



# Anti-melanogenic effect of Moju through inhibition of tyrosinase activity

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## Abstract

**Background** Moju is a traditional rice beverage local to Jeonju with an alcohol content of 1–2%. Moju is made by boiling makgeolli with several kinds of medicinal herbs, such as ginger, jujube and cinnamon. The raw materials used in Moju are well known for their physiological and functional effects. Although Moju is made with functional raw materials, the operational role of Moju has not yet been reported.

**Objectives** The aim of this study was to identify the anti-melanogenic effects of Moju in B16F10 melanoma cells and explore the potential mechanisms.

**Results** In this study, we investigated the antioxidant activity and anti-melanogenic effect of Moju. Moju showed no toxicity to HEK293T or B16F10 cells. The antioxidant activity of Moju was confirmed by its ability to increase radical scavenging activity. Moju decreased tyrosinase activity in a concentration-dependent manner. At the cellular level, Moju reduced melanin synthesis and the expression of proteins involved in melanin synthesis at concentrations of 100, 250, and 500 µg/mL in B16F10 cells. In addition, Moju inhibited the phosphorylation of extracellular signal-regulated kinase (ERK).

**Conclusions** These results provide evidence that Moju has antioxidant activity and anti-melanogenic effect that occur through regulation of the ERK pathway. Although further research is needed to elucidate the specific mechanism and functional components, the ability of Moju to inhibit melanin synthesis by altering tyrosinase activation suggest that it can be used as a functional whitening ingredient.

**Keywords** Moju · Anti-melanogenesis · TRP-1 · TRP-2 · MITF · ERK

## Introduction

Skin pigmentation is a major physiological defense mechanism of skin cells against various detrimental effects, such as ultraviolet (UV)-induced nuclear DNA damage (Solano 2020). Such pigmentation is due to the specialized pigment melanin.

Melanogenesis (the synthesis of melanin) occurs in melanocytes, which are specialized pigment-producing cells that produce melanin as pigment granules (Hida et al. 2020). Melanin is derived from tyrosine through a synthetic process regulated by the enzyme tyrosinase (Park et al. 2020). The

final product produces a variety of brown and black pigments that give animal skin and fur their unique color. Thus, melanin is composed of a mosaic of eumelanins (brown–black pigments) and pheomelanins (yellow–red pigments) (Kawakami and Fisher 2011). Exploration of the melanin synthesis pathway offers an opportunity to understand the regulation of melanogenesis and how it dictates the degree of pigmentation to ultimately find a cure for hypopigmentation disorders (Ali et al. 2017). Melanin synthesis is regulated by enzymes including tyrosinase, tyrosinase-related protein-1 (TRP-1) and TRP-2 (Uto et al. 2022). These enzymes are regulated by Microphthalmia-associated transcription factor (MITF), a critical factor that regulates skin pigmentation and melanocyte differentiation, proliferation, and survival critical transcriptional regulator of melanin synthesis (Pillaiyar et al. 2017). Members of the MAP kinase family, including ERK, JNK, and p38 MAPK (Sim et al. 2022), play important roles in melanogenesis (Huang et al. 2013). ERK stimulates MITF transcription via the transcription factor,

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BRN2 (Wellbrock et al. 2008), and activation of the p38 and ERK MAPK pathways activates MITF, which subsequently induces expression and activation of tyrosinase in zebrafish embryos and B16F10 cells (Shin and Lee 2013). On the other hand, phosphorylated ERK has also been shown to induce the phosphorylation and subsequent degradation of MITF phosphorylation, thereby reducing melanin synthesis (Jang et al. 2009).

Although skin pigmentation plays an essential role in physiological UV protection, abnormal pigmentation can lead to several dermatological disorders, such as age spots, melasma, freckles, skin cancer and actinic keratosis (Del Bino et al. 2018; Lee et al. 2018). Therefore, identifying natural compounds that can modulate or inhibit melanogenesis will be useful for pharmaceutical and biomedical purposes. In this study, we showed that Moju has antioxidant activity and an effective whitening effect. Several inhibitors of melanogenesis have been discovered in recent years, such as hydroquinone, kojic acid, arbutin, magnesium ascorbyl phosphate, licorice extract, aloesin, azelaic acid, soybean extract and niacinamide (Chang 2012; Mahajan et al. 2022).

Moju is made by boiling makgeolli with several kinds of traditional medicinal ingredients, such as ginger, jujube and cinnamon. The raw materials used in Moju are well known for their physiological and functional properties. Ginger has great biological and pharmacological potential in the prevention and treatment of several diseases, such as colds, nausea and arthritis (Anh et al. 2020; Mashhadi et al. 2013). Jujube has been widely used as food and herbal medicine due to its antioxidative effects, hematopoietic functions, and anticancer activities (Alsayari and Wahab 2021; Chen and Tsim 2020; Lu et al. 2021; Tahergorabi et al. 2015). It has also been reported that cinnamon has antioxidant, antidiabetic, anticancer, lipid-lowering, and cardiovascular disease-reducing effects (Hamidpour et al. 2015; Shang et al. 2021). Although Moju is made with these functional raw materials, the functional role of Moju has not yet been reported.

In this study, we identified that Moju has antioxidant activity and effective whitening function by inhibiting the melanin synthesis pathway.

## Materials and methods

### Sample preparation

Moju was purchased from Jeonju Jujo (Jeonju, Korea). Moju was composed of purified water, rice (Jeonju, Korea), sugar, jujube (Jeonju, Korea), ginger concentrate (ginger 93.5%, Korea), cinnamon, rice grains, modified nuruk, refined enzyme, and yeast in ethanol (less than 1%). The nutrients were reported to be 10.7 g of carbohydrates, 10.7 g of sugars, and 1.2 g of protein. After Moju was centrifuged at

3000 rpm for 10 min at 4 °C, the supernatant was freeze dried.

### Cell culture

Murine melanoma B16F10 and human embryonic kidney 293 T (HEK293T) cells were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured as previously described (Kim et al. 2022). In brief, the cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA, USA) with 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA) and penicillin/streptomycin (100 U/mL) (Invitrogen, Carlsbad, CA, USA) at 37 °C under a humidified atmosphere containing 5% CO<sub>2</sub>.

### Cell viability assay

Cells were treated with Moju for 24 h, and cell viability was then estimated using MTS ([3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt) (Promega, Madison, USA). Ten microliters of MTS solution was added to each well, and the cells were incubated for an additional 4 h. The optical density at 490 nm was measured by a microplate spectrophotometer (Multiskan Go, Thermo Scientific, Waltham, MA, USA). The control cells were considered to be 100% viable.

### DPPH radical scavenging activity

DPPH radical scavenging activity was measured using the Blois method with modifications. Different concentrations of sample were added to 0.2 mM DPPH solution. Thereafter, the solution was mixed and incubated for 30 min at room temperature in the dark. The absorbance was measured at 517 nm using a microplate spectrophotometer (Multiskan Sky; Thermo Fisher Scientific, Waltham, MA, USA). The DPPH radical scavenging activity was calculated using the following formula: DPPH scavenging activity (%) =  $[(Abs_{control} - Abs_{sample}) / (Abs_{control})] \times 100$ .

### ABTS radical scavenging activity

A standard Trolox equivalent antioxidant capacity (TEAC) assay was performed to further assess radical scavenging activity. Briefly, the ABTS solution was prepared by mixing 7.4 mM aqueous ABTS with potassium persulfate (2.6 mM) in the dark at room temperature for 24 h. To evaluate the antioxidant activity, this solution was diluted with ethanol to reach an absorbance of  $0.70 \pm 0.02$  at 734 nm. Different concentrations of sample were mixed with the ABTS solution, and the absorbance was measured at 734 nm using a microplate spectrophotometer (Multiskan Sky; Thermo

Fisher Scientific, Waltham, MA, USA). The ABTS scavenging activity was calculated using the following formula:  $\text{ABTS scavenging activity (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \times 100$ . Each experiment was carried out in triplicate, and the results are expressed as the mean % ABTS scavenging activity  $\pm$  SD.

### Measurement of total phenolic content (TPC) and total flavonoid content (TFC)

TPC in Moju was determined using Folin–Ciocalteu's reagent. Briefly, 100  $\mu\text{L}$  of sample was reacted with 50  $\mu\text{L}$  of Folin–Ciocalteu's reagent and 500  $\mu\text{L}$  of DW for 5 min at room temperature. After mixing with 600  $\mu\text{L}$  of 2%  $\text{Na}_2\text{CO}_3$ , the mixture was allowed to incubate for 30 min at 37  $^\circ\text{C}$ . Next, the absorbance at 765 nm was measured and recorded using a microplate spectrophotometer (Multiskan Sky; Thermo Fisher Scientific, Waltham, MA, USA). The results are expressed as milligrams of tannic acid eq. per gram (mg TAE/g). TFC was estimated using the aluminum colorimetric assay. Briefly, 100  $\mu\text{L}$  of sample was mixed with 100  $\mu\text{L}$  of 10%  $\text{AlCl}_3$ . The solution was allowed to stand for 5 min at room temperature. Next, the absorbance at 415 nm was measured using a microplate spectrophotometer (Multiskan Sky; Thermo Fisher Scientific, Waltham, MA, USA). The TFC of the sample is expressed as milligrams of quercetin eq. per gram (mg QE/g).

### Tyrosinase activity assay

Tyrosinase from mushrooms (25,000 units) was used for the tyrosinase activity bioassay in this study. The reaction mixture consisted of 80  $\mu\text{L}$  of 67 mM sodium phosphate buffer (pH 6.8), 40  $\mu\text{L}$  of sample solution, 40  $\mu\text{L}$  of mushroom tyrosinase (125 units), and 40  $\mu\text{L}$  of 10 mM 3,4-dihydroxy-L-phenylalanine (L-DOPA). Tyrosinase activity was determined by measuring the optical density at 492 nm with a microplate reader after incubation for 10 min at 37  $^\circ\text{C}$ . The inhibitory activity is expressed as percent inhibition at the end point of the reaction according to the equation.

### Measurement of melanin content

B16F10 melanoma cells were seeded in a 6-well plate at a density of  $5 \times 10^5$  cells/well and incubated for 24 h at 37  $^\circ\text{C}$ . After washing the cells 2 times with PBS, the medium was replaced with fresh DMEM (phenol red-free) containing 10% FBS and various concentrations of extracts (100–500  $\mu\text{g}/\text{mL}$ ), which was followed by treatment with forskolin (10  $\mu\text{M}$ ). The cells were incubated for 3 days, and kojic acid (500  $\mu\text{M}$ ) was administered to cells as a positive control. Following treatment, 200  $\mu\text{L}$  aliquots of the supernatants were placed in 96-well plates, and the optical

density (OD) was measured at 475 nm using a microplate spectrophotometer (Multiskan Sky; Thermo Fisher Scientific, Waltham, MA, USA). The amount of extracellular melanin was determined using the melanin (Sigma, MO, USA) standard curve.

### Western blot analysis

Whole-cell lysates of the cultured cells were obtained and separated using sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and Western blot analysis was performed as described previously (Kim et al. 2022). The primary and secondary antibodies used for the Western blot analyses were purchased from Cell Signaling Technology Inc. (Beverly, MA, USA).

### Statistical analysis

The data are expressed as the means  $\pm$  SDs, and all statistical analyses were performed with Sigmaplot v14.0 software (Systat Software Inc., San Jose, CA, USA). Statistical analysis was applied to identify differences, followed by one-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) post hoc tests. A value of  $p < 0.05$  was considered significant.

## Results

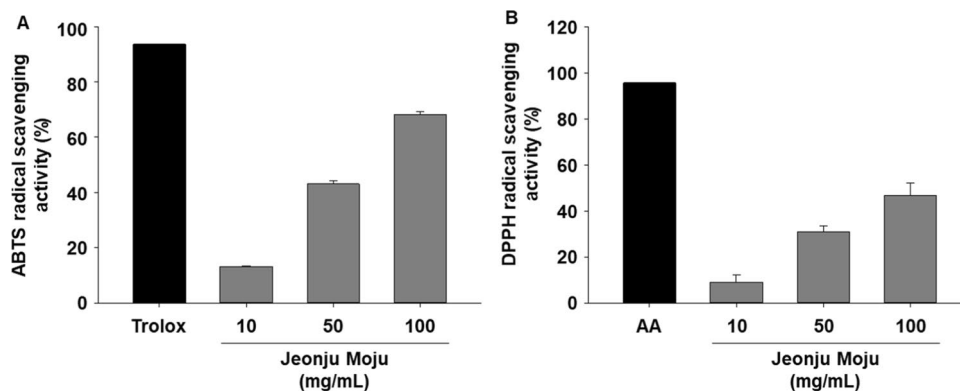
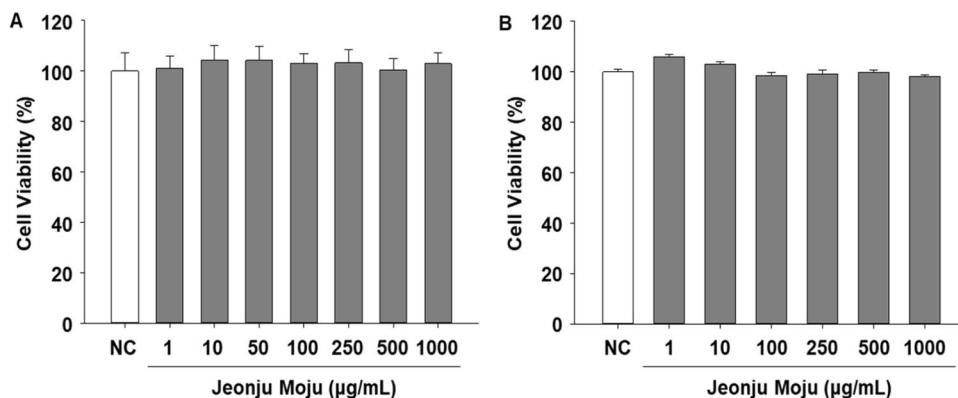
### The toxicity of Moju to human and mouse cell lines

First, we determined the toxicity of Moju to human HEK293T and mouse B16F10 cells by MTS assay. As shown in Fig. 1, there was no cytotoxicity at the administered concentration of Moju.

### The effect of Moju on antioxidant activities

We examined the effect of Moju on antioxidant activity by performing ABTS and DPPH radical scavenging assays. Antioxidant activity is defined as the ability of a given compound or mixture to reduce pro-oxidants or reactive species, including free radicals (Munteanu and Apetrei 2021). Many methods are available for antioxidant activity estimation. Among them, the ABTS and DPPH assays are widely used for the assessment of the antioxidant capacities of natural products. Both of these methods involve spectrophotometric techniques and are based on the quenching of stable colored radicals; moreover, both assays can determine the radical scavenging abilities of antioxidants even when the antioxidant is present in complex biological mixtures, such as plant or food extracts (Bibi Sadeer et al. 2020). Therefore, in this study, we used these two methods and found

**Fig. 1** Viability of HEK293T and B16F10 cells after treatment with Moju. **A** Viability of HEK293T cells treated with Moju. **B** Viability of B16F10 cells treated with Moju. Cells were treated with various doses of Moju for 24 h, and their viability was determined by MTS assay. Values are presented as the means  $\pm$  SDs of three independent experiments



**Fig. 2** Antioxidant effects of Moju. **A** ABTS and **B** DPPH radical scavenging activities were detected as described in the “Materials and methods” section. Trolox and ascorbic acid (AA) were assayed at a final concentration of 0.5 mg/mL. The results are expressed as

the percent of inhibition compared to Trolox and AA. Values are the means  $\pm$  SDs of three independent experiments. ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH: 2,2-diphenyl-1-picrylhydrazyl

**Table 1** TPC and TFC in Moju

	TPC and TFC
TPC	126.56 $\pm$ 0.88 mg TAE/100 g
TFC	7.87 $\pm$ 0.38 mg QE/100 g

that Moju increased the ABTS and DPPH radical scavenging activities (Fig. 2). In addition, we examined the effect of Moju on the concentrations of antioxidant molecules by determining the TPC and TFC. The TPC in Moju was found to be 126.56  $\pm$  0.88 mg TAE/100 g, and the TFC was 7.87  $\pm$  0.38 mg QE/100 g (Table 1).

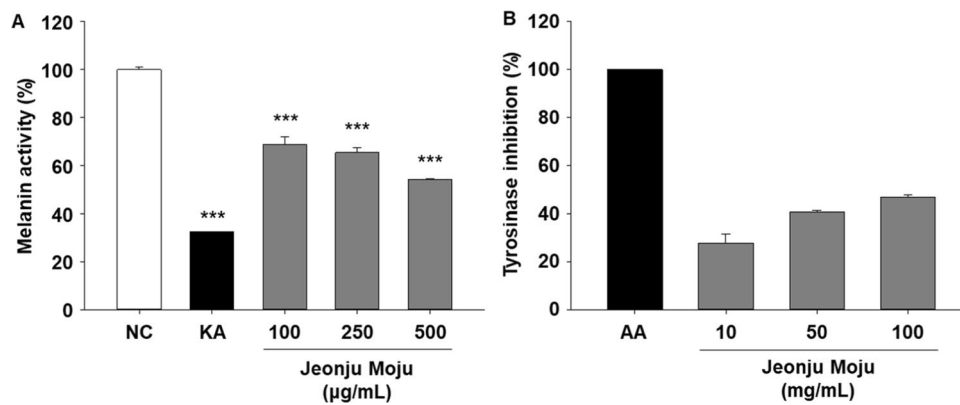
### The effect of Moju on melanin synthesis and tyrosinase activity

We examined the effect of Moju on melanin synthesis activity and found that it inhibited melanin synthesis in B16F10 cells at concentrations of 100, 250, and 500  $\mu$ g/mL (Fig. 3A). Melanin synthesis is regulated by enzymes, such

as tyrosinase, TRP-1 and TRP-2, with tyrosinase being an enzyme that acts early in the melanin biosynthesis pathway. Therefore, we determined whether Moju inhibits tyrosinase activity, and our results showed that the activity of this enzyme was inhibited by treatment with Moju in a dose-dependent manner (Fig. 3B).

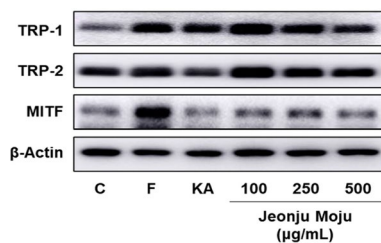
### Effect of Moju on the expression of melanin synthesis signaling pathway proteins

We examined the effect of Moju on the expression of melanin synthesis-related proteins. The expression of melanin synthesis enzymes, including TRP-1 and TRP-2, was induced by forskolin but suppressed by treatment with Moju at concentrations of 100, 250, and 500  $\mu$ g/mL. In addition, the expression of MITF, which is a transcription factor that regulates TRP-1, TRP-2 and tyrosinase levels, was dramatically decreased after treatment with Moju (Fig. 4). From these results, we suggest that Moju inhibits melanogenesis by regulating melanin synthesis signaling proteins.

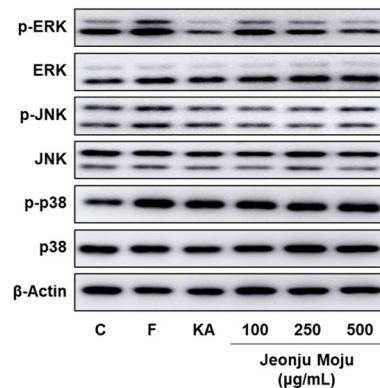


**Fig. 3** Inhibitory effect of Moju on melanin content and tyrosinase activity. **A** Melanin content in B16F10 cells. Cells were exposed to 10  $\mu$ M forskolin (F) in the presence of various doses of Moju (100, 250, and 500  $\mu$ g/mL) or 500  $\mu$ M kojic acid (KA). Relative melanin contents were measured 3 days after treatment with Moju. **B** The relative activity of mushroom tyrosinase to oxidize 3,4-dihydroxypheny-

lalanine (L-DOPA) was measured after treatment with various doses of Moju (10, 100, and 250 mg/mL). Ascorbic acid (AA) was used as a positive control. The results are expressed as percentages of tyrosinase inhibition. Values are the means  $\pm$  SDs of three independent experiments. \*\*\* $p$  < 0.001 versus the NC group



**Fig. 4** Effect of Moju on the expression of TRP-1, TRP-2 and MITF. Cells were treated with 10  $\mu$ M forskolin (F) in the presence of various doses of Moju (100, 250, and 500  $\mu$ g/mL) or 500  $\mu$ M kojic acid (KA) at 37  $^{\circ}$ C for 3 days. Then, cell lysates were prepared and used for Western blotting to determine the protein expression of TRP-1, TRP-2 and MITF. Equal amounts of total protein were resolved by SDS-PAGE.  $\beta$ -Actin was employed as an internal reference



**Fig. 5** Effect of Moju on the MAPK signaling pathway. Cells were treated with 10  $\mu$ M forskolin (F) in the presence of various doses of Moju (100, 250, and 500  $\mu$ g/mL) or 500  $\mu$ M kojic acid (KA) at 37  $^{\circ}$ C for 3 days. Then, cell lysates were prepared and used for Western blotting to determine the expression of the proteins in the MAPK signaling pathway. Equal amounts of total protein were resolved by SDS-PAGE.  $\beta$ -Actin was employed as an internal reference

### Involvement of the MAPK pathway in Moju-mediated melanogenesis inhibition

It has been reported that MAPKs are major intracellular signaling molecules that are critical for pigmentation and are known to induce melanin synthesis. To further investigate the effects of Moju on anti-melanogenic pathways, we examined the MAPK-related pathway. Our results in B16F10 cells showed that the phosphorylation of ERK was significantly decreased by Moju, whereas phosphorylation of p38 and JNK was not affected (Fig. 5). From these results, we conclude that Moju effectively works against melanogenesis by downregulating ERK phosphorylation and thereby inhibiting MITF-regulated melanogenic proteins.

### Discussion

In this study, we evaluated the antioxidant and anti-melanogenic effects of Moju. The antioxidant activity of Moju was confirmed using nonenzymatic DPPH and ABTS radicals. Natural products and their corresponding products have shown significant potential as therapeutic agents for oxidative stress-induced pathogenesis, and polyphenolic and flavonoid compounds are known to exert beneficial properties (Pandey and Rizvi 2009; Pietta 2000). Moju is made from several functional materials; however, its operational activity has not yet been reported. Therefore, in



this study, we investigated the activity of Moju, especially its anti-melanogenic effect. First, to identify the cytotoxicity of Moju in human and mouse cell lines, we used the human HEK293T and mouse B16F10 cells. We showed that Moju is not cytotoxic at concentrations up to 1 mg/mL in these two cell lines. We then investigated whether Moju has whitening activity in the B16F10 cell line. For screening of anti-melanogenic effects, B16F10 murine melanoma cells were widely used, probably because they are relatively easy to culture in vitro and share most of the melanogenic mechanisms of normal human melanocytes (Fang et al. 2022). Therefore, we studied the anti-melanogenic effect of Moju and found that Moju inhibited melanin synthesis in the B16F10 cell line. Indeed, melanin synthesis is regulated by enzymes, such as tyrosinase, TRP-1 and TRP-2. Tyrosinase acts early in the melanin biosynthesis pathway and hinders activity that interferes with melanin synthesis. We demonstrated that Moju inhibits tyrosinase activity and downregulates the expression of TRP-1 and TRP-2 in B16F10 cells. These melanin synthase enzymes (tyrosinase, TRP-1 and TRP-2) are regulated by MITF, an important transcriptional regulator of melanin synthesis. In this study, we showed that Moju suppressed the expression of MITF at concentrations of 100, 250, and 500 µg/mL, suggesting that Moju inhibits melanin synthesis by downregulating MITF, which thereby inhibits tyrosinase, TRP-1 and TRP-2.

MITF expression can be regulated by mitogen-activated protein kinases (MAPKs), including ERK, p38 and JNK (Huang et al. 2013; Shin and Lee 2013). Indeed, studies have shown that MAPKs are major intracellular signaling molecules that are critical for pigmentation and are known to induce melanin synthesis (Koike and Yamasaki 2020). After ligand-mediated MC1R activation in melanocytes, ERK phosphorylation can increase the transcriptional activity of MITF (Herraiz et al. 2011). Other reports have also shown that p38 phosphorylation increases MITF expression levels, whereas phosphorylation of JNK downregulates melanin synthesis (Saha et al. 2006; Ye et al. 2011). Our results in B16F10 cells showed that the phosphorylation of ERK was significantly decreased by Moju, whereas phosphorylation of p38 and JNK was not affected, indicating that the reduction in the anti-pigmentation effect of the Moju extract is mediated by inhibition of ERK phosphorylation. Consistent with our results, stimulation of melanogenesis by UV irradiation is associated with phosphorylation/activation of ERK but not JNK or p38 in human melanocytes (Lee et al. 2018). To better understand the mechanism of this anti-pigmentation effect, further in-depth validation studies on the role of the ERK pathway in melanogenesis are required in the future.

The raw materials of Moju are known to have pharmaceutical and nutraceutical activities. Ginger, the dried rhizome of the plant *Zingiber officinale* Roscoe (Zingiberaceae), has

been utilized as a folk remedy for thousands of years and is a commonly used spice worldwide (Angelopoulou et al. 2022). Some regulatory authorities classify ginger as a safe herbal supplement, and it has been used in both complementary and alternative medicine formulations for cold, fever, and headache management, as an appetite stimulant, and as an antiviral, antibacterial, choleric, antidiarrheal, expectorant and antiemetic compound (Anh et al. 2020; Mashhadi et al. 2013). More than 200 compounds have been identified in ginger (Mao et al. 2019), and 6-shogaol has been reported to protect human melanocytes against oxidative stress through activation of the Nrf2-antioxidant response element signaling pathway (Yang et al. 2020). Jujube has shown great potential as a food and traditional medicine in many countries (Lu et al. 2021). Several studies have demonstrated that jujube has a wide range of pharmacological activities in the cardiovascular and nervous systems as well as antioxidant, anti-inflammatory and anticancer properties (Alsayari and Wahab 2021; Chen and Tsim 2020; Lu et al. 2021). In addition, clinical studies have reported that jujube is effective and safe for treating human skin hyperpigmentation (Aafi et al. 2022). Moreover, cinnamon (Lauraceae) has been traditionally used as a food flavoring and remedy for various ailments (Hamidpour et al. 2015), and its extracts have antioxidant, anti-inflammatory, astringent, digestive, antiseptic, thermostimulant, carminative, blood purifying, antifungal, antiviral, antibacterial, and immunomodulatory properties (Hamidpour et al. 2015; Shang et al. 2021). A wide range of phytochemical compounds are found in cinnamon (Kumar et al. 2019), and interestingly, cinnamic acid from cinnamon has skin whitening effects that occur by inhibiting the activity and expression of tyrosinase within melanocytes (Kong et al. 2008).

We predicted that there are several active components of Moju, including ginger, jujube and cinnamon, but further studies are needed to identify the functional components. However, this study suggests that Moju effectively inhibits melanin synthesis through its effects on tyrosinase activity, and thus Moju can be used as a functional whitening raw material. In future studies, we will identify the functional ingredients of Moju for investigation in a clinical study to approve the individually recognized raw materials.

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**Author contributions** PMH, KHL and KSY wrote the paper and analyzed the data. KHL, LSH and NEM contributed to performing the experiments and data analysis. OBJ, KSY and PMH contributed to research planning.

**Data availability** The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Conflict of interest** Ha-Rim Kim declares no competing interests. Seung-Hyeon Lee declares no competing interests. Eun-Mi Noh declares no competing interests. Boung-Jun Oh declares no competing interests. Seon-Young Kim declares no competing interests. Mi Hee Park declares no competing interests.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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## References

- Aafi E, Shams Ardakani MR, Ahmad Nasrollahi S, Mirabzadeh Ardakani M, Samadi A, Hajimahmoodi M, Naeimifar A, Pourjabbar Z, Amiri F, Firooz A (2022) Brightening effect of *Ziziphus jujuba* (jujube) fruit extract on facial skin: a randomized, double-blind, clinical study. *Dermatol Ther* 35:e15535
- Ali SA, Naaz I, Zaidi KU, Ali AS (2017) Recent updates in melanocyte function: the use of promising bioactive compounds for the treatment of hypopigmentary disorders. *Mini Rev Med Chem* 17:785–798
- Alsayari A, Wahab S (2021) Genus *Ziziphus* for the treatment of chronic inflammatory diseases. *Saudi J Biol Sci* 28:6897–6914
- Angelopoulou E, Paudel YN, Papageorgiou SG, Piperi C (2022) Elucidating the beneficial effects of ginger (*Zingiber officinale* Roscoe) in Parkinson's disease. *ACS Pharmacol Transl Sci* 5:838–848
- Anh NH, Kim SJ, Long NP, Min JE, Yoon YC, Lee EG, Kim M, Kim TJ, Yang YY, Son EY et al (2020) Ginger on human health: a comprehensive systematic review of 109 randomized controlled trials. *Nutrients* 12:157
- Bibi Sadeer N, Montesano D, Albrizio S, Zengin G, Mahomoodally MF (2020) The versatility of antioxidant assays in food science and safety-chemistry, applications, strengths, and limitations. *Antioxidants (Basel)* 9:709
- Chang T-S (2012) Natural melanogenesis inhibitors acting through the down-regulation of tyrosinase activity. *Materials* 5:1661–1685
- Chen J, Tsim KWK (2020) A review of edible Jujube, the *Ziziphus jujuba* fruit: a health food supplement for anemia prevalence. *Front Pharmacol* 11:593655
- Del Bino S, Duval C, Bernerd F (2018) Clinical and biological characterization of skin pigmentation diversity and its consequences on UV impact. *Int J Mol Sci* 19:2668
- Fang CL, Goswami D, Kuo CH et al (2022) Angelica dahurica attenuates melanogenesis in B16F0 cells by repressing Wnt/ $\beta$ -catenin signaling. *Mol Cell Toxicol*. <https://doi.org/10.1007/s13273-022-00250-0>
- Hamidpour R, Hamidpour M, Hamidpour S, Shahlari M (2015) Cinnamon from the selection of traditional applications to its novel effects on the inhibition of angiogenesis in cancer cells and prevention of Alzheimer's disease, and a series of functions such as antioxidant, anticholesterol, antidiabetes, antibacterial, antifungal, nematocidal, acaracidal, and repellent activities. *J Tradit Complement Med* 5:66–70
- Herraiz C, Journé F, Abdel-Malek Z, Ghanem G, Jiménez-Cervantes C, García-Borrón JC (2011) Signaling from the human melanocortin 1 receptor to ERK1 and ERK2 mitogen-activated protein kinases involves transactivation of cKIT. *Mol Endocrinol* 25:138–156
- Hida T, Kamiya T, Kawakami A, Ogino J, Sohma H, Uhara H, Jimbow K (2020) Elucidation of melanogenesis cascade for identifying pathophysiology and therapeutic approach of pigmentary disorders and melanoma. *Int J Mol Sci* 21:6129
- Huang HC, Chou YC, Wu CY, Chang TM (2013) [8]-Gingerol inhibits melanogenesis in murine melanoma cells through down-regulation of the MAPK and PKA signal pathways. *Biochem Biophys Res Commun* 438:375–381
- Jang JY, Lee JH, Kang BW, Chung KT, Choi YH, Choi BT (2009) Dichloromethane fraction of *Cimicifuga heracleifolia* decreases the level of melanin synthesis by activating the ERK or Akt signaling pathway in B16F10 cells. *Exp Dermatol* 18:232–237
- Kawakami A, Fisher DE (2011) Key discoveries in melanocyte development. *J Invest Dermatol* 131:E2–4
- Kim HR, Noh EM, Lee SH, Lee SR, Kim DH, Lee NH, Kim SY, Park MH (2022) *Momordica charantia* extracts obtained by ultrasound-assisted extraction inhibit the inflammatory pathways. *Mol Cell Toxicol*. <https://doi.org/10.1007/s13273-022-00320-3>
- Koike S, Yamasaki K (2020) Melanogenesis connection with innate immunity and toll-like receptors. *Int J Mol Sci* 21:9769
- Kong YH, Jo YO, Cho CW, Son D, Park S, Rho J, Choi SY (2008) Inhibitory effects of cinnamic acid on melanin biosynthesis in skin. *Biol Pharm Bull* 31:946–948
- Kumar S, Kumari R, Mishra S (2019) Pharmacological properties and their medicinal uses of *Cinnamomum*: a review. *J Pharm Pharmacol* 71:1735–1761
- Lee A, Kim JY, Heo J, Cho DH, Kim HS, An IS, An S, Bae S (2018) The inhibition of melanogenesis via the PKA and ERK signaling pathways by *Chlamydomonas reinhardtii* extract in B16F10 melanoma cells and artificial human skin equivalents. *J Microbiol Biotechnol* 28:2121–2132
- Lu Y, Bao T, Mo J, Ni J, Chen W (2021) Research advances in bioactive components and health benefits of jujube (*Ziziphus jujuba* Mill.) fruit. *J Zhejiang Univ Sci B* 22:431–449
- Mahajan VK, Patil A, Blicharz L, Kassir M, Konnikov N, Gold MH, Goldman MP, Galadari H, Goldust M (2022) Medical therapies for melasma. *J Cosmet Dermatol* 21:3707–3728
- Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, Li HB (2019) Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe). *Foods* 8:185
- Mashhadi NS, Ghiasvand R, Askari G, Hariri M, Darvishi L, Mofid MR (2013) Anti-oxidative and anti-inflammatory effects of ginger in health and physical activity: review of current evidence. *Int J Prev Med* 4:S36–42
- Munteanu IG, Apetrei C (2021) Analytical methods used in determining antioxidant activity: a review. *Int J Mol Sci* 22:3380
- Pandey KB, Rizvi SI (2009) Plant polyphenols as dietary antioxidants in human health and disease. *Oxid Med Cell Longev* 2:270–278
- Park J, Jung H, Jang B, Song HK, Han IO, Oh ES (2020) D-Tyrosine adds an anti-melanogenic effect to cosmetic peptides. *Sci Rep* 10:262
- Pietta PG (2000) Flavonoids as antioxidants. *J Nat Prod* 63:1035–1042
- Pillaiyar T, Manickam M, Namasivayam V (2017) Skin whitening agents: medicinal chemistry perspective of tyrosinase inhibitors. *J Enzyme Inhib Med Chem* 32:403–425

- Saha B, Singh SK, Sarkar C, Bera R, Ratha J, Tobin DJ (2006) Activation of the Mitf promoter by lipid-stimulated activation of p38-stress signalling to CREB. *Pigment Cell Res* 19:595–605
- Shang C, Lin H, Fang X, Wang Y, Jiang Z, Qu Y, Xiang M, Shen Z, Xin L, Lu Y et al (2021) Beneficial effects of cinnamon and its extracts in the management of cardiovascular diseases and diabetes. *Food Funct* 12:12194–12220
- Shin SH, Lee YM (2013) Glyceollins, a novel class of soybean phytoalexins, inhibit SCF-induced melanogenesis through attenuation of SCF/c-kit downstream signaling pathways. *Exp Mol Med* 45:e17
- Sim WJ, Ahn J, Lim W, Son DJ, Lee E, Lim TG (2022) Anti-skin aging activity of eggshell membrane administration and its underlying mechanism. *Mol Cell Toxicol*. <https://doi.org/10.1007/s13273-022-00291-5>
- Solano F (2020) Photoprotection and skin pigmentation: melanin-related molecules and some other new agents obtained from natural sources. *Molecules* 25:1537
- Tahergorabi Z, Abedini MR, Mitra M, Fard MH, Beydokhti H (2015) “*Ziziphus jujuba*”: a red fruit with promising anticancer activities. *Pharmacogn Rev* 9:99–106
- Uto T, Tung NH, Shoyama Y (2022) Hirsutanone isolated from the bark of *Alnus japonica* attenuates melanogenesis via dual inhibition of tyrosinase activity and expression of melanogenic proteins. *Plants (basel)* 11:1875
- Wellbrock C, Rana S, Paterson H, Pickersgill H, Brummelkamp T, Marais R (2008) Oncogenic BRAF regulates melanoma proliferation through the lineage specific factor MITF. *PLoS ONE* 3:e2734
- Yang L, Yang F, Teng L, Katayama I (2020) 6-Shogaol protects human melanocytes against oxidative stress through activation of the Nrf2-antioxidant response element signaling pathway. *Int J Mol Sci* 21:3537
- Ye Y, Wang H, Chu JH, Chou GX, Yu ZL (2011) Activation of p38 MAPK pathway contributes to the melanogenic property of apigenin in B16 cells. *Exp Dermatol* 20:755–757

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