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Inhibitory Effect of Phenolic Compounds on Vascular Endothelial Growth Factor-Induced Retinal Endothelial Permeability and Angiogenesis

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ABSTRACT: Age-related macular degeneration (AMD), often triggered by endothelial barrier disruption through vascular endothelial growth factor (VEGF), is a leading cause of blindness. This study investigated the inhibitory effects of phenolic compounds on VEGF-induced endothelial cell proliferation, migration, angiogenesis, and permeability using human retinal microvascular endothelial cells (hRECs). Thirty-seven polyphenolic compounds were selected from various databases based on their antioxidant properties, abundance in food, and solubility. These compounds significantly reduced migration, tube formation, and endothelial permeability in VEGF-stimulated hRECs. Notably, formononetin, eriodictyol, biochanin A, and p-coumaric acid were more effective in suppressing VEGF-induced angiogenesis and endothelial permeability than lutein. Molecular docking simulations revealed that formononetin, eriodictyol, and biochanin A had relatively lower binding energies with VEGF receptor 2 (VEGFR2) than lutein and sorafenib. These findings highlight the potential of phenolic compounds to be used as VEGFR2 inhibitors and an alternative strategy for preventing AMD.

Keywords: angiogenesis, macular degeneration, polyphenols, vascular permeability

INTRODUCTION

Age-related macular degeneration (AMD), which usually occurs in individuals over the age of 65, is a major condition causing blindness. According to previous studies, risk factors including smoking, family history, alcohol consumption, malnutrition, age, race, and sex can significantly increase the prevalence of AMD. Recently, the Centers for Disease Control and Prevention announced that approximately 19.8 million (12.6%) Americans aged 40 and older have been diagnosed with AMD. In particular, 1.49 million (0.94%) were living with vision-threatening diseases (Thomas et al., 2021; Division of Diabetes Translation, 2022). Therefore, considering the increasing population aged over 65, research aimed at preventing and treating AMD is urgently needed.

AMD manifests in two primary forms (i.e., dry AMD and wet AMD), which can be classified based on the presence or absence of pathologic neovascularization and vascular leakage. Compared with cataract and glaucoma, wet AMD is closely associated with progressive vascular leakage induced by vascular endothelial growth factor (VEGF) (Jiménez-Gómez et al., 2022). In pathological conditions, VEGF-A levels within the vitreous humor are significantly elevated, thereby affecting the vasculature of the choroid and retina. VEGF-A, the most important risk factor for AMD progression, binds with VEGF receptor 2 (VEGFR2) on the endothelial cell surface, initiating proliferation, differentiation, angiogenesis, and endothelial permeability (Li et al., 2016). Despite the successful application of anti-VEGF agents in patients with wet AMD, several challenges persist, including non-responsiveness, adverse effects, and the burden of repeated intravitreal injections (Ehlken et al., 2014; Yang et al., 2016; Wallsh and Gallemore, 2021).

Several preclinical and clinical studies have elucidated the considerable antioxidant effects of fruits and vegetables rich in carotenoid compounds and polyphenols within the human physiology. These bioactive compounds are recognized for their significant potential in mitigating and

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managing AMD (Mrowicka et al., 2022; He et al., 2023). Particularly, lutein and zeaxanthin, which are the main macular carotenoids and xanthophylls, play pivotal roles in AMD as radical scavengers and antioxidants (Mrowicka et al., 2022). Moreover, these carotenoids were examined in the Age-Related Eye Disease Study, which supported the efficacy of dietary supplements in preventing and treating AMD (Stahl, 2020). However, these lipophilic carotenoids and xanthophylls are associated with potential risks and concerns, including their efficacy in translocating to the retinal microvasculature via the circulatory system, excessive accumulation, and inhibitory effects on AMD (Lawrenson and Grzybowski, 2015; Black et al., 2020).

Phenolic compounds, which are characterized by single or multiple aromatic rings, are secondary metabolites that are synthesized as part of normal plant metabolism. They are the most abundant ingredients in fruits, legumes, vegetables, tea, wine, and coffee and are categorized into subgroups comprising phenolic acids, flavonoids, stilbenes, lignans, and tannins (Alara et al., 2021). Unlike carotenoids, which are largely insoluble in water, phenolic compounds are generally recognized for their diverse solubility in water and organic solvents. This benefit could be associated with a potentially enhanced delivery of phenolics through the blood-retina barrier. Moreover, various classes of phenolic compounds present in foods exhibit specific biological properties, including antioxidant activity and ability to suppress inflammation, edema, cancer, angiogenesis, osteoporosis, and cardiovascular diseases (Munin and Edwards-Lévy, 2011; Kim, 2018). However, most previous studies investigated the inhibitory effects of carotenoids, including lutein and zeaxanthin, on AMD, and systemic studies on the suppressive effect of phenolic compounds in AMD models are lacking.

In this study, we examined the inhibitory effects of phenolic compounds on VEGF-induced endothelial cell proliferation, permeability, and angiogenesis. Additionally, we compared these effects with those of sorafenib, an angiogenesis inhibitor, and lutein, a food ingredient approved by the Food and Drug Administration for treating AMD. Considering the challenges associated with sorafenib and lutein, our study suggests that phenolic compounds could offer a promising alternative strategy for mitigating VEGF-induced endothelial permeability in AMD.

MATERIALS AND METHODS

Preparation of phenolic compounds

Syringic acid, (−)-epicatechin gallate, (−)-epigallocatechin, (−)-epicatechin, vanillic acid, catechin, protocatechuic acid, sinapinic acid, resveratrol, luteolin, gallic acid, naringenin, ferulic acid, apigenin, caffeic acid, formononetin, biochanin A, galangin, p-coumaric acid, isorhamnetin, kaempferol, myricetin, daidzein, glycitein, tangeretin, hesperetin, cyanidin chloride, genistein, naringin, fisetin, quercetin, narirutin, tannic acid, eriodictyol, sorafenib, and lutein were purchased from MedChemExpress. Malvidin, peonidin, and petunidin were bought from Cayman Chemical Co.. Phenolic compounds and chemicals were diluted with dimethyl sulfoxide for *in vitro* experiments at a concentration of 20 – 40 mM.

Chemicals and materials

The following chemicals and materials were used: EBM-2 medium (Lonza) with fetal bovine serum (FBS, Lonza), Matrigel (Corning), VEGF (Peprotech), Trypsin-EDTA (Thermo Fisher Scientific), 24-well Transwell insert culture unit (0.4 μm pore size, Transwell, SPL Inc.), Sulforhodamine B (SRB, Sigma Aldrich), and fluorescein isothiocyanate (FITC)-dextran (MedChemExpress).

Cell culture

Primary human retinal microvascular endothelial cells (hRECs, Catalog# ACBRI181) were obtained from Cell Systems. hRECs were cultured in EGM-2 MV Microvascular Endothelial Cell Growth Medium-2 supplemented with growth factors and 5% FBS (Lonza Inc.). hRECs were maintained at 37°C in a moisturized condition and 5% CO_2. When adherent hRECs reached approximately 90% confluence, subculture was performed, and the passage number was limited to 12.

Cell viability assay

We conducted an SRB assay to assess the cytotoxic effects of phenolics. Briefly, hRECs were seeded at a concentration of 3×10^4 cells/well in 48-well tissue culture plates and incubated in a CO_2 incubator for 24 h. Once the cells reached full confluence, they were treated with various concentrations of phenolics, sorafenib, and lutein for 24 h. Following treatment, the cell culture medium was removed, and 250 μL of 10% trichloroacetic acid was added to fix viable cells at 4°C for 1 h. After fixation, the cells were stained with 0.4% SRB at room temperature for 1 h and then washed with 1% acetic acid five times to remove unbound SRB reagent. Thereafter, the SRB reagent bound to fixed cells was extracted using 10 mM Tris, and the absorbance was measured at 538 nm using a microplate reader (Agilent Technologies, Inc.).

Wound healing migration assay

hRECs were spread on 24-well plates at a density of 1×10^5 cells/well and cultured until they reached 100% confluence. The monolayer of cells was wounded using

the SPLScar Scratcher (Catalog# 201925, SPL Inc.) to induce uniform linear scratches. Then, the detached cells were washed away with phosphate-buffered saline, and the cells were treated with modified EGM-2 (2% FBS and no growth factors) containing specified concentrations of phenolics, sorafenib, and lutein for 2 h. Subsequently, the cells were stimulated with 50 ng/mL of VEGF, and images were captured after incubation for 12 h. The cell migration was normalized to untreated wells, which were set as 100%.

Capillary-like tube formation analysis

To prepare a thin collagen layer, 65 μL of Matrigel solution (8–10 mg/mL) was added to each well in a 96-well plate and then incubated at 37°C for 1 h to allow gel formation. Subsequently, 2×10^4 hRECs were seeded onto the Matrigel layer in the 96-well plate and cultured for 2 h in EGM containing 2% FBS without growth factors, supplemented with 2.5 μM of phenolics and lutein and 1 μM of sorafenib. After incubation, 50 ng/mL of VEGF was added, and the cells were further incubated for 10 h. Capillary-like tube formation was visualized and photographed using an inverted microscope (×40).

Transwell permeability assay

A 24-well Transwell insert culture unit was used to assess the effects of phenolics, sorafenib, and lutein on VEGF-induced endothelial permeability. Before seeding hRECs, the upper surface of the Transwell insert was coated with Matrigel (4–6 mg/mL) to create a thin collagen layer. Subsequently, the cells were seeded onto the upper surface of the inserts at a density of 1.5×10^5 cells/well and incubated in a CO_2 incubator for 48 h. Then, phenolics, sorafenib, and lutein were applied to the upper well for 2 h. Next, 50 ng/mL of VEGF and 40 μg/mL of FITC-dextran (10 kDa) were added into the upper well for 8 h. Permeabilized FITC-dextran was collected from the bottom well at 30 min, 1 h, and 8 h. The fluorescence intensity was measured at an excitation wavelength of 498 nm and emission wavelength of 517 nm using a fluorescence microplate reader (Agilent Technologies, Inc.).

Transendothelial electrical resistance (TEER) analysis

A 24-well Transwell insert culture unit was used to investigate the effects of phenolics, sorafenib, and lutein on VEGF-induced alterations in barrier integrity. hRECs were seeded onto the Transwell insert following the same procedure as the Transwell permeability assay. After pretreatment with phenolics, sorafenib, and lutein for 2 h, 50 ng/mL of VEGF was added to the upper chamber to stimulate the cells. The TEER was measured using an EVOM3 resistance meter (WPI Inc.) by placing one electrode inside the Transwell upper chamber and the other electrode in the bottom chamber. The results were expressed in units of ohms (Ω)×cm^2, and TEER changes were normalized relative to the control.

Molecular docking simulation

The three-dimensional (3D) structures of VEGFR2 [Protein Data Bank (PDB) ID: 4ASD] and various phenolic compounds were obtained from the PDB and PubChem database, respectively: lutein [compound identifier (CID): 5281243], sorafenib (CID: 216239), narirutin (CID: 442431), naringin (CID: 442428), hesperetin (CID: 72281), formononetin (CID: 5280378), resveratrol (CID: 445154), glycitein (CID: 5317750), eriodictyol (CID: 440735), naringenin (CID: 439246), peonidin (chloride) (CID: 441773), biochanin A (CID: 5280373), catechin (CID: 9064), isorhamnetin (CID: 5281654), (−)-epicatechin gallate (CID: 107905), petunidin (chloride) (CID: 441774), tangeretin (CID: 68077), malvidin (chloride) (CID: 159287), (−)-epicatechin (CID: 72276), luteolin (CID: 5280445), quercetin (CID: 5280343), fisetin (CID: 5281614), (−)-epigallocatechin (CID: 72277), myricetin (CID: 5281672), cyanidin chloride (CID: 128861), apigenin (CID: 5280443), kaempferol (CID: 5280863), daidzein (CID: 5281708), genistein (CID: 5280961), galangin (CID: 5281616), caffeic acid (CID: 689043), ferulic acid (CID: 445858), sinapinic acid (CID: 637775), p-coumaric acid (CID: 637542), syringic acid (CID: 10742), vanillic acid (CID: 8468), gallic acid (CID: 370), and protocatechuic acid (CID: 72). Simulations were performed using AutoDock Vina software (https://vina.scripps.edu/) to screen for binding energies (kcal/mol), and possible binding sites were visualized with PyMOL software (https://www.pymol.org/). Molecular dynamics were calculated using the AMBER03 force field for refinement.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 9.0.1 (GraphPad Software). Statistical significance was considered at $P<0.05$. Group comparisons were made using a *t*-test or one-way analysis of variance. If the data did not show Gaussian distribution by D'Agostino-Pearson test or variances were unequal by the Brown-Forsythe test, the data were log-transformed. Pearson's correlation analysis was used to test the statistical relationship. The results are shown as mean±standard deviation.

RESULTS

Classification and selection of phenolics

To select phenolic compounds for our study, we utilized the "Phenol-Explorer database" and the "USDA Database for the Flavonoid Content of Selected Foods ver. 3.3"

(Rothwell et al., 2013; Haytowitz et al., 2018). We defined subcategories of hydroxybenzoic acids, hydroxycinnamic acids, flavonols, flavones, flavanols, flavanones, anthocyanidins, and isoflavonoids, which are subclasses of phenolic acids and flavonoids (Fig. 1). Resveratrol and tannic acid were included as representative stilbenes and tannins, respectively. These subcategories collectively account for approximately 85% of the 505 kinds of polyphenolic compounds in the database. Considering their abundance in food, confirmed antioxidant effectiveness, and partly water-soluble chemical properties, 37 polyphenolic compounds were selected for investigation.

Cytotoxicity of phenolic compounds, sorafenib, and lutein in human retinal microvascular endothelial cells (hRECs)

We conducted the SRB assay at concentrations ranging from 5 μM to 40 μM to identify non-cytotoxic concentrations of phenolic compounds, sorafenib, and lutein in hRECs. We observed significant cytotoxicity in 13 phenolic compounds and sorafenib when treated at concentrations above 10 μM for 24 h (Fig. 2). However, treatment with 5 μM of phenolic compounds and lutein, except for sorafenib, did not yield statistically significant differences compared with the control group. Given that sorafenib exhibited cytotoxicity at concentrations above 5 μM, we determined 1 μM as the nontoxic concentration of sorafenib via SRB assay (data not shown). Consequently, we selected 5 μM as the concentration for 37 phenolics and lutein and 1 μM for sorafenib in subsequent experiments using hRECs.

Inhibitory activity of phenolic compounds, sorafenib, and lutein on vascular endothelial growth factor (VEGF)-induced endothelial cell migration

Various growth factors, including VEGFs, angiopoietins, EGFs, and FGFs, regulate angiogenesis through proliferation, sprouting, and tube formation of endothelial cells in several physiological conditions (Otrock et al., 2007). Wound healing assay was performed on hRECs to investigate whether phenolics show any inhibitory effect on VEGF-induced endothelial cell migration. Compared with the control, VEGF treatment markedly facilitated the proliferation and migration of hRECs, resulting in a decrease in the relative wound area. However, pretreatment with 13 phenolics, including vanillic acid, catechin, sinapinic acid, resveratrol, formononetin, biochanin A, galangin, p-coumaric acid, cyanidin chloride, fisetin, and eriodictyol, sorafenib, and lutein, significantly suppressed VEGF-induced proliferation and migration in hRECs. Among them, vanillic acid, sinapinic acid, formononetin, biochanin A, galangin, cyanidin chloride, and eriodictyol exhibited remarkable inhibition of cell migration compared with lutein. Sorafenib also effectively blocked the migration of hRECs (Fig. 3).

Suppressive effect of phenolic compounds, sorafenib, and lutein on VEGF-induced capillary-like tube formation

We performed an analysis of capillary-like tube formation under VEGF stimulation to further examine the antiangiogenic effect of phenolics in hRECs. The number of capillary-like tubes was significantly higher in the VEGF-treated group than in the control group. However, pretreatment with 13 phenolic compounds, including (−)-epicatechin, ferulic acid, caffeic acid, p-coumaric acid, myricetin, glycitein, peonidin, tangeretin, genistein, and others, significantly reduced the number of tube structures under VEGF stimulation. In particular, groups treated with myricetin, glycitein, peonidin, tangeretin, or genistein showed even lower VEGF-associated tube formation than those treated with lutein (Fig. 4). Treatment with sorafenib, an angiogenesis inhibitor, significantly

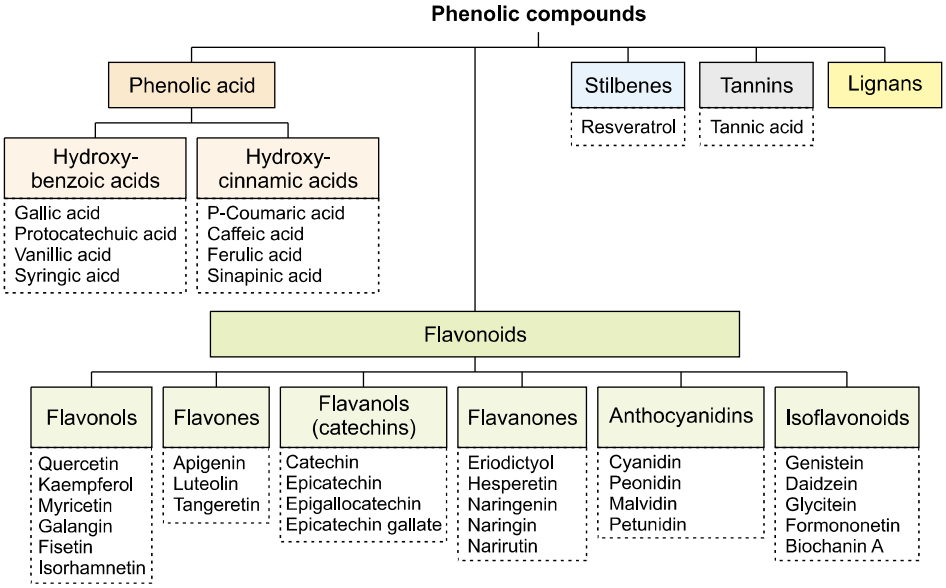

Fig. 1. Classification of phenolic compounds.

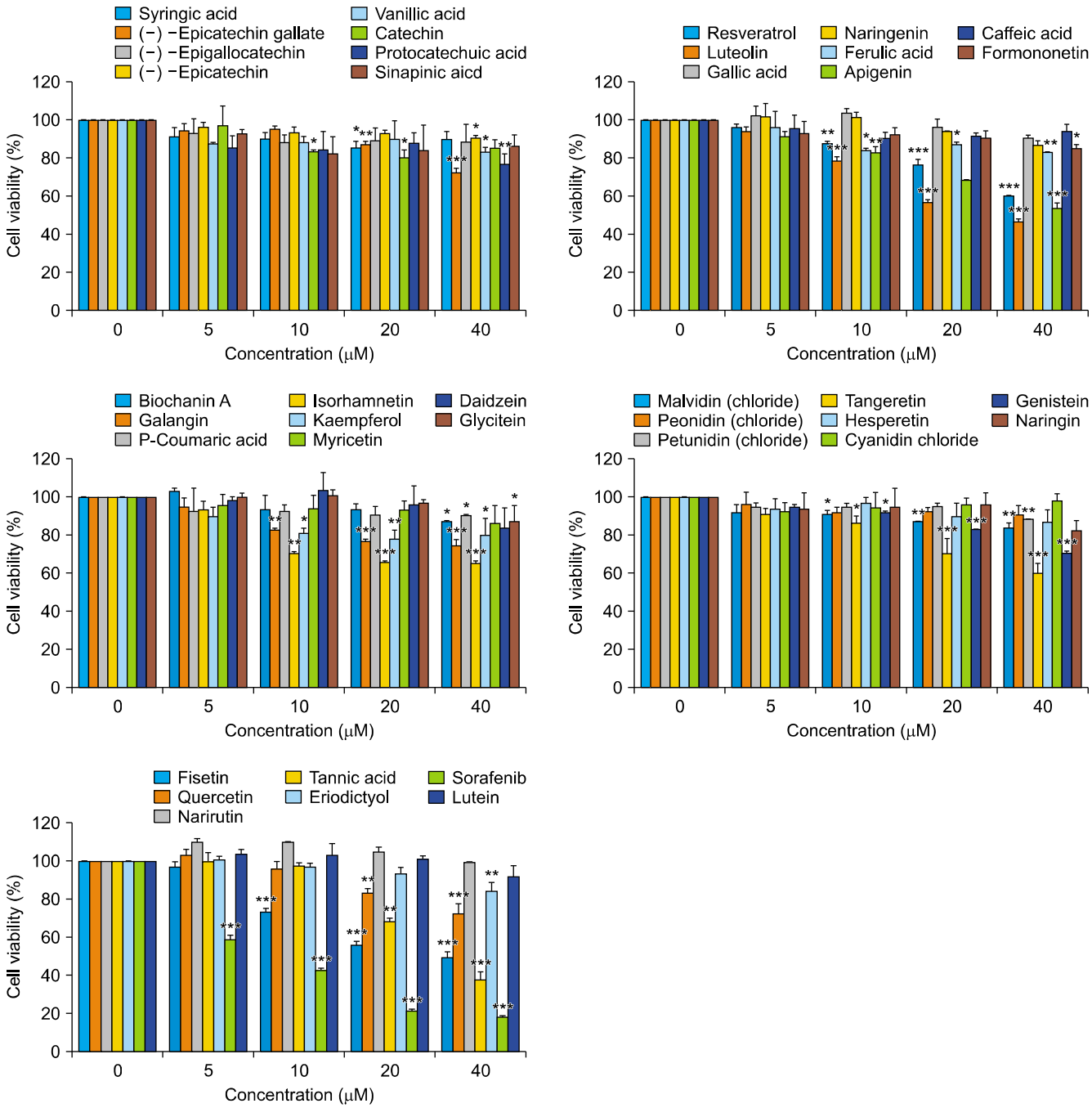

Fig. 2. Effects of phenolic compounds on the viability of human retinal microvascular endothelial cells. The cytotoxicity of phenolic compounds was confirmed using sulforhodamine B assay. The cell viability was normalized as a percentage of the control. Statistical significance was marked as ***P<0.001, **P<0.005, and *P<0.05 (n=3).

suppressed VEGF-induced tube formation of hRECs compared with the control and VEGF groups, whereas treatment with lutein did not suppress VEGF-induced tube formation.

Permeability regulation of phenolic compounds, sorafenib, and lutein in hRECs

On the endothelial cell surface, VEGF-VEGFR2 (ligand-receptor) binding promptly initiates downstream signaling pathways involved in angiogenesis, proliferation, and endothelial cell permeability. Endothelial barrier disruption morphologically indicates focal gaps and junctional separations, resulting in fluid and/or blood leakage (Claesson-Welsh et al., 2021). We conducted a Transwell permeability assay using FITC-dextran (10 kDa) to assess whether the 37 phenolics are associated with the inhibition of VEGF-induced endothelial permeabilization. Compared with the control, VEGF treatment significantly increased the permeability of FITC-dextran across the endothelial monolayer after 30 min, 1 h, and 8 h. However, pretreatment with phenolic compounds substantially suppressed VEGF-induced endothelial permeability at

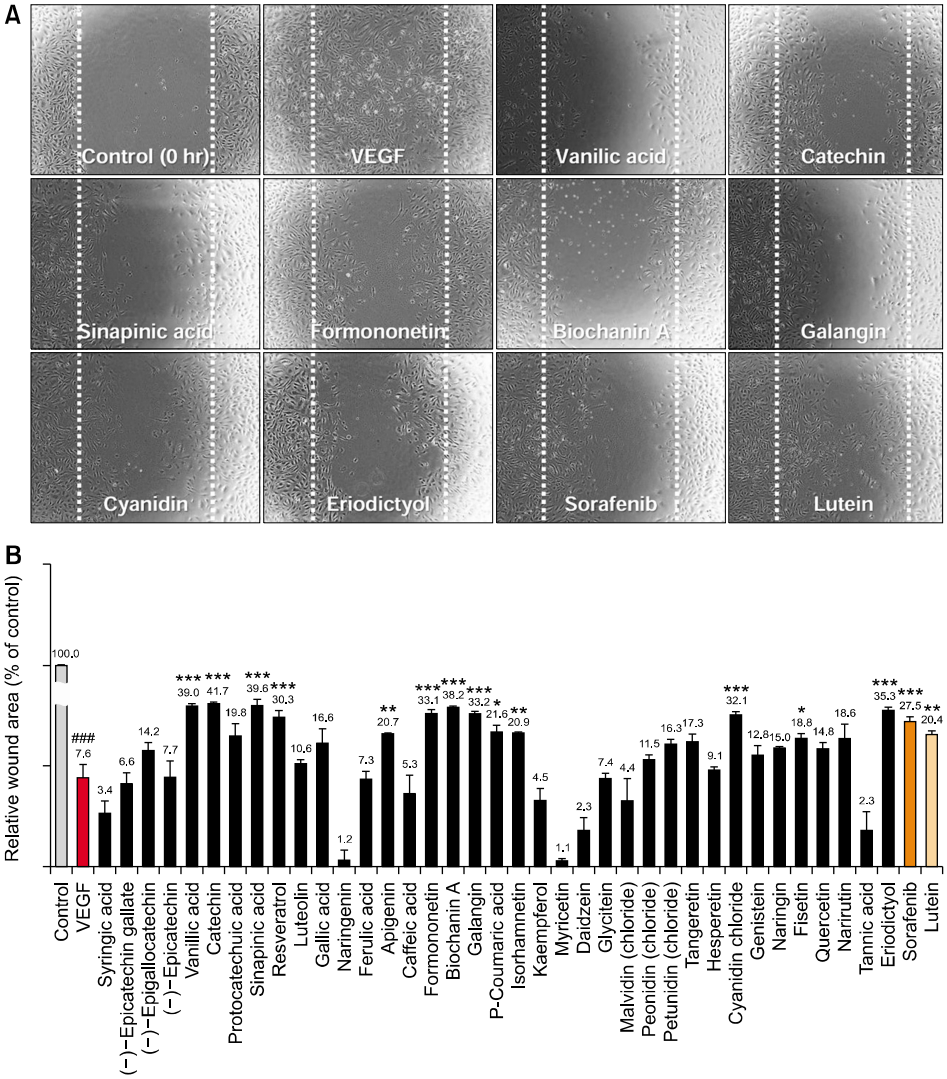

Fig. 3. Effects of phenolic compounds on the migration of human retinal microvascular endothelial cells. The inhibitory effect of phenolic compounds on migration was assessed by wound healing assay. Relative wound healing was normalized as a percentage of the control. (A) Morphological changes, (B) relative wound area. Statistical significance was compared with the control group at $^{###}P<0.001$ and with the vascular endothelial growth factor (VEGF) group at $^{***}P<0.001$, $^{**}P<0.005$, and $^{*}P<0.05$ (n=3).

30 min, 1 h, and 8 h. In particular, treatment with 30 phenolics, 33 phenolics, and all 37 phenolics exhibited statistical significance compared with the VEGF group at 30 min, 1 h, and 8 h of VEGF stimulation, respectively (Fig. 5).

Protective effect of phenolic compounds, sorafenib, and lutein on VEGF-induced endothelial barrier disruption

TEER is a quantitative marker of the tightness and integrity of the endothelial barrier, and it can be measured by placing electrodes inside and outside of a Transwell. To screen the effect of phenolics, the average of normalized TEER levels was calculated at 30 min, 1 h, and 8 h. Similar to the Transwell permeability assay, VEGF stimulation markedly disrupted the integrity of the endothelial barrier, as evidenced by a decrease in TEER values. Fig. 6 shows that some phenolic compounds, including apigenin, formononetin, biochanin A, p-coumaric acid, eriodictyol, and others, significantly contributed to maintaining endothelial barrier integrity. Although statistical significance was not observed, treatment with apigenin, formononetin, biochanin A, and p-coumaric acid exhibited higher TEER values compared to treatment with lutein, with increases of 4.0%, 4.5%, 3.2%, and 7.7%, respectively.

Molecular interactions between phenolic compounds and VEGF receptor 2 (VEGFR2)

VEGFR2 molecular docking simulations were analyzed using AutoDock Vina, and the possible binding sites were visualized using PyMOL software. Supplementary Fig. 1 shows the 3D modeling of the possible interactions between phenolic compounds and VEGFR2. The VEGFR2 binding affinities of phenolic compounds, indicating lower energy for interaction, were arranged in the following order: lutein> sorafenib> narirutin> naringin> hesperetin> formononetin> resveratrol> glycitein> eriodictyol> naringenin> peonidin> biochanin A> catechin> isorhamnetin> (−)-epicatechin gallate> petunidin> tangeretin> malvidin> (−)-epicatechin> luteolin> quercetin> fisetin> (−)-epigallocatechin> myricetin> cyanidin chloride> apigenin> kaempferol> daidzein> genis-

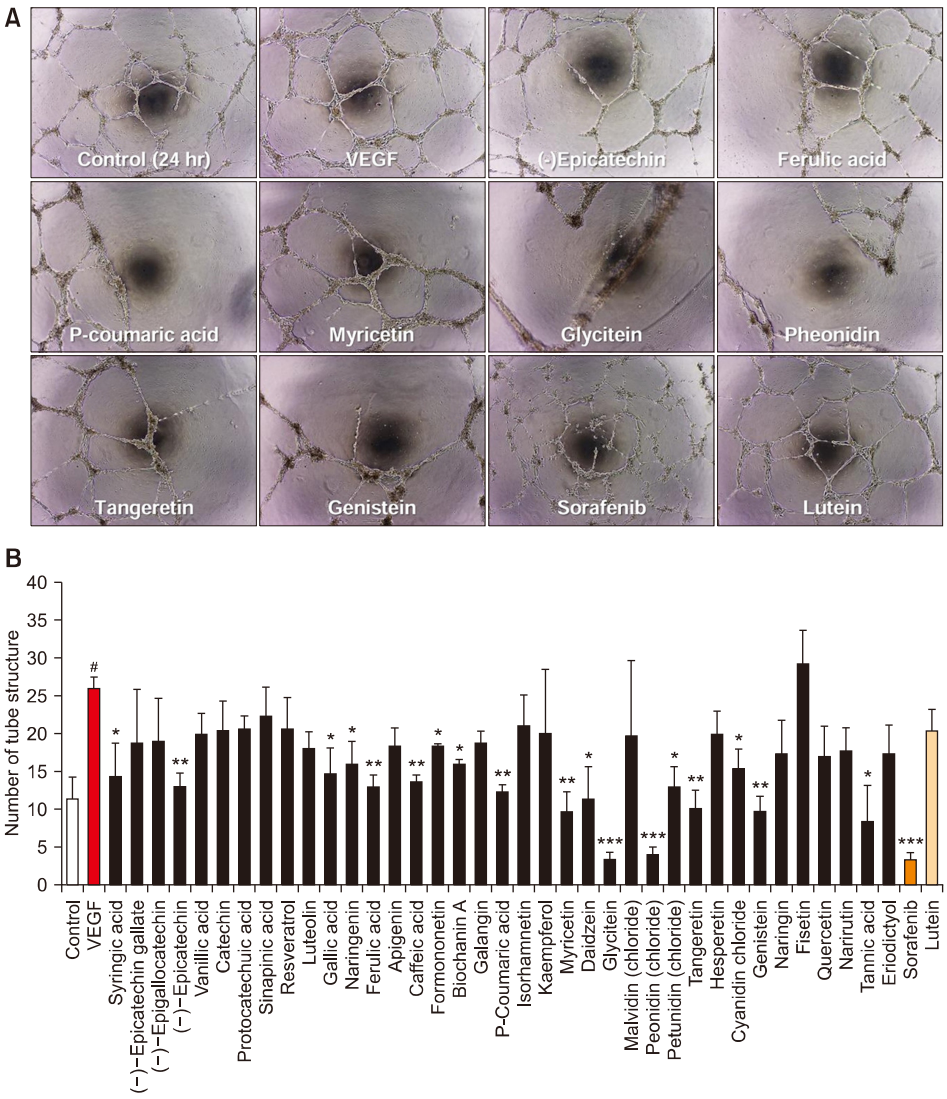

Fig. 4. Effects of phenolic compounds on the capillary-like tube formation of human retinal microvascular endothelial cells. The inhibitory effect of phenolic compounds on migration was investigated by capillary-like tube formation assay. The number of tube structures was counted manually. (A) Morphological changes, (B) number of tube structures. Statistical significance was compared with the control group at $^{\#}P<0.05$ and with the vascular endothelial growth factor (VEGF) group at $^{***}P<0.001$, $^{**}P<0.005$, and $^{*}P<0.05$ (n=3).

tein> galangin> caffeic acid> ferulic acid> sinapinic acid> p-coumaric acid> syringic acid> vanillic acid> gallic acid> protocatechuic acid (Table 1).

DISCUSSION

Several studies have uncovered the novel physiological activities of nutrients and natural compounds. In the present study, we investigated the nutritional significance of polyphenols compared with lutein and angiogenesis inhibitors by assessing their effect on VEGF-induced angiogenesis and permeability in hRECs.

In chronic vascular disorders including wet AMD, VEGF plays a significant role as a major regulator of angiogenesis and vascular leakage. Its effects are mediated through various mechanisms, including proliferation, migration, and increased vascular permeability in retinal endothelial cell (Spyridopoulos et al., 2002). In the present study, we found that lutein, an essential carotenoid known for its preventive effects against wet AMD, significantly inhibited the migration and endothelial permeability of hRECs stimulated by VEGF, without inducing cytotoxicity (Fig. 3, 5, and 6). However, treatment with lutein did not substantially affect VEGF-stimulated capillary-like tube formation (Fig. 4). These results suggest that while lutein shows potential as an inhibitor of proliferation, migration, and endothelial permeability in hRECs, it may not be as effective in blocking capillary formation. These findings are further supported by a previous study demonstrating lutein's ability to inhibit platelet-derived growth factor-induced migration of retinal pigment epithelial cells (Su et al., 2014). Keegan et al. (2020) also reported that cotreatment with lutein and zeaxanthin synergistically attenuated VEGF-induced proliferation, migration, and angiogenesis in hRECs more effectively than lutein treatment alone. Furthermore, VEGF-mediated angiogenesis and vascular permeability were not consistently regulated by parallel pathways. Specifically, the inhibition of the protein kinase C pathway was associated with the induction of VEGF-induced vascular permeability and the suppression of VEGF-mediated angio-

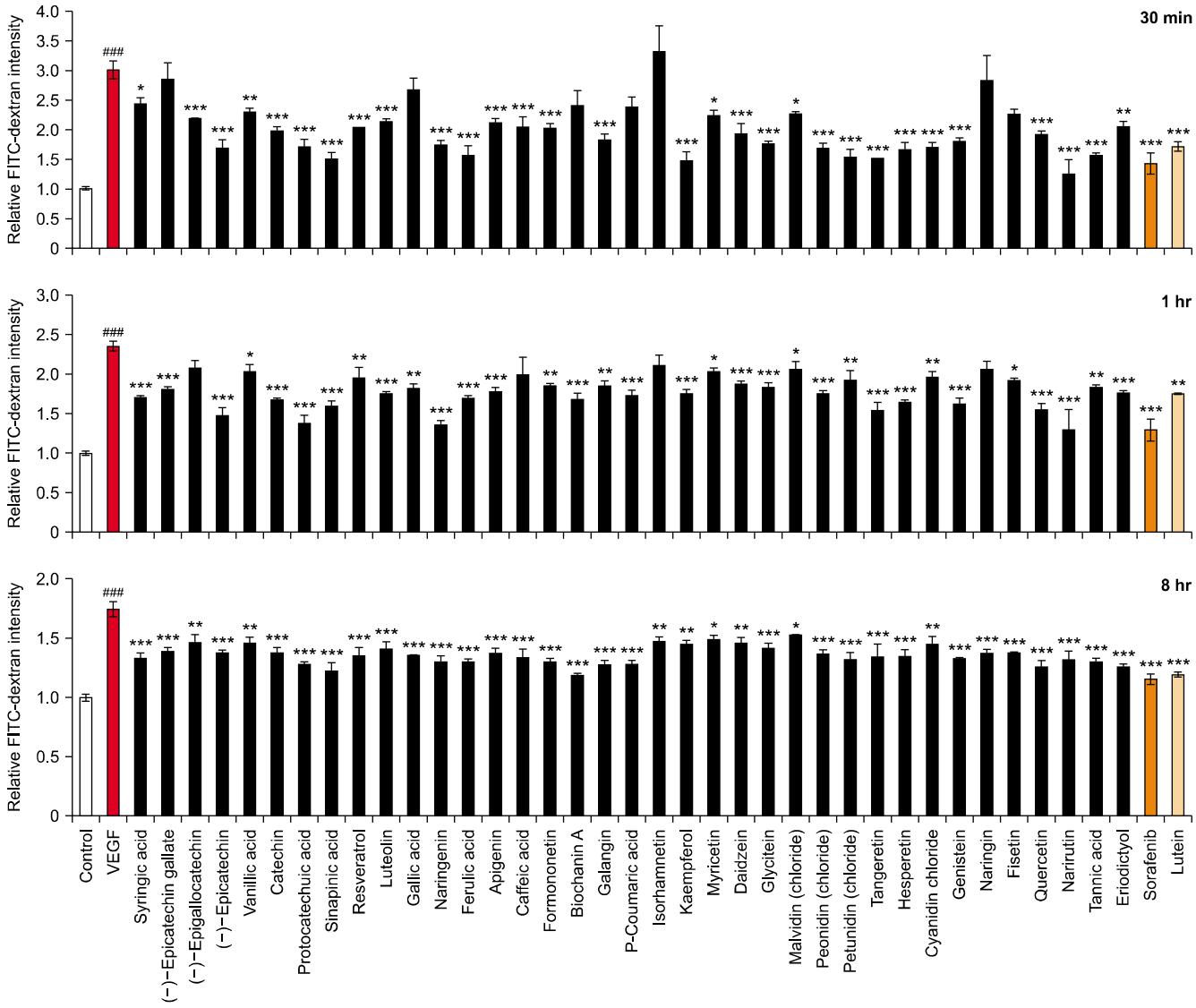

Fig. 5. Effects of phenolic compounds on the monolayer permeability in human retinal microvascular endothelial cells. The monolayer permeability demonstrates an increase of FITC-dextran level. The relative FITC-dextran intensity was normalized by control. Statistical significance was compared with the control group at $^{###}P<0.001$ and with the vascular endothelial growth factor (VEGF) group at $^{***}P<0.001$, $^{**}P<0.005$, and $^{*}P<0.05$ (n=3). FITC, fluorescein isothiocyanate.

genesis (Spyridopoulos et al., 2002). Thus, while lutein may be identified as an ingredient that protects the retinal microvasculature from VEGF-induced vascular leakage, a more comprehensive understanding is needed regarding its role in VEGF-stimulated angiogenesis.

VEGF ligand binding to VEGFR2 immediately activates downstream cascades, including the phosphorylation of VEGFR2, SRC, and VE-cadherin at cell-cell junctions within 3 min. VEGFR2 signaling persistently disrupts human retinal endothelial cell junctions, resulting in decreased endothelial barrier function from 30 min to 12 h (Cho et al., 2023). In the present study, pretreatment with phenolic compounds significantly suppressed endothelial permeability after VEGF stimulation (Fig. 5 and 6). Notably, syringic acid, (−)-epicatechin, catechin, protocatechuic acid, sinapinic acid, ferulic acid, biochanin A, p-coumaric acid, tangeretin, hesperetin, genistein, quercetin, narirutin, and eriodictyol showed significant differences ($P<0.001$) compared with the VEGF group after 1 h of VEGF cotreatment, as assessed by Transwell permeability assay. The TEER assay demonstrated that ferulic acid, apigenin, caffeic acid, formononetin, biochanin A, galangin, p-coumaric acid, and eriodictyol significantly inhibited ($P<0.01$ and $P<0.001$) VEGF-enhanced endothelial permeability. Commonly, formononetin, biochanin A, eriodictyol, and p-coumaric acid appeared to protect hRECs from VEGF-stimulated endothelial permeability and were also effective in suppressing VEGF-induced cell migration and capillary-like tube formation (Fig. 2 and 3). The anti-permeability effects of formononetin, biochanin A, p-coumaric acid, and eriodictyol on VEGF-stimulated hRECs were confirmed in the present study, whereas their antiangiogenic effects have been supported by previous studies. Formononetin

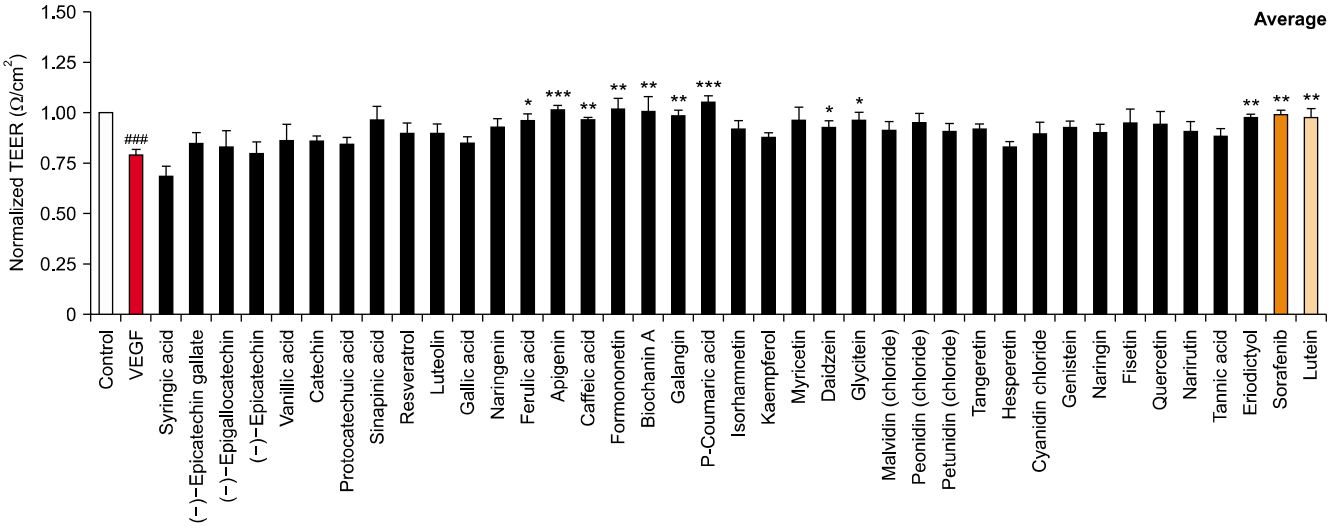

Fig. 6. Effects of phenolic compounds on transendothelial electrical resistance (TEER) in human retinal microvascular endothelial cells. The monolayer permeability demonstrates a decreased TEER (Ω/cm^2) level. The average TEER values from 15 min to 8 h were normalized by control. Statistical significance was compared with the control group at $^{###}P<0.001$ and with the vascular endothelial growth factor (VEGF) group at $^{***}P<0.001$, $^{**}P<0.005$, and $^{*}P<0.05$ (n=3).

has been reported to exert an anti-neovascularization effect in a hypoxia-induced diabetic retinopathy model through the regulation of the HIF-1/VEGF signaling pathway (Wu et al., 2016). Similarly, biochanin A administration has been shown to improve and delay the progression of diabetic retinopathy by decreasing VEGF levels in retinal tissue (Mehrabadi et al., 2018). Other studies have observed the antiangiogenic effects of p-coumaric acid and eriodictyol in endothelial cells (Kong et al., 2013; Huang et al., 2024). Interestingly, these phenolics exhibited more effective antiangiogenic activity and endothelial permeability inhibition compared with lutein. Therefore, phenolic compounds contribute to the reduction of VEGF-induced migration, angiogenesis, and endothelial permeability, with some demonstrating superior efficacy to lutein in *in vitro* tests.

Recent literature suggests the physiological significance of inhibiting receptor or enzyme functions by blocking functional domains using polyphenols (Islam et al., 2015). In the present study, the possible interaction sites and binding energies (kcal/mol) of 37 phenolic compounds, lutein, and sorafenib were investigated using molecular docking simulations (AutoDock Vina) targeting VEGFR2. As shown in Table 1, the binding energies of lutein and sorafenib to VEGFR2 were −11.28 and −11.00 kcal/mol, respectively, suggesting functional impairment of the VEGFR2 protein (Wang et al., 2019). Furthermore, formononetin, eriodictyol, and biochanin A, which exhibited excellent inhibitory effects on angiogenesis and endothelial permeability, showed low binding energies with VEGFR2 (−8.11, −7.96, and −7.81 kcal/mol, respectively). These findings are consistent with previous research indicating that targeting VEGFR2 functions is a potentially promising strategy for reducing endothelial permeability in AMD models (Xiu et al., 2024). Although lutein was identified as having the lowest VEGFR2 binding energy (−11.28 kcal/mol) by molecular docking simulation, its antiangiogenic activity was not fully controlled under VEGF stimulatory conditions. By contrast, p-coumaric acid had a high binding energy of −5.7 kcal/mol, yet considerable antiangiogenic and anti-endothelial permeability effects were observed in the present study. Taken together, these data suggest that VEGF-induced endothelial permeability and angiogenesis can be controlled by potential VEGFR2 inhibitors, including sorafenib, lutein, and certain polyphenols. However, further investigations are needed to determine whether VEGFR2 downstream signaling pathways are also affected by these phenolic compounds in hRECs.

Overall, this study revealed that phenolic compounds, including formononetin, eriodictyol, and biochanin A, could be a novel strategy to attenuate VEGF-induced angiogenesis and endothelial permeability, especially in light of the reported concerns regarding lutein and antiangiogenic drugs. Although the molecular mechanisms of these promising candidates need to be further elucidated, the data presented herein offer promising evidence and basic information for AMD prevention.

FUNDING

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Table 1. AutoDock Vina analysis of vascular endothelial growth factor receptor 2-polyphenol complexes

Phenolic compounds	Binding energy (kcal/mol)
Lutein	−11.28±0.11
Sorafenib	−11.00±0.00
Narirutin	−9.12±0.63
Naringin	−8.95±0.30
Hesperetin	−8.15±0.39
Formononetin	−8.11±0.80
Resveratrol	−8.11±0.63
Glycitein	−8.00±0.88
Eriodictyol	−7.96±0.48
Naringenin	−7.93±0.32
Peonidin (chloride)	−7.85±0.41
Biochanin A	−7.81±0.74
Catechin	−7.78±0.69
Isorhamnetin	−7.73±0.51
(−)-Epicatechin gallate	−7.55±0.65
Petunidin (chloride)	−7.53±0.35
Tangeretin	−7.50±0.00
Malvidin (chloride)	−7.40±0.34
(−)-Epicatechin	−7.35±0.38
Luteolin	−7.33±0.69
Quercetin	−7.31±0.77
Fisetin	−7.23±0.73
(−)-Epigallocatechin	−7.18±0.27
Myricetin	−7.08±0.79
Cyanidin chloride	−7.03±0.68
Apigenin	−6.80±0.60
Kaempferol	−6.76±0.79
Daidzein	−6.42±0.89
Genistein	−6.27±0.73
Galangin	−6.23±0.69
Caffeic acid	−6.01±0.48
Ferulic acid	−6.00±0.57
Sinapinic acid	−5.75±0.42
P-Coumaric acid	−5.70±0.57
Syringic acid	−5.47±0.24
Vanillic acid	−5.41±0.27
Gallic acid	−5.26±0.22
Protocatechuic acid	−5.23±0.29

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: HDC. Analysis and interpretation: DYK, SMH, JSC, SBL. Data collection: DYK, SMH, JSC. Writing the article: HDC, SMH, SBL. Critical revision of the article: JSC, SBL. Final approval of the article: all authors. Statistical analysis: HDC. Obtained funding: HDC. Overall responsibility: HDC.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3746/pnf.2024.29.3.321

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