-treated B16F10 melanoma cells by assessing melanin content and expression levels of MITF, TYR, TRP-1, and TRP-2. The reported findings provide insight into the potential of *p*-acetoxycinnamyl alcohol as a natural skin-lightening agent for cosmetic and dermatological applications.

Materials and methods

Plant materials

A. officinarum was obtained from Vunique Co. (Eumseong, Korea). The voucher specimens were deposited at the Department of Beauty and Cosmetics, Far East University, Eumseong, Republic of Korea.

Equipment and reagents

The ^{1}H- and ^{13}C-NMR spectra of *p*-acetoxycinnamyl alcohol were determined using an Avance 600 MHz NMR spectrometer (Bruker, Rheinstetten, Germany). Dulbecco's modified Eagle medium (DMEM) was purchased from WELGENE (Daegu, Korea). Fetal bovine serum (FBS), penicillin, streptomycin, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2 H-tetrazolium bromide (MTT), kojic acid, and other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). All other reagents were of analytical grade.

Extraction, fractionation, and isolation

Fifty grams of dried and pulverized *A. officinarum* was dispersed in 1 L of distilled water (5%, *w/v*) and incubated at room temperature for 17 h with continuous stirring. After extraction at 70 °C for 6 h, the mixture was filtered through a Whatman No. 4 filter paper under suction. The resulting filtrate was then subjected to liquid-liquid extraction with methylene chloride in a 1:1 (*v/v*) ratio. This fractionation process was repeated thrice using a separatory funnel to ensure maximum extraction efficiency. The collected fractions were concentrated to 10 mL by rotary evaporation. The concentrated fractions were further separated by open-column chromatography using a silica gel column (1 × 10 in.), with methylene chloride as the eluent. Eluates were collected in 5 mL fractions, and specific fractions were selected based on thin-layer chromatography analysis. The solvent was removed from the purified fractions via rotary evaporation. The dried residues were dissolved in 100 mL purified water and lyophilized to obtain the final powdered extract (141 mg/50 g).

p-Acetoxycinnamyl alcohol (4-((1E)-3-hydroxyprop-1-en-1-yl)phenyl acetate; CAS No. 94723-93-0); melting point 68–70 °C [29]: ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 7.39 (1 H, d, J = 8.6 Hz, H-2), 7.39 (1 H, d, J = 8.6 Hz, H-6), 7.05 (1 H, d, J = 8.6 Hz, H-3), 7.05 (1 H, d, J = 8.6 Hz, H-5), 6.60 (1 H, d, J = 16.2 Hz, H-7), 6.33 (1 H, td, J = 5.6, 16.2 Hz, H-8), 4.32 (1 H, d, J = 5.4 Hz, H-9), 2.30 (3 H, s, H-11). ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 169.5 (C-10), 150.1

(C-4), 134.5 (C-1), 130.1 (C-7), 128.8 (C-8), 127.5 (C-2, C-6), 121.7 (C-3, C-5), 63.7 (C-9), 21.2 (C-11).

Molecular docking

In the molecular docking study, the active site of the target receptor was identified based on previously reported ligand-binding pockets [30] and further refined through a combination of search algorithms and energy scoring functions to predict the optimal binding conformation of *p*-acetoxycinnamyl alcohol. The docking software AutoDock Vina (1.2.1) [31] was used to investigate the potential interactions between p-acetoxycinnamyl alcohol and the target proteins tyrosinase (TYR) and microphthalmia-associated transcription factor (MITF). The SMILES configuration of *p*-acetoxycinnamyl alcohol was retrieved from the PubChem database (pubchem.ncbi.nlm.nih.gov). This was subsequently typed in the search tool of SwissTargetPrediction database (www.swisstargetprediction.ch) to get the three-dimensional structure in protein data bank (PDB) format. The crystal structures of TYR (PDB ID: 2y9x) and MITF (PDB ID: 4ati) were obtained from the RCSB PDB (www.rcsb.org). All necessary input files were prepared using the AMDock Tools package (1.5.2) [32]. For the docking simulations, a grid box encompassing the active site was defined with dimensions of 119 Å × 119 Å × 151 Å and a grid spacing of 1.0 Å. The Lamarckian genetic algorithm was used for docking with the following parameters: population size of 150; 250,000 energy evaluations; mutation rate of 0.02; crossover rate of 0.80; and 100 independent docking runs. A semiflexible docking protocol was used in this study. The docking results were clustered using a positional root-mean-square deviation (RMSD) threshold of 2.0 Å. Binding poses with an RMSD value below 2.0 Å were considered reliable. The ligand with the lowest binding free energy was identified as the most favorable candidate for interaction with the target protein. The binding energy of the ligand was compared with that of the native TYR ligand tropolone [33]. The docking outcomes were visualized using the PyMOL molecular graphics system (www.pymol.org).

Cell culture

B16F10 melanoma cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin (P/S) in a humidified incubator at 37 °C and a 5% CO_2 atmosphere.

Cell viability assay

B16F10 melanoma cells were seeded in 96-well plates (1×10^4 cells/well) and incubated for 24 h in DMEM supplemented with 10% FBS and 1% P/S at 37 °C and a 5% CO_2 atmosphere. Cells were then treated with *p*-acetoxycinnamyl alcohol (0–500 μg/mL) for 24 h. After treatment, 0.5 mg/mL MTT reagent was added and incubated for 3 h. Formazan crystals were dissolved in DMSO, and the absorbance was measured at 570 nm. The untreated group (0 concentration) is the positive control for normal cell growth. Cell viability was calculated as a percentage of the control.

Measurement of melanin content

B16F10 melanoma cells were seeded at a density of 1×10^5 cells/well in a 12-well plate and incubated for 24 h. Melanin production was induced by treating the cells with IBMX (200 μM). The cells were then treated with *p*-acetoxycinnamyl alcohol at concentrations of 0, 10, 20, 50, and 100 μg/mL, with kojic acid (100 μg/mL) as a positive control. After 48 h of incubation, the culture medium was collected and centrifuged at 12,000 rpm for 10 min at 4 °C to remove the cell debris. The supernatant (150 μL) was transferred to a 96-well plate, and the absorbance was measured at 475 nm using a microplate reader.

RNA extraction and quantitative real-time PCR (qRT-PCR)

B16F10 melanoma cells were seeded in a 6-well plate (7×10^4 cells/well) and incubated for 24 h. After treatment, the total RNA was extracted using a GneAmp Kit (Perkin Elmer, Foster City, CA, USA) according to the manufacturer's protocol. RNA concentration and purity were assessed using a microplate reader VICTOR 3X (Perkin Elmer, Waltham, MA, USA). qRT-PCR was performed using a CFX Connect Real-Time PCR Detection System (Bio-Rad, Hercules, CA, USA). The primer sequences used for TYR, MITF, TRP-1, TRP-2, and GAPDH (housekeeping gene) are listed in Table 1.

Statistical analysis

All experiments were conducted in triplicate ($n = 3$), and the results are presented as the mean ± standard error of the mean (SEM). Statistical analyses were performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc multiple comparison test. Statistical significance was set at $p < 0.05$.

Table 1 Primer sequences used for real-time qPCR

Gene	Upstream	Downstream
MITF	5′-GTA TGA ACA CGC ACT CTC TCG A-3′	5′-CTT CTG TCA TAC TGC TC-3′
TYR	5′-CAT TTT TGA TTT GAG TGT CT-3′	5′-TGT GGT AGT CGT CTT TGT CC-3′
TRP-1	5′-GCT GCA GGA GCC TTC TTT CTC-3′	5′-AAG ACG CTG CAC TGC TGG TCT-3′
TRP-2	5′-GGA TGA CCG TGA GCA ATG GCC-3′	5′-CGG TTG TGA CCA ATG GGT GCC-3′

Results and discussion

Identification of *p*-acetoxycinnamyl alcohol

Separation of *A. officinarum* Hance yielded multiple compounds, of which *p*-acetoxycinnamyl alcohol was isolated by extraction and column chromatography. NMR spectroscopy was used to confirm the structure of *p*-acetoxycinnamyl alcohol. The ^{1}H-NMR spectrum revealed characteristic peaks at δ 7.39 (H-2 and H-6), 7.05 (H-3 and H-5), 6.60 (H-7), 6.33 (H-8), 4.32 (H-9), and 2.30 (H-11), indicating the presence of aromatic and aliphatic protons. The ^{13}C-NMR data supported the presence of eleven carbon signals, confirming the successful isolation of the compound with significant resonance shifts at δ 169.5 (C-10), 150.1 (C-4), and others [13]. The chemical structure was shown on Fig. 1.

Molecular docking analysis with MITF and TYR

Molecular docking simulations were conducted to explore how *p*-acetoxycinnamyl alcohol interacts with MITF (Fig. 2). The results indicate that *p*-acetoxycinnamyl alcohol binds to the active site of MITF (highlighted in green), specifically within the DNA-binding domain. Figure 2 depicts several hydrogen bonds between *p*-acetoxycinnamyl alcohol and key MITF residues, including Arg216 and Asn219. Additionally, interactions between the nucleotides DA-7 and DC-6 are evident, suggesting that *p*-acetoxycinnamyl alcohol influences the transcriptional activity of MITF by altering its DNA-binding ability. These findings indicate that *p*-acetoxycinnamyl alcohol is a potential MITF inhibitor, as it may interfere with the ability of MITF to bind to DNA. Molecular docking simulations were also performed to investigate the binding mechanism of TYR. Figure 2 shows that *p*-acetoxycinnamyl alcohol likely interacts with the active site pocket of TYR and forms a typical hydrogen bond with MET319 and a π-donor hydrogen bond with HIS367. This may have important implications for its role in regulating melanogenesis and other biological pathways associated with MITF and TYR.

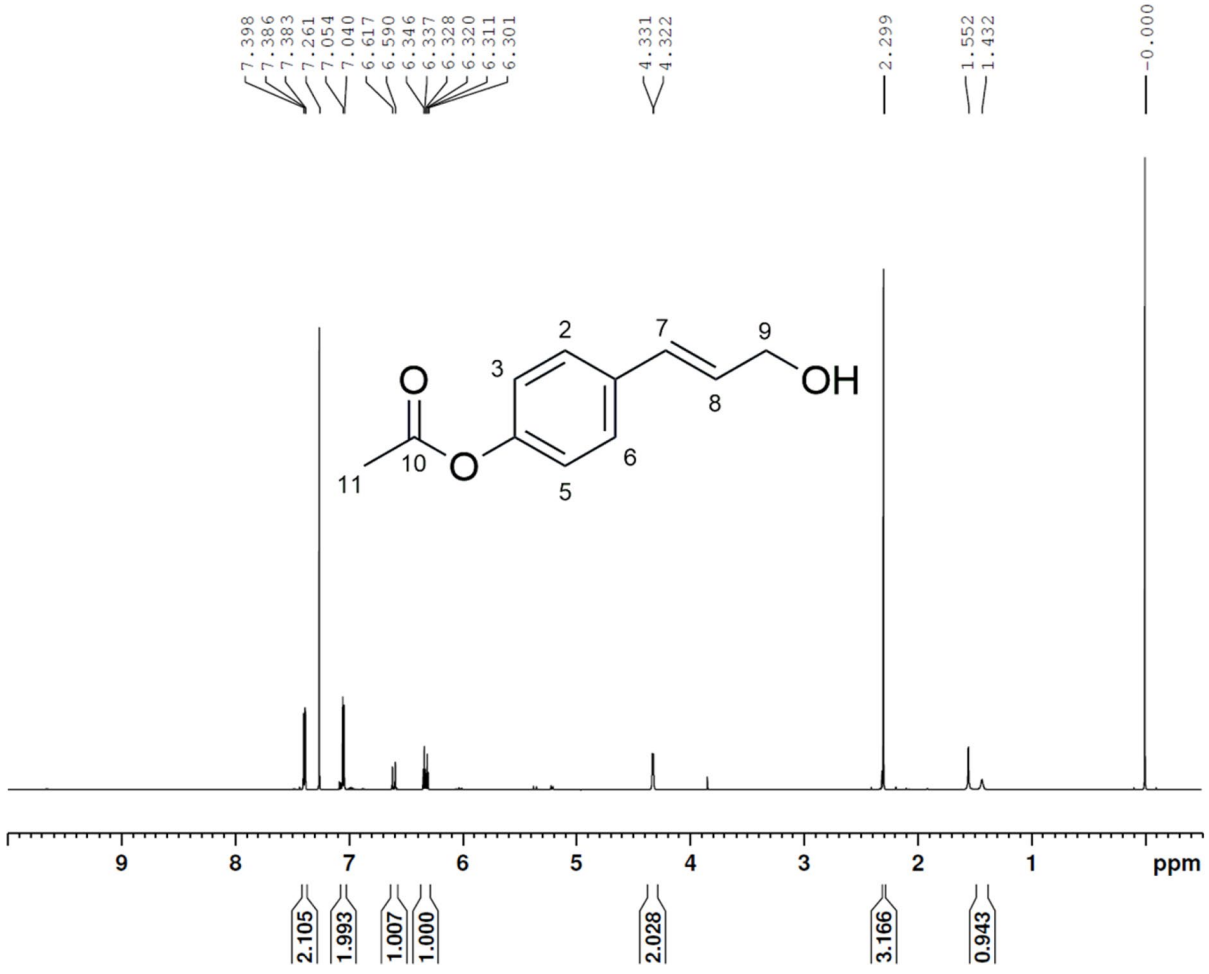

Fig. 1 Chemical structure and ^{1}H-NMR spectrum of *p*-acetoxycinnamyl alcohol

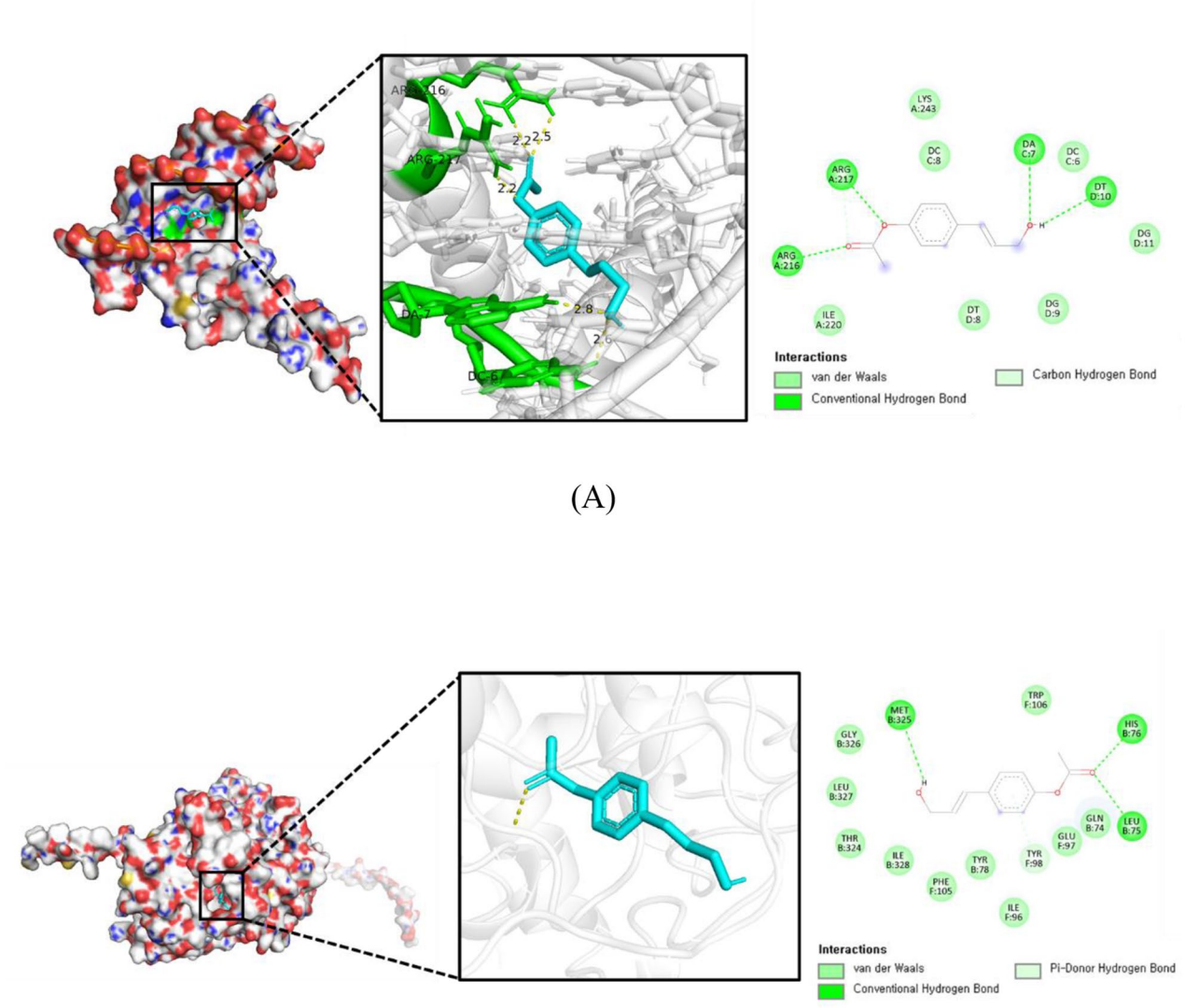

Fig. 2 Molecular docking simulation of interactions between TYR, MITF, and various compounds. Computed surface structure of TYR interacting with MITF (**A**) and TYR (**B**). The grey sticks show the catalytic core residues of TYR. *p*-Acetoxycinnamyl alcohol is presented mainly as a cyan stick structure and situated in the catalytic core domain of TYR. The dashed yellow line represents hydrogen bonding

Inhibitory effects on tyrosinase activity using TYR and L-DOPA

The inhibitory effects of *p*-acetoxycinnamyl alcohol on TYR activity were evaluated at varying concentrations (10–1000 μg/mL) using TYR and L-DOPA as substrates. The results consistently demonstrated dose-dependent inhibition of TYR with increasing concentrations, leading to progressively higher levels of inhibition (Fig. 3). At low concentrations (10–50 μg/mL), the inhibition of TYR activity was minimal, showing only a slight reduction compared to the control group. This suggests that low doses of *p*-acetoxycinnamyl alcohol have negligible effects on TYR-mediated melanin biosynthesis. At concentrations above 100 μg/mL, the inhibitory effect was more pronounced, indicating a threshold concentration at which *p*-acetoxycinnamyl alcohol significantly suppressed TYR activity. At 1000 μg/mL, *p*-acetoxycinnamyl alcohol exhibited the highest level of inhibition; however, its effect was lower than that of kojic acid (50 μg/mL), which served as a positive control and showed the strongest inhibitory effect on TYR activity when both tyrosine and L-DOPA were used as substrates. Nonetheless, statistical analysis confirmed that concentrations ≥ 100 μg/mL exhibited significant inhibition compared to the

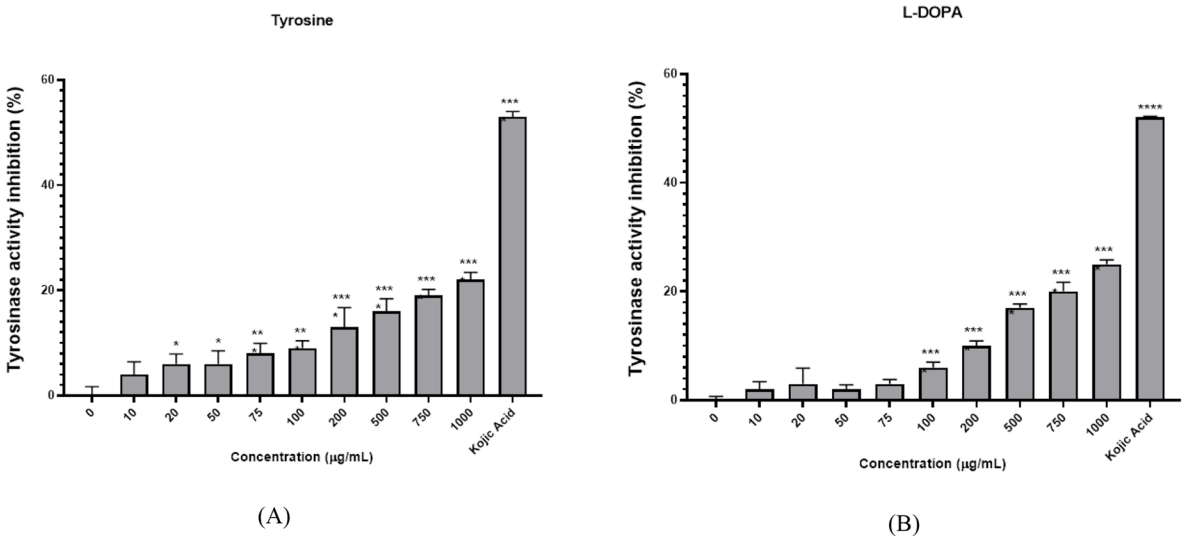

Fig. 3 Inhibitory effects of *p*-acetoxycinnamyl alcohol using tyrosine (**A**) and L-DOPA (**B**) as substrates. TYR activity inhibition was assessed at various concentrations (10–1000 μg/mL) of *p*-acetoxycinnamyl alcohol

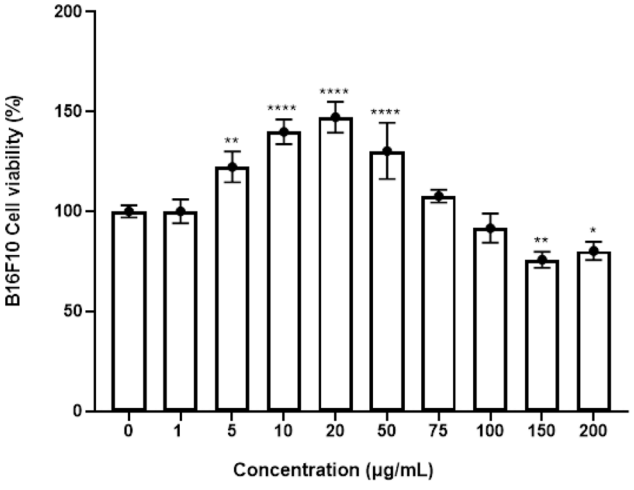

Fig. 4 Effects of *p*-acetoxycinnamyl alcohol on B16F10 cell viability. B16F10 melanoma cells were treated with the test compound at various concentrations (0, 1, 5, 10, 20, 50, 75, 100, 150, 200, and 500 μg/mL) for 24 h. Cell viability was measured using the EZ-Cytox assay, and results are expressed as a percentage of the untreated control (0 μg/mL)

control group, supporting its potential as a melanogenesis inhibitor.

Cytotoxicity and cell proliferation in B16F10 cells

These results suggested that *p*-acetoxycinnamyl alcohol may serve as a potential melanogenesis inhibitor by directly suppressing TYR activity. However, to confirm their suitability for cosmetic and dermatological applications, it is essential to assess their cytotoxic effects on melanocytes. To this end, we evaluated the cytotoxic effects of *p*-acetoxycinnamyl alcohol in B16F10 cells at various concentrations (0, 1, 5, 10, 50, 75, 100, 150, 200, and 500 μg/mL) using cell viability assays (Fig. 4). Cell viability significantly increased within a concentration range of 5–50 μg/mL, indicating a proliferative effect, with the highest stimulation observed at 10 and 20 μg/mL ($p < 0.0001$). No cytotoxicity was detected at concentrations up to 100 μg/mL. However, at concentrations of ≥ 150 μg/mL, cell viability was significantly reduced ($p < 0.05$, at 150 μg/mL, and $p < 0.01$ at 200 μg/mL), suggesting dose-dependent cytotoxicity. These findings indicate a biphasic effect of *p*-acetoxycinnamyl alcohol, where it promotes cell proliferation at low concentrations, but exerts cytotoxic effects at high doses.

These results are consistent with those of prior studies showing that specific natural compounds can boost cell viability at low concentrations by activating cell growth-related pathways without any cytotoxic effects; however, cytotoxicity occurs at high concentrations [34, 35]. The proliferative effects in B16F10 cells indicate that *p*-acetoxycinnamyl alcohol possibly modulates the cell metabolic machinery by acting on the oxidative stress response. Its cytotoxicity at high concentrations can be explained by the induction of apoptosis, oxidative stress damage, or interference with crucial signaling pathways. Further studies are required to elucidate the precise mechanisms underlying this biphasic response.

Effect of *p*-acetoxycinnamyl alcohol on melanogenesis

To assess the effect of *p*-acetoxycinnamyl alcohol on melanogenesis, B16F10 cells were treated with IBMX (200 μM) to induce melanin production. IBMX treatment significantly increased melanin secretion compared to the untreated control ($p < 0.0001$), confirming its role in stimulating melanogenesis (Fig. 5). Melanin secretion varies depending on the concentration of *p*-acetoxycinnamyl alcohol. At 10, 20, and 50 μg/mL, melanin secretion was

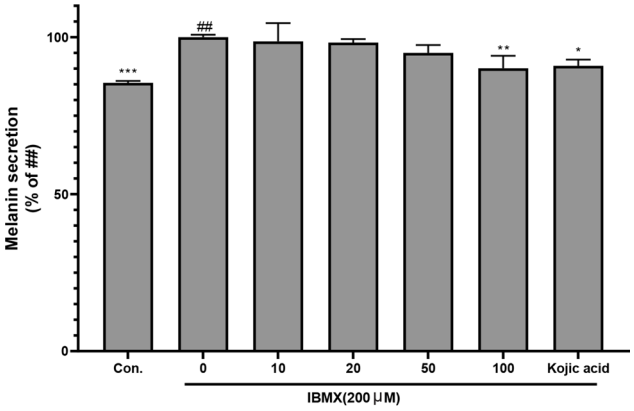

Fig. 5 Effects of *p*-acetoxycinnamyl alcohol on melanin secretion in IBMX-treated B16F10 melanoma cells. B16F10 melanoma cells were treated with IBMX (200 μM) to induce melanin production and then treated with the test compound at various concentrations (10, 20, 50, and 100 μg/mL) for 48 h. Kojic acid (100 μg/mL) was used as a positive control. Melanin secretion was quantified by measuring absorbance at 475 nm, and results are expressed as a percentage relative to the IBMX-treated group

significantly higher than in the untreated control group ($p < 0.0004$, $p < 0.0003$, and $p < 0.0003$, respectively), suggesting a stimulatory effect at these low concentrations. However, at 100 μg/mL, melanin secretion was significantly reduced compared to the IBMX-treated control ($p < 0.0001$), indicating a suppressive effect at high concentrations. The augmented melanin production at lower concentrations (10–50 μg/mL) is likely due to increased cell proliferation, as revealed by greater viability of the B16F10 cells (Fig. 4). In contrast, the decrease at 100 μg/mL likely occurred due to depression of melanogenic enzyme gene expression, such as MITF, TYR, TRP-1, and TRP-2 (Fig. 6), instead of decreased cell viability. Kojic acid (100 μg/mL), a well-established skin-lightening agent, also significantly inhibited melanin secretion ($p < 0.0001$), demonstrating a comparable effect to *p*-acetoxycinnamyl alcohol at 100 μg/mL.

These results suggest that *p*-acetoxycinnamyl alcohol exerts a biphasic effect on melanin production, promoting melanin synthesis at low concentrations and inhibiting melanin production at high doses. This dual effect may be attributed to its regulatory influence on melanogenic signaling pathways. At low concentrations, it may upregulate TYR activity and other melanogenic enzymes, whereas at high concentrations, it may suppress MITF, a key regulator of melanogenesis. Similar biphasic effects have been reported for other natural compounds, where pro- and anti-oxidative responses vary depending on the dosage.

Transcriptional regulation of melanogenic genes

To investigate the molecular mechanisms underlying the observed effects on melanogenesis, qRT-PCR was performed to quantify the expression of key melanogenic genes, including MITF, TYR, TRP-1, and TRP-2. IBMX treatment significantly upregulated MITF expression ($p < 0.0001$) (Fig. 6). Treatment with 10, 20, and 50 μg/mL *p*-acetoxycinnamyl alcohol significantly downregulated MITF ($p < 0.001$ for all conditions), with a stronger suppression observed at 100 μg/mL ($p < 0.0001$). Kojic acid (100 μg/mL) similarly reduced MITF expression ($p < 0.0001$), indicating that *p*-acetoxycinnamyl alcohol exerts melanogenesis-inhibitory effects comparable to those of kojic acid. Treatment with IBMX significantly increased TYR mRNA expression ($p < 0.01$). Treatment with 10, 20, and 50 μg/mL *p*-acetoxycinnamyl alcohol resulted in moderate reductions ($p < 0.05$, $p < 0.05$, and $p < 0.01$, respectively), whereas at 100 μg/mL, TYR expression was significantly suppressed ($p < 0.001$). Treatment with IBMX significantly increased the mRNA expression of TRP-1 and TRP-2 ($p < 0.0001$ and $p < 0.01$, respectively). Treatment with 10, 20, and 50 μg/mL *p*-acetoxycinnamyl alcohol significantly reduced TRP-1 expression ($p < 0.001$ for 10 and 50 μg/mL; $p < 0.01$ for 20 μg/mL), and at 100 μg/mL, TRP-1 expression was almost completely suppressed ($p < 0.0001$). Similarly, TRP-2 expression was significantly reduced at 10 and 20 μg/mL ($p < 0.01$), with further suppression at 50 μg/mL ($p < 0.001$) and 100 μg/mL ($p < 0.001$; the strongest inhibition), comparable to that exhibited by kojic acid.

Discussion of biphasic effects of *p*-acetoxycinnamyl alcohol on melanogenesis and its potential as a skin-whitening agent

Suppression of MITF and its downstream targets (TYR, TRP-1, and TRP-2) at 100 μg/mL suggests that *p*-acetoxycinnamyl alcohol inhibits melanogenesis through transcriptional regulation. Because MITF is a master regulator of melanogenic gene expression, its downregulation leads to decreased TYR activity and melanin biosynthesis. Similar findings have been reported for other natural compounds that interfere with the MITF signaling axis to reduce pigmentation [36, 37]. The inhibitory effect observed at 100 μg/mL supports the potential use of *p*-acetoxycinnamyl alcohol as a natural depigmenting agent.

The findings of this study demonstrate that *p*-acetoxycinnamyl alcohol exerts a biphasic effect on B16F10 melanoma cells, stimulating melanin production at low concentrations and suppressing it at high doses. The melanogenesis-inhibitory effects at high concentrations were associated with significant downregulation of MITF, TYR, TRP-1, and TRP-2, comparable to the effects of kojic acid. These findings suggest that *p*-acetoxycinnamyl alcohol is a natural inhibitor of melanogenesis, making it a promising candidate for cosmetic and dermatological applications.

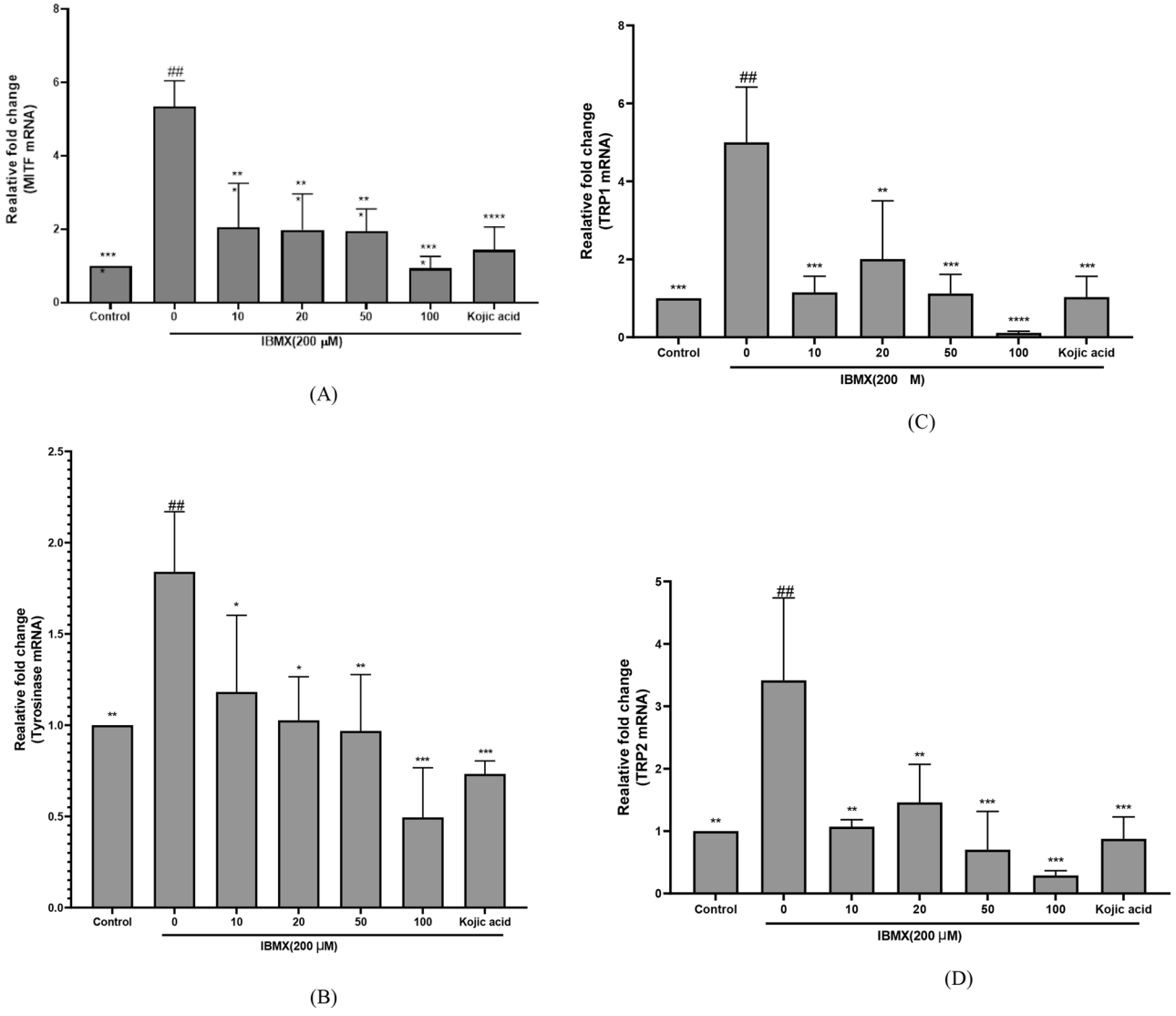

Fig. 6 Effects of the test compound on MITF (**A**), TYR (**B**), TRP-1 (**C**), and TRP-2 (**D**) mRNA expression in IBMX-treated B16F10 melanoma cells. B16F10 melanoma cells were treated with IBMX (200 μM) to stimulate melanogenesis and subsequently treated with the test compound at various concentrations (10, 20, 50, and 100 μg/mL) for 48 h. Kojic acid (100 μg/mL) was used as a positive control. MITF, TYR, TRP-1, and TRP-2 mRNA expression levels were quantified using qRT-PCR and normalized to the control group

Herein, *A. officinarum*-derived *p*-acetoxycinnamyl alcohol inhibited melanogenesis in a dose-dependent manner through both enzymatic and transcriptional regulation. It efficiently inhibited melanin production in IBMX-treated B16F10 cells, especially at 100 μg/mL, in which efficacy was similar to that of kojic acid, a well-known skin-whitening compound. The suppression of melanin production was correlated with the inhibition of the melanogenic enzymes TYR, TRP-1, and TRP-2, which are transcriptionally regulated by MITF [38]. This indicates that *p*-acetoxycinnamyl alcohol operates mainly via MITF-mediated signaling pathways [39].

In contrast to the direct action of some whitening agents on TYR enzyme activity, the activity of *p*-acetoxycinnamyl alcohol is multidimensional, entailing transcriptional inhibition of upstream regulators, revealing that it is a multitarget skin-lightening compound [40, 41]. Furthermore, the hydrogen-bonding interactions of the compound with MITF and TYR, as ascertained by molecular docking, confirmed its ability to obstruct DNA binding or enzymatic function at the molecular level.

p-Acetoxycinnamyl alcohol elicited a biphasic response, stimulating melanogenesis at low concentrations but inhibiting it at high concentrations, a response described for other plant chemicals such as glabridin and resveratrol [28]. These dual responses must be considered when establishing the desired dose for application in cosmetics [42].

Taken together, these results indicate that *p*-acetoxycinnamyl alcohol is a natural candidate for correcting

hyperpigmentation [22]. Its potency, which is similar to that of kojic acid, combined with its broad mechanism of action, suggests that it can be used as a therapeutic agent in next-generation skin-whitening treatments [43]. Further studies, including acute and chronic safety assessments, are necessary to confirm its potential clinical relevance and cosmetic applicability.

Moreover, further investigations, including mechanistic analyses, are required to elucidate the precise regulatory pathways involved in the melanogenesis-inhibitory effects of *p*-acetoxycinnamyl alcohol. Additionally, exploring its potential interactions with other skin-lightening agents may provide insights into its efficacy as a therapeutic compound for hyperpigmentation disorders.

Conclusion

The findings of this study demonstrate that *p*-acetoxycinnamyl alcohol from *A. officinarum* effectively inhibits melanogenesis in IBMX-treated B16F10 melanoma cells by downregulating MITF, a key transcription factor that regulates melanogenic enzymes. MITF suppression led to a dose-dependent decrease in TYR, TRP-1, and TRP-2 expression, ultimately reducing melanin biosynthesis. The biphasic dose-response observed in this study, stimulatory at low concentrations and inhibitory at higher doses, suggests that *p*-acetoxycinnamyl alcohol may serve as a dosage-sensitive agent for regulating melanogenesis. Additionally, cytotoxicity analysis revealed a biphasic effect, where low concentrations (5–50 μg/mL) promoted cell viability, whereas high concentrations (≥ 150 μg/mL) induced cytotoxicity. At 100 μg/mL, *p*-acetoxycinnamyl alcohol significantly inhibited melanin production, showing effects comparable to those of kojic acid, a well-known skin-lightening agent. In conclusion, *p*-acetoxycinnamyl alcohol derived from *A. officinarum* demonstrates significant potential as a natural melanogenesis inhibitor, operating via both enzymatic and transcriptional pathways. Its biphasic effects on melanocyte viability and melanin production underline the importance of dose optimization for future cosmetic and dermatological applications. Further clinical evaluations are necessary to confirm its efficacy, safety, and long-term effects on pigmentation control.

Acknowledgements
Not applicable.

Author contributions
C-DL: Column chromatography and molecular docking. HL: qRT-PCR and TYR activity. H-DL: melanin analysis and NMR assignment. SLL: data curation and cell viability. IYB: resources and funding acquisition. SHL: supervision and writing manuscript. All authors have read and agreed to the published version of the manuscript.

Funding
This work was supported by the Technology Development Program (RS-2023-00227008), funded by the Ministry of SMEs and Startups (MSS, Korea).

Data availability
All data generated or analyzed during this study are included in this published article.

Declarations

Competing interests
The authors declare no competing interests. Sanghyun Lee is an Editor of Applied Biological Chemistry. Editor status has no bearing on editorial consideration.

Received: 18 April 2025 / Accepted: 25 May 2025
Published online: 30 May 2025

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