INTRODUCTION

Flavonoids, which are present in most plants and used as useful ingredients in foods, pharmaceuticals, and functional cosmetics owing to biological properties that include antioxidant activities and low cytotoxicities, are biosynthesized in response to various environmental stimuli (Winkel-Shirle, 2002; Kootstra, 1994; Weidmann, 2012).

Derivatives of dihydroquercetin (DHQ), a type of flavonoid found in nature, appear in various forms that include free and glycosylated phenol ethers, as well as esters (Kiehlmann, Slade, 2003). Their chemical structures contain two benzene rings (A, B) and a heterocyclic ring (C), with a C6-C3-C6 carbon structure (Koroteev et al., 2015). Dihydroquercetin, (2R,3R)-2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-2,3-dihydrochromen-4-one, is a polyphenol that is naturally abundant in plants (e.g., milk thistle, onion, citrus, tamarind seeds, and Douglas fir bark) and has numerous pharmacological benefits, including anti-cancer therapeutic (Topal et al., 2016; Pew, 1948; Lee et al., 2007), anti-inflammatory (Garcia-Lafuente et al., 2009), anti-ageing (Lee et al., 2012), and antioxidant (Potapovich, Kostyuk, 2003) properties. Moreover, DHQ has been reported to inhibit tyrosinase, while simultaneously increasing tyrosinase protein levels (An et al., 2008); it has also been used in depigmentation drugs, whitening cosmetics, health-care products, and...
food additives (Vega-Villa et al., 2009; Yang et al., 2011). However, because of its instability under thermal and oxidizing conditions, and low solubility, DHQ is difficult to use in dermocosmetic and nutraceutical applications. There are reports of highly lipophilic quercetin derivatives synthesized using oleic, linoleic, and linolenic acids, and partial esterification was found to help maintain the antioxidant properties of these compounds (Mainini et al., 2013). Some natural and semisynthetic quercetin derivatives exhibit improved biological activities compared to quercetin (Dok-Go et al., 2003). Acetylation of the hydroxyl groups in quercetin has been shown to improve the cell-proliferation inhibiting properties of quercetin (Iwase et al., 2001).

Skin is vulnerable to a variety skin disorders in response to Gram positive bacteria, including skin pathogens such as Staphylococcus aureus and Propionibacterium acnes, Gram negative bacteria, such as Escherichia coli and Pseudomonas aeruginosa, and fungi, such as Candida albicans (Orchard, van Vuuren, 2017). These skin-resident microorganisms negatively affect the skin, and synthetic preservatives are often used in cosmetics to kill these microbes. However, due to possible skin-irritation and safety issues, research and development into natural antimicrobial agents and preservatives that are harmless to the human body is important (Ham et al., 1997; Jun et al., 2000; Marples, 1974). In this respect, alkaloids, steroids, flavonoids, coumarins, quinones, phenols and polyphenols, glycoproteins, carbohydrates, terpenes, and a variety of essential oils exhibit important antimicrobial properties (Leite et al., 2006).

This study examined the antimicrobial and antioxidant effects of acetyl and butyryl derivatives of DHQ synthesized using acetic anhydride and butyryl chloride and investigated their thermal stabilities and the influence of partial esterification.

**MATERIAL AND METHODS**

**Material**

DHQ (I: ≥99.5%) was purchased from Zhengzhou Feng Yao Agricultural Science and Technology Co. (China). Acetic anhydride (≥99.8%), anhydrous pyridine (≥99.5%), and butyryl chloride (≥99.0%) were purchased from Acros Organics (USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), and luminol were purchased from Sigma-Aldrich (Korea). The remaining chemicals were of reagent grade and were used without further purification.

**Characterization methods**

NMR spectra were recorded using an Avance-DPX 500 MHz NMR spectrometer (Bruker, Germany). Mass spectra were acquired using a quadrupole time-of-flight mass spectrometer (Q-TOF, Waters MS Technologies, UK). Silica gel (70–230 mesh, Merck, Germany) was used for open-column chromatography (CC). Thin-layer chromatography (TLC) was performed using silica gel 60 F254 TLC plates (Merck, Germany), and high-performance liquid chromatography was performed using an Agilent Technologies 1200 Series chromatograph (Agilent Technologies Inc., USA).

**Preparing the dihydroquercetin derivatives**

3,3',4',5,7-Pentaacetyldihydroquercetin (2) The title compound was prepared by a modified literature procedure (Mattarei et al., 2010). DHQ (1.0 g, 3.28 mmol, 1 equiv.) was heated in a mixture of acetic anhydride (6.75 g, 65.6 mmol, 20 equiv.) and pyridine (15 mL) for 5 h at 80 °C. The resulting precipitate was collected by filtration and partitioned between ethyl acetate (100 mL) and distilled water (30 mL). The combined organic layers were dried over anhydrous MgSO4 and the solvent was evaporated under vacuum to provide the title compound as a white solid (1.6 g, 95%). 1H-NMR (500 MHz, δ, dimethyl sulfoxide (DMSO)-d6) 7.53 (dd, J=8.4 Hz, J=1.85 Hz, 1H), 7.50 (d, J= 1.85 Hz, 1H), 7.35 (d, J= 8.3 Hz, 1H), 6.9 (d, J= 2.1 Hz, 1H), 6.8 (d, J= 2.1 Hz,1H), 5.95 (d, J= 12.4 Hz, 1H), 5.83 (d, 1H, J= 12.35 Hz), 2.29 (t, 12 H), 1.96 (s, 3H). 13C-NMR (125 MHz, δ, DMSO-d6) 185.3, 168.5, 168.4, 168.1, 168.0, 162.0, 156.2, 150.7, 142.6, 141.7, 133.9, 126.1, 123.8, 123.2, 111.5, 110.3, 109.2, 79.2, 72.7, 20.8, 20.6, 20.3, 20.2, 19.9. MS (ESI-MS): 515.09 [M+H] + (calcd. for C25H22O12: 514.11).
3,3',4',7-Tetraacetyldihydroquercetin (3) The title compound was prepared by a modified literature procedure (Mattarei et al., 2010). DHQ (1.0 g, 3.28 mmol, 1 equiv.) was dissolved in CH$_2$Cl$_2$ (20 mL) and pyridine (5 mL), followed by the dropwise addition of acetic anhydride (1.67 g, 16.4 mmol, 5.0 equiv.) at room temperature (RT) over 3 h while stirring. The solvent was evaporated under reduced pressure and the residue was partitioned between ethyl acetate (100 mL) and distilled water (30 mL). The combined organic layers were dried over anhydrous MgSO$_4$ and evaporated under vacuum. TLC was performed to establish cyclohexane/ethyl acetate (3:2 v/v) as the appropriate eluent. The crude product was then purified by silica gel column chromatography using this eluent to provide the title compound as a white powder (0.75 g, 48.3%).

$^1$H-NMR (500 MHz, δ, DMSO-d$_6$) 11.21 (s, 1H), 7.52 (dd, J=10.4, 2 Hz, 1H), 7.48 (d, J=1.95 Hz, 1H), 7.36 (d, J=8.3 Hz, 1H), 6.45 (d, J=2 Hz, 1H), 6.43 (d, J=2 Hz, 1H), 6.10 (d, J=12.15 Hz, 1H), 5.82 (d, J=12.15 Hz, 1H), 2.29 (m, 9H), 1.98 (s, 3H). $^{13}$C-NMR (125 MHz, δ, DMSO-d$_6$) 192.0 168.6, 168.1, 168.0, 168.0, 161.7, 161.4, 158.2, 142.6, 141.8, 133.9, 126.0, 123.8, 123.2, 105.0, 103.6, 102.0, 79.1, 72.2, 20.8, 20.3, 20.2, 19.5. MS (ESI-MS): 473.10 [M+H]$^+$ (calcd. for C$_{23}$H$_{21}$O$_{11}$: 473.10), 495.09 [M+Na]$^+$, (calcd. for C$_{23}$H$_{20}$O$_{11}$Na: 495.09).

7,3',4'-Triacetyldihydroquercetin (4) The title compound was prepared by a modified literature procedure (Kiehlmann, 1999). DHQ (1.0 g, 3.28 mmol, 1 equiv.) was stirred in 60 mL of acetic anhydride for 7 h at room temperature. The solvent was evaporated under reduced pressure and the residue was partitioned between ethyl acetate (100 mL) and distilled water (30 mL). The combined organic layers were dried over anhydrous MgSO$_4$ and evaporated under vacuum. TLC was performed to establish benzene/acetone (4:1 v/v) as the appropriate eluent. The crude product was then purified by silica gel column chromatography with this eluent to afford the title compound as a white powder (0.81 g, 54%).

$^1$H-NMR (500 MHz, δ, DMSO-d$_6$) 11.3 (s, 1H), 7.38 (dd, J=10.5, 2.1 Hz, 2H) 7.31 (d, J=2.05 Hz, 1H), 7.26 (m, 1H), 6.38 (d, J=2.05 Hz, 1H), 6.32 (d, J=2.05 Hz, 1H), 5.78 (d, J=12.1 Hz, 1H), 5.42 (d, J=12.1 Hz, 1H), 2.53 (t, 6H), 2.35 (t, 2H), 1.75 (m, H), 1.65 (m, 6H), 1.05 (m, 9H), 0.86 (t, 3H). $^{13}$C-NMR (125 MHz, δ, DMSO-d$_6$) 199.2 171.9, 171.9, 171.9, 159.2, 143.2, 142.4, 133.5, 125.4, 124.0, 123.0, 105.1, 104.3, 104.3, 102.2, 80.6, 72.4, 36.3, 36.0, 36.0, 35.6, 18.5, 18.5, 18.4, 18.4, 13.8, 13.8, 13.7, 13.5. MS (ESI-MS): 431.09 [M+H]$^+$ (calcd. for C$_{21}$H$_{19}$O$_{10}$: 431.09), 453.07 [M+Na]$^+$, (calcd. for C$_{21}$H$_{18}$O$_{10}$Na: 453.09).

7,3',4',7-Tetrabutyryldihydroquercetin (5) The title compound was prepared by a modified literature procedure (Mainini et al., 2013). DHQ (0.4 g, 1.33 mmol, 1 equiv.) was dissolved in anhydrous dioxane (15 mL). Butyryl chloride (0.58 mL, 7.34 mmol, 5.0 equiv.) and anhydrous pyridine (0.58 mL, 7.34 mmol, 5.0 equiv.) were added dropwise, and the mixture was stirred at RT for 4 h. A precipitate formed upon standing for 12 h, which was collected by filtration. The collected solid was partitioned between ethyl acetate (100 mL) and distilled water (30 mL). The combined organic layers were dried over anhydrous MgSO$_4$ and evaporated under vacuum. The crude product was purified by silica gel column chromatography with hexane/dioxane (2:1 v/v) as the eluent to give the title compound as a white powder (0.51 g, 64.5%).

$^1$H-NMR (500 MHz, δ, DMSO-d$_6$) 11.3 (s, 1H), 7.38 (dd, J=10.5, 2.1 Hz, 2H) 7.31 (d, J=2.05 Hz, 1H), 7.26 (m, 1H), 6.38 (d, J=2.05 Hz, 1H), 6.32 (d, J=2.05 Hz, 1H), 5.78 (d, J=12.1 Hz, 1H), 5.42 (d, J=12.1 Hz, 1H), 2.53 (t, 6H), 2.35 (t, 2H), 1.75 (m, H), 1.65 (m, 6H), 1.05 (m, 9H), 0.86 (t, 3H). $^{13}$C-NMR (125 MHz, δ, DMSO-d$_6$) 192.9 171.9, 171.9, 171.9, 159.2, 143.2, 142.4, 133.5, 125.4, 124.0, 123.0, 105.1, 104.3, 102.2, 80.6, 72.4, 36.3, 36.0, 36.0, 35.6, 18.5, 18.5, 18.4, 18.4, 13.8, 13.8, 13.7, 13.5. MS (ESI-MS): 607.21 [M+Na]$^+$, (calcd. for C$_{31}$H$_{36}$O$_{11}$Na: 607.21).

Chemical stability Each compound was dissolved in polyethylene glycol 400 (PEG-400, 2 wt%). The solutions were stored at 60 °C and changes in color were noted on a weekly basis.

DPPH radical-scavenging assay DPPH was used to measure the antioxidant activities of the acetyl derivatives of DHQ (Kim et al., 2012). For these experiments, sample solutions (1 mL) with different concentrations (400, 200, 100, 50, 25, and 12.5 μg/mL in DMSO) of the DHQ derivatives were prepared and added to 2.5 mL of ethanol each. Subsequently, 0.5 mL of 0.2
mM DPPH solution was added, and the resulting solution was allowed to react in the dark for 1 h. After the reaction was complete, the absorbance of the solution at 517 nm was measured using a spectrophotometer (Benchmark Plus, Bio-Rad, USA). All experiments were performed in triplicate. Ascorbic acid was used as the control, while methanol was used as the blank. The DPPH-radical scavenging activities were converted to IC₅₀ values, which represent the 50% inhibition concentrations.

The DPPH radical scavenging activity was calculated as follows:

Scavenging effect (%) = \( \frac{(A - B)}{A} \times 100 \)

where A is the absorbance at 517 nm of the blank and B is the absorbance at 517 nm of the test sample.

**Ferric reducing ability of plasma (FRAP) assay**

The FRAP-assay of Benzie and Strain (1996) was followed; this method is used to assess the antioxidant activities of the DHQ derivatives. The FRAP reagent was prepared by adding 2.5 mL of 20 mM ferric chloride (FeCl₃) and 5 mL of 10 mM TPTZ in 40 mM HCl solution and 25 mL of acetate buffer (300 mM, pH 3.6) heated to 37 °C. After adding 0.03 mL of each sample under each set of conditions, and 0.09 mL of distilled water to 0.9 mL of the prepared FRAP reagent, the mixture was allowed to react for 10 min at 37 °C. The absorbance at 593 nm was then measured using the above-mentioned spectrophotometer. Distilled water used instead in the blank instead of the test sample, while ascorbic acid was used as the control. After constructing a calibration curve for FeSO₄ based on repeated measurements at concentrations of 10, 25, and 50 μg/mL, the measured absorbance of each test sample was converted into concentration. All experiments were performed in triplicate.

**Antimicrobial activity**

Antimicrobial activity was assessed using both disc and minimum inhibitory concentration (MIC) methods. *C. albicans* (ATCC10231), *E. coli* (ATCC8739), *P. acnes* (ATCC6919), *P. aeruginosa* (ATCC9027), and *S. aureus* (ATCC6538), fungal and bacterial strains were used to assess antimicrobial activities; they were procured from the Korean Culture Center of Microorganisms (KCCM, Seoul, Korea). *P. acnes* was incubated in reinforced clostridial medium and reinforced clostridial agar (Becton, Dickinson and Company, USA) for 48–72 h at 37 °C in the presence of CO₂. *E. coli* and *P. aeruginosa* were incubated in a 37 °C incubator using Mueller Hinton broth and Mueller Hinton agar (Becton, Dickinson and Company, USA), while *B. subtilis* and *S. aureus* were incubated for 24 h in a 37 °C and 30 °C incubators, respectively, using tryptic soy broth (TSB, Becton, Dickinson and Company, USA) and tryptic soy agar (TSA, Sigma-Aldrich Co., USA).
Antioxidant and antimicrobial properties of dihydroquercetin esters

Disc method (Lehrer et al., 1991) After incubating up to the mid-logarithmic phase (OD at 570 nm = 0.1, $5 \times 10^7$ colony forming units (CFU/mL) in TSB, 100 μL of the cell broth was applied to sterilized TSA medium. A paper disc (8 mm diameter) was subsequently placed in the plate and 50 μL of each sample was absorbed onto the disc to achieve a concentration of 2 or 4 mg/disc. After incubation for 24 h in a 37 °C incubator, activity was determined from the size of the clear zone formed around each disc.

MIC The MICs of each test sample was determined by adding 0.95 mL of cell suspension ($1 \times 10^4$ CFU/mL broth) to a 48-well plate, to which 0.05 mL of each the test sample was added. The test samples were two-fold diluted with DMSO. The test samples were dissolved in DMSO to final concentrations of 10,000, 5,000, 2,500, 1,250, 625, 312, 156, 78, and 39 μg/mL for each strain. 1,2-Hexanediol and methylparaben, which are often used as preservatives in cosmetics, were used as controls. After 24 h of incubation (48–72 h for P. acnes), the wells were visually inspected and the minimum concentration at which cell proliferation was not observed was taken to be the MIC. In addition, experiments were also conducted using a blank (treated only with broth and DMSO as the solvent) and a growth control (treated with cell solution and DMSO) (Rodriguez-Tudela et al., 2003).

Statistical analysis

All results presented the averages of at least three independent experiments. Data expressed as means ± SDs and analyzed by one-way analysis of variance (ANOVA), followed by Tukey’s test; p<0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Synthesis of the dihydroquercetin derivatives

The derivatives of DHQ, which contains five hydroxyl groups, were synthesized according to previous literature methods (Figure 1) (Mainini et al., 2013; Mattarei et al., 2010; Kiehlmann, 1999). The acetyl derivatives of DHQ were prepared by carefully adjusting the equivalences/volumes of acetic anhydride, the reaction temperature, and reaction time. The yield of the desired products depended on the purification process used.
The molecular weights of the acetyl derivatives of DHQ were determined by Q-TOF mass spectrometry, which confirmed the formation of the penta-, tetra-, and tri-acetylated DHQ derivatives by their molecular ions. The structures of the compounds were identified by $^1$H and $^{13}$C NMR spectroscopy. The single free hydroxyl group of 3,3',4',7-tetraacetyldihydroquercetin (3) was identified by the shape and chemical shift (11.21 ppm, DMSO-$d_6$) of the peak corresponding to the hydroxyl proton (Rietjens et al., 2001), as well as the differences in the chemical shifts of the ring protons of 3,3',4',5,7-pentaacetyldihydroquercetin (2) and 3,3',4',7-tetraacetyldihydroquercetin (3) (Mattarei et al., 2010). The $^1$H NMR chemical shifts of the ring protons in 3 were observed at 6.45 ppm (H-6) and 6.43 ppm (H-8), which are downfield shifted compared to those in the spectrum of 2, in which H-6 and H-8 resonate at 6.94 and 6.81 ppm, respectively. Meanwhile, the chemical shifts of H-2', H-5', and H-6' in the B ring of 2 are very similar to those of 3, despite

**FIGURE 1** - Preparation of the dihydroquercetin derivatives. Reagents and conditions: 2: DHQ (1 g, 3.28 mmol), acetic anhydride (120 mL), pyridine, 80 °C, 5 h.; 3: DHQ (1 g, 3.28 mmol), acetic anhydride (5 equiv.), pyridine, RT, 3 h; 4: DHQ (1 g, 3.28 mmol), acetic anhydride (60 mL), RT, 7 h; 5: DHQ (0.41 g, 1.32 mmol), butyryl chloride (0.58 mL, 7.34 mmol), pyridine, RT, 4 h.
the H-6 and H-8 chemical shifts being noticeably different. The $^{13}$C NMR data reveal that C-4 (192 ppm) in 3,3',4',7-tetraacetyldihydroquercetin (3) resonates at a higher frequency than C-4 (185 ppm) in 2; hence, we determined that the hydroxyl group at C-5 in 3 was non-acetylated (Yang et al., 2011). The hydroxyl group at C-5 in 7,3',4'-triacetyldihydroquercetin (4) was also not acetylated, as confirmed by the chemical shifts of H-6, H-8, and C-5 in the $^1$H and $^{13}$C NMR spectra. By comparing the chemical shifts of H-2', H-5', and H-6' (7.55, 7.49, and 7.35 ppm, respectively) in the spectrum 2 with those 4 (7.47, 7.36, and 7.35 ppm, respectively) we determined that the hydroxyl groups at the 3'- and 4' positions in 4 were acetylated.

### Chemical stabilities

The chemical stabilities of the DHQ derivatives were determined by separately dissolving the derivatives in PEG-400 (2 wt%) and storing the solutions at 60 °C for 2 weeks. Over time, the color of the DHQ solution became intensely yellow, while the solutions containing the DHQ derivatives showed relatively little change in color (Figure 2). These results revealed that the stabilities of these compounds toward thermal oxidation depend on the number of substituted hydroxyl groups.

#### TABLE I - Chemical shifts (in DMSO-d6) of the aromatic protons of acetylated dihydroquercetins and chemical shift differences ($\Delta$δ) relative to 2 in parentheses

<table>
<thead>
<tr>
<th>Compound</th>
<th>$\delta$(H6)</th>
<th>$\delta$(H8)</th>
<th>$\delta$(H-2')</th>
<th>$\delta$(H-5')</th>
<th>$\delta$(H-6')</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>6.94</td>
<td>6.81</td>
<td>7.55</td>
<td>7.49</td>
<td>7.35</td>
</tr>
<tr>
<td>3</td>
<td>6.45(-0.5)</td>
<td>6.43(-0.38)</td>
<td>7.52(-0.03)</td>
<td>7.48(-0.01)</td>
<td>7.36(+0.01)</td>
</tr>
<tr>
<td>4</td>
<td>6.40(-0.54)</td>
<td>6.37(-0.44)</td>
<td>7.47(-0.08)</td>
<td>7.36(-0.13)</td>
<td>7.35(0)</td>
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</tbody>
</table>

**FIGURE 2** - Visual assessments of the stabilities of the dihydroquercetin derivatives
DPPH radical-scavenging assay

DPPH was used to measure the antioxidant activities of the acetyl derivatives of DHQ (Kim et al., 2012). Among ROS generated by UV radiation, radicals such as hydroxyl (OH) and superoxide (O2•-) promote skin aging by oxidizing cell-membrane lipids, proteins, and DNA. Antioxidants, such as l-ascorbic acid, (+)-α-tocopherol, and flavonoids act as a hydrogen donors that quench lipid radicals generated by ROS, thereby terminating chain reactions (Denisov, Afanas’ev, 2005). Hence, radical scavenging is very important for suppressing skin aging through the prevention of cell damage.

The antioxidant activities of DHQ derivatives (2–5) were examining using DPPH radicals. The DPPH radical-inhibition assay results reveal that 7,3',4'-triacetyldihydroquercetin (4) (IC50 56.67 ± 4.79 μg/mL) exhibit superior antioxidant activity to 3,3',4',7-tetraacetyl dihydroquercetin (3) (IC50 160.89 ± 10.55 μg/mL), 3,3',4',7-tetrabutyldihydroquercetin (5) (IC50 204.41 ± 11.88 μg/mL), and 3,3',4',5,7-pentaacetyldihydroquercetin (2) (IC50 239.88 ± 14.96 μg/mL). However, the DHQ derivatives are less active as antioxidants than DHQ (IC50 32.41 ± 3.35 μg/mL) and L-ascorbic acid (IC50 47.17 ± 4.19 μg/mL) (Figure 3).

FRAP assay

The FRAP assay uses the reducing power of an antioxidant to reduce the ferric tripyridyltriazine (Fe3+TPTZ) complex to its ferrous analogue (Fe2+TPTZ). The antioxidant activities of the DHQ derivatives (2–5) were assessed by the FRAP assay, with absorbance at 593 nm measured using a spectrophotometer. DHQ (6.23 ± 0.34 mM at 50 μg/mL) exhibited a higher antioxidant activity than 3,3',4',7-tetraacetyl dihydroquercetin (3) (2.20 ± 0.29 mM at 50 μg/mL) and 3,3',4',5,7-pentaacetyldihydroquercetin (2) (0.85 ± 0.05 mM at 50 μg/mL), and among the DHQ derivatives, 7,3',4'-triacetyldihydroquercetin (4) (4.17 ± 0.69 mM at 50 μg/mL) exhibited the highest FRAP value. However, all derivatives exhibited lower antioxidant activities than the control (l-ascorbic acid; FRAP value: 5.8 ± 0.73 mM at 1 mM).
Antioxidant and antimicrobial properties of dihydroquercetin esters

Various types of ROS are generated in the Fe$^{3+}$-EDTA/H$_2$O$_2$ system, including ·OH, O$_2^-$, and H$_2$O$_2$, which react to promote luminol into its excited state. Light is emitted when excited luminol eventually returns to its ground state; this process is referred to as “chemiluminescence”. At this time, if an antioxidant, such as a phenolic compound that can scavenge ROS, is present, the chemiluminescence intensity is reduced through the elimination or inhibition of ROS. The reduction in the chemiluminescence intensity is a direct measure of the ROS-scavenging activity (total antioxidant capacity) of the antioxidant (Ha et al., 2017).

According to our study, the DHQ derivatives (2–5) exhibited ROS scavenging activities (OSC$_{50}$ values) of 57.6 ± 3.22, 28.2 ± 1.12, 10.77 ± 0.86, and 72.93 ± 4.57 μg/mL. Among the DHQ derivatives, 7,3’,4’-triacetyldihydroquercetin (4) exhibited the highest antioxidant activity. However, all derivatives showed lower anti-oxidant activities than DHQ and l-ascorbic acid. The ROS scavenging activities of the DHQ derivatives follow the order: 4 > 3 > 5 > 2 (Figure 5). These results are similar to those determined by the DPPH and FRAP assays. The results reveal that the antioxidant activities of these phenolic compounds depend on the presence and positions of free hydroxyl groups, as well as steric freedom (Topal et al., 2016).
Antimicrobial activity

Commonly known skin-residing bacteria include *S. aureus*, *P. aeruginosa*, *E. coli*, and *P. acnes*. In particular, *S. aureus*, which can induce purulent infections and is the cause of atopic dermatitis, has been found in 90% of patients with atopic dermatitis (Raimer, 2000). *P. acnes* is the main pathogen responsible for acne, and is an anaerobic bacterium usually found inside the skin or in the sebaceous glands. *P. acnes* induces inflammation by producing pro-inflammatory cytokines and promotes the formation of open comedones, papulopustules, nodules, and cysts, while *P. aeruginosa* can cause meningitis and sepsis (Yousif, Dabbagh, 2016).

The antimicrobial activities of the DHQ derivatives were assessed against two bacterial strains and a fungus, namely Gram (+) *S. aureus* and *P. acnes*, Gram (-) *E. coli* and *P. aeruginosa*, and the fungus *Candida albicans*, using the disc diffusion assay, the results of which are summarized in Table II. DHQ exhibited high antimicrobial activities against *S. aureus* and *E. coli*, no antimicrobial activity against *C. albicans*, and higher antimicrobial activity than methylparaben against *P. acnes*. The results confirm that DHQ is antimicrobial against *S. aureus*, *P. acnes* and *E. coli*. Derivatives 2, 3, and 5, which are penta- and tetraesters, exhibited no antimicrobial activities against all five strains. Moreover, derivative 4, which is a triester, showed high antimicrobial activities against *S. aureus* and *P. acnes*, while it was inactive activities against *E. coli* and *C. albicans*.
The agar-dilution method was used to determine the MICs of DHQ and 7,3',4'-triacetyldihydroquercetin (4), both of which exhibited excellent antimicrobial activities in the disc-diffusion assay. The concentration of each microbial strain was adjusted to $1 \times 10^7$ CFU, while methylparaben and 1,2-hexanediol were used as controls. The test samples were two-fold diluted with DMSO. After 20 mL of medium containing 2 mL of the test sample was injected onto a petri dish, the test strain was smeared on the plate medium and incubated. These experiments reveal that, among the skin-residing microorganisms, DHQ showed the highest antimicrobial activity against *P. acnes* (MIC, 625 μg/mL), approximately 4-times higher than that of methylparaben (2,500 μg/mL) (Table III). DHQ also showed the same antimicrobial activity against Gram (+) *S. aureus* as methylparaben, but its antimicrobial activity against Gram (-) *E. coli* was lower by a factor of two compared to that of methylparaben, and higher by a factor of two compared to 1,2-hexanediol. Meanwhile, 7,3',4'-triacetyldihydroquercetin (4) showed antimicrobial activity against *P. acnes* similar to that of methylparaben, but higher activity against *S. aureus* than 1,2-hexanediol. Moreover, it was inactive against *E. coli* and *P. aeruginosa*.

### TABLE II - Antimicrobial activities of dihydroquercetin derivatives against microorganisms (paper disc)

<table>
<thead>
<tr>
<th>control conc. (mg/disc)</th>
<th>conc. of dihydroquercetin derivative (mg/disc)</th>
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<tbody>
<tr>
<td>DMSO&lt;sup&gt;1)&lt;/sup&gt; methylparaben</td>
<td>DHQ&lt;sup&gt;2)&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>-</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>-</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>-</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>-</td>
</tr>
<tr>
<td><em>P. acnes</em></td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>1)</sup>Dimethyl sulfoxide.  <sup>2)</sup>Dihydroquercetin.  <sup>3)</sup>No inhibition.
CONCLUSION

The chemical stabilities of acetylated and butyrylated dihydroquercetin derivatives were found to depend on the number free hydroxyl groups (or esters) present, with 3,3’,4’,4,7-pentaacetyldihydroquercetin (2) found to be the most stable acetylated dihydroquercetin. The triacetylated DHQ, 7,3’,4-triacetyldihydroquercetin (4), exhibited potent antioxidant activity, with an IC\textsubscript{50} value of 56.67 ± 4.79 μg/mL (DHQ: 32.41 ± 3.35 μg/mL) using the 1,1-diphenyl-2-picrylhydrazyl assay. Among the derivatives, the triacetyldihydroquercetin 4 showed the highest ROS scavenging activity in the FRAP assay, but lower activity than that of the parent compound (DHQ). The antioxidant activities determined by the DPPH, FRAP, and ROS scavenging assays are similar. The results reveal that the antioxidant activities of these phenolic compounds depend on the presence and positions of free hydroxyl groups, as well as steric freedom.

In terms of antimicrobial activity, DHQ and 7,3’,4’-triacetyldihydroquercetin were found exhibit antimicrobial activities against \textit{S. aureus} and \textit{P. acnes} in the paper-disc assay. MIC (minimum inhibitory concentration) experiments reveal that DHQ exhibited the highest antimicrobial activity at 625 μg/mL against \textit{P. acnes}, 2,500 μg/mL against \textit{S. aureus}, and 5,000 μg/mL against \textit{E. coli}. These results suggest that DHQ and acetylated dihydroquercetin derivatives can be used as complex antioxidant and antimicrobial agents that may find use in industrial and engineering applications such as novel topical antioxidant and antibacterial pharmaceutical and/or cosmetic products.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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Antioxidant and antimicrobial properties of dihydroquercetin esters


Received for publication on 18th March 2020
Accepted for publication on 21st September 2020