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Research Article

Effect of extraction solvents on antioxidant and skin-whitening potentials of defatted *Camellia* seed cakes

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Abstract
Defatted *Camellia japonica* L. seed cake is an important byproduct during the manufacture of *Camellia* seed oil. The present study evaluated the influence of two extraction solvents on the total contents of phenol and flavonoid, antioxidant activity and skin-whitening effect capable of inhibiting the biosynthesis of melanin of defatted Camellia seed cakes, a byproduct from Camellia oil production. The antioxidant capacities of 100% methanol and 70% ethanol extracts were analysed using radical scavenging (1,1-diphenyl-2-picrylhydrazyl, O_2-, H_2O_2 and NO), SOD-like, ferrous ion chelating and reducing power assays. The total phenolic and flavonoid contents were further determined by the Folin-Ciocalteu method. Moreover, intracellular antityrosinase activity and melanin contents were evaluated in human malignant melanoma cells (SK mel-100). Ethanol extracts of defatted Camellia seed cake extracts exhibited higher phenolic (4097 mg gallic acid equivalents/100 g) and flavonoid (2899 mg rutin equivalents/100 g) contents with higher superoxide (IC50 = 1.9 mg/mL), nitric oxide (IC50 =1.6 mg/mL) radical scavenging, ferrous ion chelating (IC50 = 2.9 mg/mL) and reducing power (IC50 = 1.8 mg/mL) activities than those of methanol. These ethanol extracts also evidenced more effective inhibitory activities of tyrosinase and melanin synthesis than methanol extracts. Therefore, the present results demonstrated that defatted Camellia seed cakes could be a valuable source of antioxidative and whitening ingredients, and ethanol was more efficient in extracting antioxidants and bioactive compounds than methanol.

Keywords: Antioxidant, Defatted *Camellia* seed cakes, Extraction solvents, Melanin and tyrosinase inhibition

INTRODUCTION

Camellia japonica L. is a popular tree species worldwide. Oil derived from the seeds of C. japonica L. has also been used across Korea and Southeast Asia for centuries (Zhang *et al,* 2022). Many studies have revealed that *C. japonica* ingredients include saponin polyphenols, proteins and polysaccharides in its seed oil. The oil has long been used in folk medicines, cosmetics, soaps, and hair oil for various pharmacological properties, such as antioxidation, anticancer, reducing blood cholesterol, promoting cardiovascular health, antisepsis, anti-inflammation, prevention of coronary heart disease, delayed progression of atherosclerosis, and immune function regulation (Ancut and Sonia, 2020; He *et al*., 2021). There has been a growing demand for Camellia oil in recent years due to its health-promoting effects. Camellia seed oil is rich in unsaturated fatty acids, mainly oleic acid and vitamin E, which are not only good for procollagen production and skin barrier function (Fam *et al*., 2022) but also have anti-inflammatory activity (Zhang *et al*., 2021). Moreover, the application of camellia seed oil as a food component is anticipated as a result of advances in cultivation techniques and refining technology (Shi *et al*., 2020). *Camellia* seed cake is an important byproduct of the *Camellia* seed shell during oil extraction process. The worldwide production of defatted seeds is nearly 800,000 tons per year (Chaydarreh, 2021; Liang, 2017; Feng *et al*., 2019). After the seed oil is pressed, the

residue is usually compressed into a cake shape. Although defatted seeds are rich in bioactive compounds, *Camellia* seed cake is primarily used as fuel or discarded without isolation and further exploitation, which not only results in agricultural wastes but also leads to environmental pollution and huge economic loss (Chaydarreh, 2021; Liang, 2017; Feng *et al*., 2019). Thus, it is important to explore an effective strategy to make full use of the resource to improve the economic value and health benefits of *Camellia* seed cake.

Solvent type and polarity during the extraction process are reported to affect the nature and amounts of secondary metabolites and the biological capacity of plants (Wang *et al*., 2019; Tsai and Lin, 2019; Liu *et al*., 2019; Ngo *et al*., 2017). Several studies have revealed the impact of various solvents on phytochemical composition contributing to antioxidant activity and reuse them by using water and alcohol, such as methanol, ethanol and propanol, as solvents (Wang *et al*., 2019; Tsai and Lin, 2019; Liu *et al*., 2019; Ngo *et al*., 2017). Among organic solvent systems, methanol is known as an efficient solvent to extract the best, but it can cause adverse health effects and cannot be directly used (Wang *et al*., 2019; Tsai and Lin, 2019; Liu *et al*., 2019; Ngo *et al*., 2017). At the same time, ethanol is a safer alternative solvent for human consumption (Wang *et al*., 2019; Tsai and Lin, 2019; Liu *et al*., 2019; Ngo *et al*., 2017).

The objective of this study was to investigate and compare the inhibitory activity of both methanol and ethanol extracts of defatted *Camellia* seed cakes on tyrosinase activity and melanogenesis in human SK mel-100 melanoma cells. The analysis of the total phenolic and flavonoid contents and antioxidant activity of these two extracts was also carried out.

MATERIALS AND METHODS

Plant materials and extraction

The defatted *Camellia* seed cakes used in this study were donated by Jeju Camellia Hill located in Andeok-myeon, Seogwipo-si, Jeju-do Province, South Korea, under the coordinates 33 ° 17' 30" N, 126 ° 29' 59" E. Methanol and ethanol extracts of defatted *Camellia* seed cakes were prepared using a previously described protocol (Kim, 2020). In brief, the seed cakes were ground into a fine powder. Seed cake powders (20 g) were extracted with 100 mL of 100% methanol or 70% ethanol for 72 h. The extract was filtered, concentrated under reduced pressure at 40 °C using a rotary evaporator (Buchi Rotavapor R-200, New Castle, DE, US) and lyophilized. The average extraction yields (fresh sample/methanol and ethanol) were 8.7 and 8.3%, respectively.

Total phenol and flavonoid contents

The total phenol and flavonoid contents were quantified according to the protocol described previously (Park and Kim, 2019). For total phenol quantitation, the extract (30 µL) was mixed with 30 µL of 95% ethanol, 150 µL of distilled water and 15 µL of Folin-Ciocalteu reagent. The mixture was allowed to react for 5 min, and 15 µL of 5% saturated Na_2CO_3 was added. After that, it was placed in the dark for 1 h, and the absorbance was measured at 725 nm with a Spectra MR microplate reader (Dynex Technologies, Inc., Chantilly, VA, US). A gallic acid standard curve was obtained from the 0-400 µL/mL concentration range. Total phenol values are expressed as gallic acid equivalents (mg/100 g of dry mass).

The total flavonoid content of the extracts was measured following the aluminum chloride colorimetric assay (Park and Kim, 2019). An aliquot (15 µL) of each extract was mixed with 4.5 µL of 5% sodium nitrite, 60 µL of distilled water and 4.5 µL of 10% aluminum chloride and left at room temperature for 5 minutes. Two milliliters of 1 M sodium hydroxide was added to the mixture and brought up to 150 µL with distilled water. The absorbance of the reaction mixture was measured at 510 nm. A rutin standard curve was obtained for the calculation of flavonoid content.

Scavenging of free radicals

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of the defatted *Camellia* seed cake extracts was measured according to the modified methods of Moon and Kim (2018). Briefly, 100 µL of freshly prepared 0.4 mM DPPH solution dissolved in methanol was added to an equal volume of each sample fraction. The reaction mixture was incubated for 10 min, and the absorbance was measured at 517 nm.

The superoxide anion scavenging activity of defatted *Camellia* seed cake extracts was based on the method described previously (Moon and Kim, 2018). Superoxide radicals were generated in 50 mM sodium carbonate buffer (pH 10.5), 3 mM xanthine, 3 mM ethylenediaminetetraacetic acid (EDTA), 0.5 mM nitroblue tetrazolium (NBT) and 0.15% bovine serum albumin solution, and a solution of defatted *Camellia* seed cake extracts was then added. For the reaction, xanthine oxidase (XO) (0.25 units/mL) was added to the mixture and incubated at room temperature for 25 min. The absorbance was measured at 560 nm in a microplate reader.

The ability of defatted *Camellia* seed cake extracts to scavenge hydrogen peroxide was determined according to a previously described method (Moon and Kim, 2018). A solution of hydrogen peroxide (20 µL, 10 mM) and 80 µL of extract were mixed with 100 µL of 100 mM phosphate buffer (pH 5.0). After incubation at 37 °C for 5 min, 30 µL of freshly prepared 1.25 mM ABTS and 30 µL of 1 U/mL peroxidase were added to the reaction mixture. The absorbance of hydrogen peroxide at 405

nm was determined spectrophotometrically 10 min later at 37 °C against a phosphate buffer blank without hydrogen peroxide.

Nitric oxide radical inhibition was measured by Griess reduction (Moon and Kim, 2018). Sodium nitroprusside in phosphate-buffered saline (PBS, 10 mM, pH 7.0) was added to the extracts, and the reaction mixtures (100 µL) were incubated at 25 °C for 3 h. Then, an equal volume of Greiss reagent was allowed to stand for 5 minutes. The absorbance of these solutions was measured at 540 nm against the corresponding blank.

The results for the scavenging activity of radicals, obtained from triplicate analyses, were expressed as IC_{50} values (mg/mL), which was the dose required to cause 50% inhibition. All samples were analysed in triplicate. L-ascorbic acid was used as a positive control.

Superoxide dismutase (SOD)-like

The SOD-like scavenging activity of defatted *Camellia* seed cake extracts was determined using the method described by Kim *et al.* (2020). Briefly, 200 µL of extracts of defatted *Camellia* seed cakes with different concentrations (0.125-2 mg/mL) was mixed with 20 µL of 7.2 mM pyrogallol solution and 260 µL of Tris-HCl buffer (50 mM Tris, 10 mM EDTA, pH 8.5). The mixture was incubated at room temperature for 10 min, and the reaction was terminated by adding 10 µL of 1 N HCl into the mixture. The autooxidation of pyrogallol was monitored at 420 nm using a microplate reader.

Ferrous ion chelation

As described earlier, the chelating ability was determined (Moon and Kim, 2018). Five microliters of freshly prepared $FeCl_2$ (2 mM) were mixed with 250 µL of defatted *Camellia* seed cake extracts. A 10 µL aliquot of 5 mM ferrozine was added to the mixture, and absorbance readings were taken after exactly 10 min at 25 °C.

Reducing power

The Fe^{3+} reducing power of defatted *Camellia* seed cake extracts was carried out as described previously (Moon and Kim, 2018). Different concentrations of the extract (200 µL, 0.125-2 mg/mL) were mixed with 200 µL of 200 mM phosphate buffer (pH 6.6) and $K_3Fe(CN)_6$ (200 µL, 1%). After incubation for 20 min at 50 °C, 200 µL of 10% trichloroacetic acid solution was added to the mixture and then centrifuged at 800 × g for 10 min. One hundred µL of the upper layer of solution was mixed with deionized water (100 µL) and $FeCl_3$ solution (20 µL, 0.1%), and the absorbance was measured at 700 nm.

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity of defatted *Camellia* seed cake extracts was carried out by a previously described method (Kim, 2017). Briefly, mushroom tyrosinase (40 µL, 110 unit/mL) was mixed with 100 µL of phosphate buffer (0.175 M, pH 6.8) and 40 µL of 10 mM tyrosine and 20 µL of different concentrated defatted *Camellia japonica* L. seed cake extracts (0.125, 0.25, 0.5, 1 and 2 mg/mL). The mixture was then incubated for 15 min at room temperature. Following incubation, the absorbance of the mixture was determined at 475 nm by using a Spectra MR microplate reader. The concentration of the extract that caused half-maximal inhibition of tyrosinase activity (IC_{50}) was obtained from a semilog plot of defatted *Camellia japonica* L. seed cake extract concentrations against the percentage of enzyme inhibition. Kojic acid was used as a positive control for the assay.

Cell culture and cytotoxicity measurement

Human melanoma SK mel-100 cells obtained from Dr. G. N. Wogan (Massachusetts Institute of Technology, MA, USA) were cultivated in high glucose Dulbecco's modified Eagle's medium (DMEM) with 10% heat-inactivated fetal bovine serum (FBS), L-glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 µg/mL). Cells were grown at 37 °C in a 5% CO_2 incubator. To determine the cytotoxicity of defatted *Camellia japonica* L. seed cakes to SK mel-100 cells, 3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays were performed using a cell proliferation kit I (Roche, Indianapolis, IN, USA) (Moon *et al.*, 2016). Briefly, at 24 h after seeding SK mel-100 cells in 96-well plates, the culture medium was replaced by a solution containing DMEM (10% FBS) with different concentrations of defatted *Camellia* seed cake extracts (0.05-0.4 mg/mL) and allowed to incubate for 48 h. Thereafter, 10 µL of MTT (5 mg/ml) was added to each well and incubated at 37 °C in the dark for 4 h. The tetrazolium crystals were then solubilized by adding 10% SDS (100 µL). After overnight incubation at 37 °C, the plate was read using a microplate reader at 550 nm. The relative percentage of cell survival was calculated by dividing the absorbance of treated cells by that of the control in each experiment.

Melanin content

SK mel-100 cells (1×10^3 cells/well) were seeded in 6-well plates with phenol-free DMEM supplemented with 10% FBS and incubated in the presence or absence of 0.1 µM α-melanocyte stimulating hormone (α-MSH). The cells were then incubated for 48 h with various concentrations (0.05-0.2 mg/mL) of defatted *Camellia* seed cake extracts. The cells were washed twice with PBS, dissolved in 1 N NaOH, incubated at 80 °C for 1 h and mixed to solubilize the melanin. The amount of melanin was measured using a microplate reader at 405 nm.

Statistical analysis

All data are presented as the mean ± standard deviation of triplicate values. Significant differences between the groups were determined by using SPSS software (SPSS Inc. Chicago, IL, USA) using a two-tailed Student's *t* test. A dose–response curve was plotted to determine IC_{50} values. Correlations among the data obtained were analysed using Pearson's correlation coefficient. A *p* value less than 0.05 and 0.01 was considered statistically significant.

RESULTS AND DISCUSSION

Contents of total phenol and flavonoid

Ethanol is a good solvent for polyphenol extraction and is safe for human consumption (Chemat *et al.*, 2019). Phenolics as well as flavonoids are commonly known as plant secondary metabolites having antioxidant and several pharmaceutical effects that have long been interesting due to their potential in biomedical and pharmaceutical applications (Tungmunnithum, 2018). In the present study, 70% ethanol and 100% methanol were used as extraction solvents to make two different extracted samples of defatted *Camellia japonica* L. seed cake. There was no significant difference between the total flavonoid contents obtained in the ethanol (35960 mg RE/100 g)- and methanol (35883 mg RE/100 g)-based extractions. However, the total phenolic content from ethanol extraction (49365 mg GAE/100 g) was significantly higher ($p < 0.05$) than that from methanol-based extraction (41044 mg GAE/100 g) (Fig. 1). This indicates that ethanol is a relatively more efficient extraction solvent for polyphenolic compounds from defatted Camellia japonica L. seed cake. Other studies reported that water in solvent increased polyphenol extraction efficiency because of different polarity compared to pure solvent (Dirar *et al*., 2019; Rajapaksha and Shimizu, 2022; Sánchez-Vallejo *et al*., 2022). Our findings are similar to those reported in a previous paper (Gema *et al*., 2020). Gema *et al.* (2020) reported that 50% aqueous ethanol was the best extraction solvent for the total phenolic content of Chinese seeds. Seeds of many plants release viscous polysaccharides called mucilage when mixed with water, which avoids the extraction of phenolic compounds (Galloway *et al*., 2020). Therefore, it is necessary to use other polar solvents, such as methanol and ethanol.

Antioxidant activities

It has been reported that the antioxidant activity in plants is influenced by phenolics and flavonoids (Tungmunnithum *et al*., 2018). The antioxidant capacities of the plant are monitored by a variety of assays with various factors, such as temperature, time, extraction solvent and test method; thus, it is necessary to consider several different methods and factors to further understand the molecular mechanisms of action of antioxidants (Tungmunnithum *et al*., 2018). The current study evaluated *in vitro* antioxidant activities of defatted *Camellia* seed cake extracts obtained using 100% methanol and 70% ethanol solvents in terms of DPPH radical scavenging activity, superoxide anion radical scavenging activity, hydrogen peroxide scavenging activity, nitric oxide scavenging activity, superoxide dismutase-like activity, ferrous ion chelating activity and reducing power activity.

Most reactive oxygen species (ROS) have a short half-life, and a high concentration of ROS induces oxidative damage to lipids, proteins, and DNA (Lushchak and Lushchak, 2021). Therefore, the strong and rapid ROS scavenging properties of the ethanol extract can actually prevent the oxidation of food and help fight human diseases caused by ROS (Sharma *et al*., 2021). As observed in Table 1, the ethanol extracts of defatted *Camellia* seed cake exhibited significantly higher superoxide and nitric oxide scavenging activities than methanol extracts ($p < 0.05$). The IC_{50} values (concentrations at which 50% of superoxide and nitric oxide are scavenged) were higher in the ethanol extract (1.92 and 1.61 mg/mL, respectively) than in the methanol extract (2.63 and 1.80 mg/mL, respectively). Ethanol extracts presented a higher total phenolic content (Fig. 1), allowing the superoxide and nitric oxide scavenging activities to increase. This may be attributable to the fact that ethanol treatment can eliminate compounds of different natures that interfere with the antioxidant activities of phenolic compounds. However, methanol and ethanol extracts showed similar scavenging capacities for DPPH and hydrogen peroxide radicals and SOD-like activity (Table 1).

The chelating effect of various concentrations (0.125-2 mg/mL) of defatted *Camellia* seed cake extracts on Fe^{2+} and ferrozine complex formation is shown in Table 1. The chelating abilities of both extracts increased with increasing concentration. The IC_{50} value of the chelating effect of the ethanol extract was 2.90 mg/mL, which was higher than that of the ethanol extract (6.57 mg/mL) ($p < 0.05$) (Table 1). Ethylenediaminetetraacetic acid (EDTA) used as a positive control was 99% at 0.5 mg/mL. The results suggest that the defatted *Camellia* seed cake extracts can inactivate free radicals and reduce the rate of production of radical species by chelating the metal catalysts involved in this production.

The ferric (Fe^{3+}) reduction capacity is an important characteristic property of phenolic antioxidants and serves as a significant indicator of potential antioxidant activity (Naji *et al*., 2020). To measure the reducing ability, the Fe^{3+}-Fe^{2+} transformation was investigated in the presence of defatted *Camellia* seed cake extracts.

Table 1. Antioxidant activity of defatted *Camellia japonica L.* seed cake extracts

Solvents	Radical scavenging (IC_{50}, mg/mL)				Superoxide dismutase-like (IC_{50}, mg/mL)	Ferrous ion chelating (IC_{50}, mg/mL)	Reducing power (EC_{50}, mg/mL)
	DPPH	Superoxide	Hydrogen peroxide	Nitric oxide			
100% MeOH	1.69 ± 0.006	2.63 ± 0.050	0.11 ± 0.006	1.80 ± 0.059	1.37 ± 0.033	0.66 ± 0.127	2.37 ± 0.084
70% EtOH	1.68 ± 0.065	1.92 ± 0.050*	0.11 ± 0.002	1.61 ± 0.014*	1.35 ± 0.008	0.29 ± 0.022*	1.76 ± 0.040*

MeOH=methanol; EtOH=ethanol. IC_{50} and EC_{50} indicate the effective concentration at which the antioxidant activity was 50% and at which the absorbance was 0.5, respectively, which was obtained by interpolation from linear regression analysis. Each value is expressed as the mean ± standard deviation (n = 3). *$p < 0.05$ compared to MeOH extract by Student's *t* test.

Table 2. Coefficients of correlation between total phenolics and antioxidant activities of defatted *Camellia japonica* L. seed cake extracts

	Solvents	Radical scavenging				Superoxide dismutase-like	Ferrous ion chelating	Reducing power
		DPPH	Hydrogen peroxide	Nitric oxide	Superoxide			
Total phenolics	100% MeOH	0.640	0.768	0.814*	0.902*	1.000*	0.850*	0.969*
	70% EtOH	0.908*	0.986*	0.397	0.973*	0.866*	0.999*	0.997*

All values are absolute values of correlation coefficients; *$p < 0.05$ is considered statistically significant.

The antioxidant properties expressed as EC_{50} are summarized in Table 1. Similar to the ferrous ion chelating activity, the reducing power of the extracts increased with increasing dosage. Significantly lower reducing activity was observed for the ethanol extract (1.76 mg/mL) than for the corresponding methanol extract (2.37 mg/mL) ($p < 0.05$) (Table 1). The highest absorbance for both extracts was 0.43−0.55 at 2 mg/mL, whereas ascorbic acid, used as a positive control, showed 0.64 at 0.25 mg/mL.

Correlation between antioxidant components and antioxidant activity

To further evaluate the correlation between the total polyphenol content and antioxidant activity of *Camellia* seed cake under different extraction conditions, Pearson`s coefficients were used and are shown in Table 2. Depending on the extraction solvent concentration, total phenolics were observed to be significantly positively correlated with DPPH and hydrogen peroxide in ethanol extract (r^2 = 0.908 and 0.986, respectively, $p < 0.05$), nitric oxide and superoxide in methanol extract (r^2 = 0.814 and 0.902, respectively, $p < 0.05$), and superoxide dismutase-like, Fe^{3+} *reducing power* and metal *chelating* on *ferrous ions activities* in both ethanol and methanol extracts were found (r^2 = 0.850-0.999, $p < 0.05$) (Table 2). In addition, the total phenolic content was weakly correlated with DPPH and hydrogen peroxide in the methanol extract (r^2 = 0.640 and 0.768, respectively) and nitric oxide in the ethanol extract (r^2 = 0.397) (Table 2). Thus, it was believed that phenolic compounds involved in *Camellia* seed cake extracts have a strong antiradical capacity and are related to their antioxidant activity (Table 2). In addition, the greater antioxidant property of the ethanol extract of defatted *Camellia* seed cake could be explained by its higher total phenolic and flavonoid contents compared to the methanol extract, as shown in Fig. 1.

Tyrosinase inhibitory activity

Tyrosinase inhibitor ingredients help prevent an over-

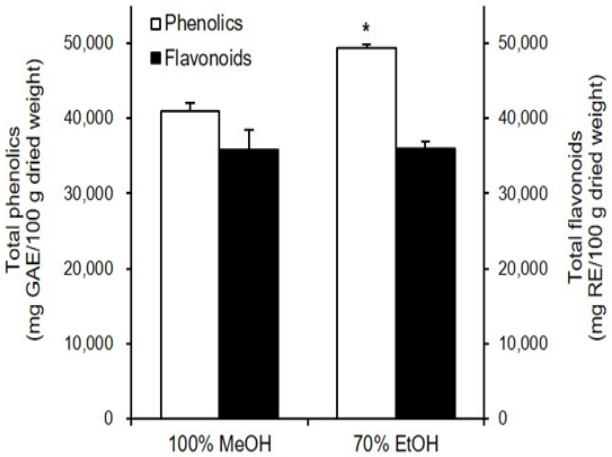

Fig. 1. *Total phenolic and flavonoid contents of defatted Camellia japonica L. seed cake extracts. Values are the mean of three replications (n=3).* *$p < 0.05$ *compared to MeOH extract by Student's t test.*

Table 3. Tyrosinase inhibitory activity (%) of defatted *Camellia japonica L.* seed cake extracts

Concentration (mg/mL)	100% MeOH	70% EtOH
0.125	2.4 ± 0.47	4.9 ± 1.65
0.25	6.7 ± 1.54	9.0 ± 0.88
0.5	15.6 ± 0.55	20.9 ± 1.75
1	24.7 ± 1.71	32.3 ± 0.34*
2	35.7 ± 0.56	48.7 ± 1.52*

All data are expressed as the mean ± SD (n=3). *$p < 0.05$ compared to MeOH extract by Student's t test.

production of melanin, which causes hyperpigmentation on the epidermis (Mukherjee *et al*., 2018). Thus, several tyrosinase inhibitors from natural and synthetic resources have been used as skin-lightening agents in the skin health, cosmetics, food and agriculture industries in recent years (Mukherjee *et al*., 2018; Shi *et al*., 2020). Nevertheless, further investigations are necessary to identify new potent tyrosinase inhibitors from natural products without adverse effects (Mukherjee *et al*., 2018; Shi *et al*., 2020). In this study, methanol and ethanol extracts of defatted *Camellia* seed cake had potent inhibitory effects on the DOPA oxidase activity of mushroom tyrosinase, and inhibitory activities increased with increases in the extract concentration (Table 3). The inhibitory effect of the ethanol extract (32.3 and 48.7%) was significantly higher than that of the methanol extract (24.7 and 35.7%) at 1 and 2 mg/mL, respectively ($p < 0.05$) (Table 3), which is consistent with the results of a previous report (Zhu *et al*., 2018). Seventy percent ethanol extracts of the seed cake of *C. oleifera* suppress melanogenesis by inhibiting the expression of tyrosinase. In the present study, kojic acid, used as a positive control, drastically reduced mushroom tyrosinase even at the lowest dose used (98.1% at 0.2 mg/mL). Furthermore, our results demonstrate that the ethanol extract of *Camellia* seed cake has a more pronounced inhibitory effect on tyrosinase activity than the methanol extract (Table 3).

Cell viability

In the present study, human melanoma SK mel-100 cells were used as an *in vitro* model. An MTT assay for cytotoxicity was employed at concentrations ranging from 0.05 to 0.4 mg/mL before further *in vitro* testing of melanin content. The viability of SK mel-100 cells is shown in Fig. 2A. It was found that up to a concentration of 0.2 mg/mL, cell viability was above 97% after treatment for 48 h, while 0.4 mg/mL resulted in a loss of viability of 34-40% ($p < 0.01$) (Fig. 2A). Thus, further experiments were conducted with extract concentrations ranging from 0.05-0.2 mg/mL.

Effects on intracellular tyrosinase activity and melanin production

The study also investigated the effects of *Camellia* seed cake extracts on the melanogenic activities of SK-mel 100 cells. As shown in Fig. 2B, all extract treatments caused significant decreases in melanin content concentration-dependent ($p < 0.01$). Moreover, inhibition of melanin synthesis is related to tyrosinase inhibition (Table 3). The inhibitory effect of ethanol extract on melanin production was much stronger than that of methanol extract, which showed melanin content (% of control) at 0.05, 0.1, and 0.2 mg/mL was 17.0 and 25.6, 15.1 and 14.3, and 10.9 and 12.7% in ethanol and methanol extracts, respectively. This indicated that the defatted *Camellia* seed cake extracts exhibited depigmenting action, making them good candidates for skin-whitening materials.

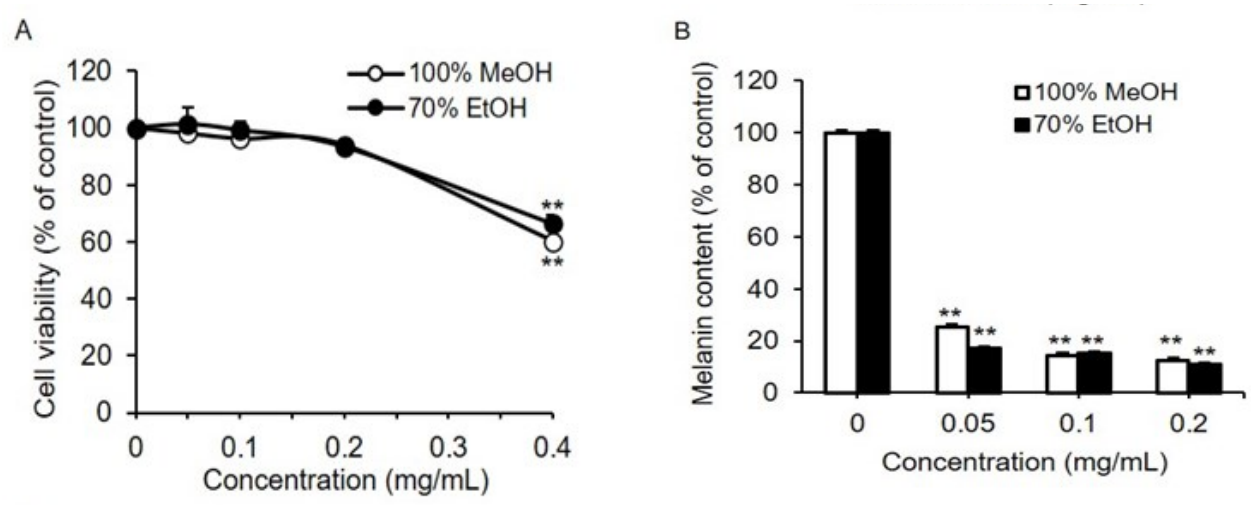

Fig. 2. *Effect of defatted Camellia japonica L. seed cake extracts on cytotoxicity (A) and melanin secretion (B) in human melanoma SK mel-100 cells. *$p < 0.05$ and **$p < 0.01$ compared to DMSO control by Student's t test.*

Conclusion

In the present research, the enhanced yield of phenolic and flavonoid compounds from defatted *Camellia* seed cakes depended on the type of extraction solvent. Seventy percent ethanol solvent was the best for the highest recovery of phenolic and flavonoid compounds in *Camellia* seed cakes. In addition, the antioxidant activity of the *Camellia* seed cake extracts was directly proportional to their phenolic and flavonoid compounds. Furthermore, at 0.2 mg/mL, the ethanol extracts reduced melanin production by 90% but had no cytotoxicity against *Camellia* seed cakes. These results suggest that *Camellia* seed cakes have great potential as bioactive compounds from natural sources for inhibiting melanogenesis and skin whitening substances.

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Conflict of interest

The authors declare that they have no conflict of interest.

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