Isolation and Characterization of Bioactive Compounds from Root of Rubus coreanus Miquel and their Antimicrobial Activity

Ha Na Jang, Ji Hoon Ha, Yoon Ju Lee, Min Min Fu, and Soo Nam Park*

Department of Fine Chemistry, Cosmetic R&D Center, Seoul National University of Science and Technology, Seoul 01811, Republic of Korea

Received: August 16, 2018 / Revised: October 18, 2018 / Accepted: November 5, 2018

Introduction

The unhealthy skin of modern people is likely to increase pathogen population by abnormal skin condition due to irregular eating habits and sleeping time and environmental stress [1, 2]. As skin pathogens that induce inflammation of the skin, there are strains such as Staphylococcus aureus (S. aureus), Propionibacterium acnes (P. acnes), Bacillus subtilis (B. subtilis), Escherichia coli (E.coli), Pseudomonas aeruginosa (P. aeruginosa). In particular, P. acnes is widely distributed in hair follicles and sebaceous glands, and is known to cause pyogenic infection and atopic dermatitis [3, 4]. S. aureus is distributed in the nasal passages and skin, leading to the formation of purulent dermatitis and pus through skin wounds [5]. B. subtilis is a cause of conjunctivitis, and P. aeruginosa causes meningitis and sepsis [6]. Especially, E. coli, P. aeruginosa and S. aureus are pathogenic strains and are regulated by the cosmetic raw. Most bio-compatible product such as cosmetics, containing rich in nutrients and water, can be contaminated with microorganisms, leading to infection of bacteria in skin and

Rubus coreanus Miquel (RCM), also known as Korean blackberry or bokbunja, is used as a South Korean traditional medicine to treat acne and inflammatory skin conditions. The antimicrobial activity of RCM root and its active compounds remain unclear. In this study, we prepared a 50% ethanol fraction, ethyl acetate fraction, and acid-treated ethyl acetate fraction (aglycone fraction) of RCM root, and evaluated antibacterial activities against the skin pathogens Staphylococcus aureus, Pseudomonas acnes, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. In a paper disc assay, all fractions of RCM root showed antimicrobial activities against the five skin pathogens. The ethyl acetate fraction displayed 6-, 12-, and 2-fold higher minimal inhibitory concentration (MIC) than the 50% ethanol fraction against S. aureus, E. coli, and P. acnes, respectively. The aglycone fraction displayed 2-fold higher MIC than methyl paraben against P. acnes, S. aureus, E. coli, and P. aeruginosa. The ethyl acetate fraction displayed a minimal bactericidal concentration (MBC) similar to that of methyl paraben, and the aglycone fraction showed 2- to 4-fold higher MBCs than those of methyl paraben. In particular, the ethyl acetate fraction was not cytotoxic and showed thermal stability after incubation at high temperatures (60–121°C). Finally, the ethyl acetate fraction was separated and four components were identified: procyanidin C, propelagonidin dimer, ellagic acid, and methyl ellagic acid acetyl pentose. The compounds showed high antibacterial activities. These results suggest that RCM root is potentially applicable as a natural preservative in cosmetics.

Keywords: Rubus coreanus Miquel root, antibacterial activity, identification, natural preservative, ellagic acid, methyl paraben

*Corresponding author
Tel: +82-2-970-6451, Fax: +82-2-972-9585
E-mail: snpark@seoultech.ac.kr

© 2019, The Korean Society for Microbiology and Biotechnology
eyes. Also, proliferation of bacteria changes in flavor, color, and viscosity of the cosmetics [7].

Preservatives are used to protect cosmetics from various problems caused by microorganisms, and parabens (such as methyl paraben) are commonly used preservatives [8]. However, a recent research indicated that parabens can act as environmental hormones, and it has raised concerns about the safety [9]. Therefore, the natural preservatives, including the anti-microorganism materials such as alkaloids, flavonoids, and phytoalexin, were developed to use in the field of cosmetics and food [10, 11].

To resolve the problem of safety using plants as traditionally to manage microbial infection or contamination, we targeted *R. coreanus* Miquel (RCM) [12–14]. RCM is a kind of *rubus* belonging to Rosaceae, which grows in South Korea, China, and Japan [15]. Especially, RCM root was registered as skin conditioning agents at International Cosmetic Ingredient Dictionary (ICID) and Cosmetic ingredient dictionary in our country. Additionally, related patents were registered to use in cosmetic in Korea [16, 17]. RCM root is reported to have the anti-inflammation effects through the reduction of production of NO and cytokines (IL-1β, IL-6 and IL-10) and expression of cyclooxygenase (COX)-2 and prostaglandin E2 (PGE2). RCM root has the antibacterial activities against resistant bacteria containing methicillin-resistant *Staphylococcus aureus* (MRSA) as dangerous skin microorganism, carbapenem-resistant *Acinetobacter baumannii* (CRAB) and *Bacillus anthracis* [10]. RCM root was studied that it improved acne skin and chronic relapsing inflammatory skin disorder were improved in clinical test. Phenolic compounds and nutrients (minerals, phosphorus, iron, potassium, organic acids, etc.) are present in RCM, which is known for various antioxidant, anticancer and the enhancement effect of immunity [12, 18]. However, the research of RCM root has not been deeply known on the antibacterial activity against skin pathogen (*S. aureus*, *P. acnes*, *B. subtilis*, *E. coli* and *P. aeruginosa*).

The purpose of this study was to evaluate the antimicrobial activity of extracts and fractions from RCM root on the paper disc, minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) methods, time-kill curve assay, and to identify the main active compound.

### Materials and Methods

#### Equipment and chemicals

An ultraviolet-visible (UV-VIS) spectrophotometer was used for Cary 50 (Varian, Australia), high-performance liquid chromatograph (HPLC, Shimadzu, Japan). A pH meter was used for Metter Toledo (Europe). Methyl paraben, the positive control in the antimicrobial activity studies was purchased from Sigma Chemical Co., USA. The solvents including ethanol, methanol, ethyl acetate, n-hexane, formic acid, acetonitrile, and dimethyl sulfoxide (DMSO) were a special grade chemical from the Daejung Chemicals & Metals Co., Ltd., Korea.

#### Plant material

The *R. coreanus* (RCM) root was purchased from Cosmetic Research and Development Center (Korea) in which all traditional Korean herbs were deposited and identified by herbal expert according to the Korean herbal pharmacopoeia. A voucher specimen of *R. coreanus* was deposited at our laboratory in Seoul University of Science and Technology (# RCM_003). The plant material has been prepared in the form of dried powder at -70°C.

#### Preparation of RCM root crude extract and fractions

Dried healthy RCM root (100 g) were successively extracted at room temperature with 1 L of 50% ethanol fraction for 24 h. The filtered ethanol fraction was then powdered using reduced pressure and enrichment using rotary evaporator. The organic and non-polar material was removed from the rest of the extract using n-hexane and then it was partitioned with ethyl acetate. In addition, the aglycone was obtained by acidic hydrolysis with ethyl acetate fraction. Ethyl acetate fraction was mixed with 5% H₂SO₄ and acetone and then refluxed, cooled, and heated with boiling water for 4 h. After the neutralization-titration reaction with 5% KOH-methanol, the powder obtained by fractionation with ethyl acetate was further dissolved in 100% ethanol. Yield of 50% ethanol fraction and ethyl acetate, aglycone fractions was 8.64, 2.68, and 0.98%, respectively. The components of all extracts were evaluated, and their antimicrobial activities were evaluated. All samples were stored at -70°C.
Bacterial strains, media and buffers

The skin flora microorganisms tested were Gram-positive bacteria including *B. subtilis* (ATCC19659), *P. acnes* (ATCC6919), *S. aureus* (ATCC6538); Gram-negative bacteria including *E. coli* (ATCC23736), *P. aeruginosa* (ATCC28369), and the yeast *P. ovale* (ATCC12078). The organisms were obtained from the Korean Culture Center of Microorganisms (KCCM). *P. acnes* was cultured in reinforced clostridial (RC) broth (Merck, Germany) using an anaerobic jar with the Gaspak system (Merck Anaerocult® Gaspak System, Germany) for 48 h at 37°C. Four strains were cultivated on Mueller-Hinton broth (Merck, Germany) for 24 h at 37°C.

Disc diffusion assay

The RCM root fractions and methyl paraben were respectively dissolved in DMSO, a negative control, to obtain a final concentration of 50 μg/ml. Briefly, a 100 μl sample of a suspension containing approximately 10^6–10^7 colony-forming units (CFU)/ml of the bacterial cells was spread onto nutrient agar. For the antibacterial test, paper disc (8 mm in diameter, Roshi Kaisha Ltd., Japan) were separately impregnated with 50 μl of 50 μg/ml plant extracts (2.5 mg/disc) and placed on the inoculated agar. For the positive control, paper disc were impregnated with the same amount (50 μl) of methyl paraben (2.5 mg/disc) dissolved in both types of solvents used. Plates were incubated at 37°C for 24–48 h for bacterial strains. Antimicrobial activity was assessed by measuring the diameter of the growth inhibition zone (IZ) in mm (including disc diameter of 8 mm) for the test organisms, compared to controls.

Broth dilution assay

The broth dilution methods including MIC and MBC assay were used to evaluate the antibacterial activity in Iso-Standards Institute (CLSI, formerly NCCLS) procedures (NCCLS, 2003). The 48-well culture plates were inoculated 10 μl of a diluted bacterial suspension containing about 10^6–10^7 CFU/ml with the dissolved samples (100 μl) in Mueller-Hinton broth or reinforced clostridial broth according to the bacterial species. Cell suspensions incubated with saline solution and without samples were used as controls. The incubation conditions were for 24 or 48 h at 37°C. The wells were re-inoculated with the sterile medium to evaluate whether the concentrations showed microbicidal effects by no observable growth in the wells treated by methyl praben.

Time-kill curve assay

Time-kill curve experiments were performed for each strain [19]. Based on results obtained from broth dilution assay, particular concentrations of RCB root ethyl acetate and aglycone fractions against various microorganisms; *B. subtilis, S. aureus, E. coli, P. aeruginosa, P. acnes*. Survival microbial counts were measured to confirm the influence of RCB root fractions in microbial suspensions over time. Subsequently, to confirm populations of bacteria incubated for a period of time, suspensions were diluted by sterilized saline, spread on RC agar medium plates and incubated for 24 h or 48 h at 37°C.

Thermal stability

The stability of the ethyl acetate fraction following heat treatment was determined by disc diffusion assay using *P. acnes*. The 50 mg/ml fraction was dissolved in DMSO was incubated at different temperatures (60, 80, and 100°C for 2 h) in a water bath and by autoclave (121°C for 15 min). The samples were slowly cooled at room temperature for 1 h, and then thermal stability based on antimicrobial activity was measured by determining the inhibition zone.

Cytotoxicity test

HaCaT cells (provided by the Kyung Hee University Skin Biotechnology Center) were incubated in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in an atmosphere of 5% CO₂. Cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT, Sigma, USA) assay. HaCaT cells were seeded at a density of 1×10⁵ cells in a 96-well plate template at 37°C in a 5% CO₂ atmosphere. Samples of RCM root were added at varying concentrations (63–1000 μg/ml) for 24 h. After washing two times, the MTT solution (2 μg/ml) was added to each well of a 96-well plate, and the samples were incubated for 1 h. The formazan crystals produced were dissolved in DMSO and quantified by measuring their optical density at 570 nm using an enzyme-linked immunosorbent assay (ELISA) reader (Tecan, Austria). These values were subtracted from the final absorbance reading to eliminate back-
Antimicrobial Activity of Rubus coreanus Miquel Root

57

March 2019 | Vol. 47 | No. 1

ground interference. The value was expressed using the following equation.

\[
\text{Cell viability (\%)} = \left( \frac{A_{\text{experiment}}}{A_{\text{control}}} \right) \times 100
\]

TLC and HPLC separations of ethyl acetate fraction components

For the phenolic-rich solids content analysis of the ethyl acetate fraction, it was dissolved in 100% ethanol and filtered through a syringe filter (Millipore 0.45-μm). The filtrate was then used for the experiments. The TLC developing solvents was a mixture of acetonitrile : 0.4% phosphoric acid : methanol : formic acid at a volume ratio of 4 : 6 : 1 : 0.1. HPLC analysis (Shimadzu, Japan) used a Shim pack VP-ODS (L: 250 mm, LD: 4.6 mm) column as a stationary phase. The analysis conditions are shown in Table 1.

Liquid Chromatography (LC)/electrospray Ionization (ESI)-MS/MS analysis

The LC/ESI-MS/MS (Thermo Scientific, USA) was used to further characterize the five components (Peak 2-5) isolated from ethyl acetate fraction of RCM root. The liquid chromatography (LC) parameters used for the separation included column specifications of UVD Spher Pur C18-E 1.8 m, 50 × 2.0 mm (Cat.-No. N0520E181UVC). The injection volume and flow rate were 5 μl and 200 μl/min, respectively. The mobile phase consisted of 0.1% formic acid (solution A) and acetonitrile (solution B). Gradient elution at a flow rate of 200 μl/min was performed as follows: total run time, 30 min; ionization of analysis was by ESI; capillary temperature, maintained at 275°C; ion source voltage, 5 kV; and sheath and aux gas, 30 and 5 units, respectively. The capillary voltage was set at 45 or -15 V in the positive or negative ionization modes.

Statistical analysis

All experiments were repeated in triplicate, and statistical analyses were performed using the student’s T-test at 5% a significant level.

Results

Antibacterial and bactericidal activities of RCM root

The antimicrobial activities of the fractions were evaluated using a disc diffusion assay against Gram-positive (B. subtilis, P. acnes, and S. aureus) and Gram-negative bacteria (E. coli and P. aeruginosa) constituting skin microflora. The 50% ethanol fraction, ethyl acetate fraction and aglycone fraction of RCM root indicated antimicrobial activities as shown the growth inhibition zones against all microorganisms (Table 2). The aglycone fraction of RCM root showed higher antibacterial activity than 50% ethanol fraction and ethyl acetate fraction, and its activity was similar to that of methyl paraben as positive control. The 50% ethanol fraction and ethyl acetate fraction has antibacterial activity although their activities were lower than methyl paraben against all microorganisms.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Strain</th>
<th>Inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50% Ethanol fraction</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>ATCC19659</td>
<td>12.50 ± 0.50</td>
</tr>
<tr>
<td>P. acnes</td>
<td>ATCC6919</td>
<td>15.67 ± 0.58</td>
</tr>
<tr>
<td>S. aureus</td>
<td>ATCC6538</td>
<td>11.17 ± 0.29</td>
</tr>
<tr>
<td>E. coli</td>
<td>ATCC23736</td>
<td>12.17 ± 0.29</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>ATCC29336</td>
<td>12.08 ± 0.14</td>
</tr>
</tbody>
</table>

Concentration of fractions was 2.5 mg/disc. Zones of inhibition include diameter of disc (8 mm). The value expressed as mean ± SD (n = 3). *No inhibition. +Positive control.

Table 1. Conditions for high-performance liquid chromatography (HPLC) analysis.

<table>
<thead>
<tr>
<th>Conditions of HPLC analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
</tr>
<tr>
<td>Detector</td>
</tr>
<tr>
<td>Detection wavelength</td>
</tr>
<tr>
<td>Flow rate</td>
</tr>
<tr>
<td>Injection volume</td>
</tr>
<tr>
<td>Mobile phase</td>
</tr>
</tbody>
</table>

Table 2. Antimicrobial activity of R. coreanus Miquel Root against microorganisms.

The value expressed as mean ± SD (n = 3). *No inhibition. +Positive control.
on paper disc assay. These results suggest that the extract and fractions of RCM root have the antimicrobial activities.

The MIC and MBC of each fraction was evaluated based on the results of the disc diffusion assay. As shown in Table 3, MICs of the 50% ethanol fraction were 0.50, 1.00, 3.00, 3.00, and 0.50% against *B. subtilis*, *P. acnes*, *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively. Especially, 50% ethanol fraction showed high antimicrobial activities against *B. subtilis* and *P. aeruginosa*. The ethyl acetate fraction and aglycone fraction indicated high antibacterial effects against all microorganism. The ethyl acetate fraction showed a 6-, 12-, and 2-fold higher MICs than the ethanol fraction against *S. aureus*, *E. coli*, and *P. acnes* respectively. However, the ethyl acetate fraction showed MICs similar or lower than methyl paraben. In contrast, the aglycone fraction indicated 2-fold higher MICs than those of methyl paraben against *P. acnes*, *S. aureus* and *P. aeruginosa*.

In the MBC evaluation, the 50% ethanol fraction indicated 1.00, 1.00, 4.00, 4.00, and 1.00% against *B. subtilis*, *P. acnes*, *S. aureus*, *E. coli*, and *P. aeruginosa*. The 50% ethanol fraction showed the same MBCs to methyl paraben against *B. subtilis* and *P. aeruginosa*. The ethyl acetate fraction indicated the MBCs similar to those of methyl paraben against *B. subtilis*, *P. acnes*, *E. coli* and *P. aeruginosa*. Surprisingly, the aglycone fraction indicated 2- to 4-fold higher MBC than those of methyl paraben against all bacterial spectra excluding *P. acnes*. The 50% ethanol fraction and fractions of RCM root exhibited a potent antimicrobial activity against *P. aeruginosa*, which is impressive because this species show high

### Table 3. MIC and MBC of extract and fraction of *R. coreanus* Miquel root against various bacteria.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC/MBC (%)</th>
<th>50% Ethanol fraction</th>
<th>Ethyl acetate fraction</th>
<th>Aglycone fraction</th>
<th>Methyl paraben</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. subtilis</em></td>
<td></td>
<td>0.50/1.00</td>
<td>0.50/1.00</td>
<td>0.25/0.25</td>
<td>0.25/1.00</td>
</tr>
<tr>
<td><em>P. acnes</em></td>
<td></td>
<td>1.00/1.00</td>
<td>0.50/0.50</td>
<td>0.13/0.50</td>
<td>0.25/0.50</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>3.00/4.00</td>
<td>0.50/1.00</td>
<td>0.13/0.25</td>
<td>0.25/0.50</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td></td>
<td>3.00/4.00</td>
<td>0.25/0.50</td>
<td>0.13/0.25</td>
<td>0.13/0.50</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td></td>
<td>0.50/1.00</td>
<td>0.50/1.00</td>
<td>0.25/0.25</td>
<td>0.50/1.00</td>
</tr>
</tbody>
</table>

![Fig. 1. HaCaT cell viability of *R. coreanus* Miquel root extract and fractions by MTT assay.](http://dx.doi.org/10.4014/mbl.1808.08009)
resistance against to all kinds of preservatives.

We attempted to determine whether the activity of the fractions is bacteriostatic or bactericidal by conducting time-kill studies against skin microflora. The used concentrations were based on the MIC or MBC results of the ethyl acetate and aglycone fractions (Table 3). The fractions and methyl paraben (positive control) were incubated at 0.25–1.00% concentrations for 24–48 h with each bacteria. Then, the colonies analysis per time were observed to determine the growth inhibition changes of samples for the time interval (Fig. 1). Overall, while the untreated negative control (DMSO) showed a substantial increase in colonies with time, sample groups indicated bacteriostatic activity at MICs and bactericidal activity at MBCs. 1% ethyl acetate fraction and 0.25 aglycone fraction immediately reduced, and killed all bacteria within 24 h except for *P. acnes*, which was bactericidal after 48 h. 0.5% ethyl acetate fraction and 0.25% methyl paraben only killed *E. coli* within 24 h. These results suggest that effective compounds exist in RCM root against various bacteria.

Cytotoxicity of RCM root extract and fractions

When preservatives are applied to human skin, their

---

**Fig. 2.** Time-kill curves of ethyl acetate and aglycone fractions of *R. coreanus* Miquel root, control (DMSO) and methyl paraben (MP) against various microorganisms; (A) *B. subtilis*, (B) *S. aureus*, (C) *E. coli*, (D) *P. aeruginosa*, (E) *P. acnes*. In the initial stages of the experiment, the microorganisms were inoculated with $10^6$–$10^7$ colony-forming units (CFU)/ml, and then treated with various samples for 24 or 48 h. Values represent means ± SD at the following times after the treatment of indicated samples in triplicate measurements.
safeties are important. To determine the cell viabilities of RCM root extract and fractions, we carried out MTT assay (Fig. 2). 50% ethanol fraction and aglycone fraction of RCM root indicated low cell viabilities. Especially, the aglycone fraction indicated high cytotoxicity at 63 μg/ml. On the other hand, ethyl acetate fraction of RCM root indicated cell growth activity rather than cytotoxicity from 63 μg/ml to 500 μg/ml. Only the ethyl acetate fraction of all revealed nontoxic activity at all tested concentrations. Methyl paraben indicated no cytotoxicity by 500 μg/ml (91% cell viability). This suggests that the RCM root ethyl acetate fraction is highly applicable for use as a natural antiseptic alternative to the synthetic methyl paraben. Therefore, we carried out the thermal stability analysis of RCM root ethyl acetate fraction.

**Thermal stability of ethyl acetate fraction of RCM root**

To apply for cosmetic, we confirm thermal stability of RCM root ethyl acetate fraction. ethyl acetate fractions heated at various temperature (60, 80, 100 and 121 ℃). Each fractions showed the same inhibition zone against *P. acnes*, which were equal to the values of the untreated samples (Fig. 3). These results indicate that the ethyl acetate fraction of RCM root is heat stable up to 121 ℃. Therefore, the constituents of the ethyl acetate fraction would maintain their original efficacy following industrial applications required heating. These results suggest that the antibacterial activities of RCM root ethyl acetate fraction are thermodynamically stable molecules, which are useful for cosmetic formulation.

**Separation and identification of antibacterial effective components analysis of ethyl acetate fraction of RCM root**

To identify the antibacterial and thermodynamically stable compounds of RCM root ethyl acetate fraction, we separated and analyzed by UV spectroscopy, HPLC and LC/ESI-MS. The HPLC data of the stable and high antibacterial effective ethyl acetate fraction separated four bands (RC-1, RC-2, RC-3 and RC-4) following determination of the UV-visible spectrum (Fig. 4). Four peaks clearly separated bands. RC-1 and RC-2 were similar to a flavonoid wavelength, which absorbed at 270 and 365 nm together, but RC-3 and RC-4 just absorbed at 270 nm such as phenolic acid. Then, their molecular weights were analyzed by LC/ESI-MS (Thermo Scientific, USA) and referenced previously reported spectroscopic information (Table 4). They were identified as follow RC-1 (procyanidin C), RC-2 (propelagonidin dimer), RC-3 (ellagic acid), and RC-4 (methyl ellagic acid acetylpentose) (Fig. 5). Finally, we confirmed that their retention times and absorption spectrums of RCs corresponded with those of the standards on HPLC analysis. These results suggest that the antibacterial activities of RCM root ethyl acetate faction are contributed by procyanidin C, propelagonidin dimer, ellagic acid, and methyl ellagic acid acetylpentose.

**Antimicrobial activity of RCM root components**

To confirm whether the components of RCM root contribute the antibacterial activities against skin microflora. We evaluated the MIC and MBC of the bioactive components to elucidate their antibacterial effects (Table 5). MICs of procyanidin C were 0.10% against *P.
antibacterial activity against \( P. \) \( \text{acnes} \), \( S. \) \( \text{aureus} \), \( E. \) \( \text{coli} \), and \( P. \) \( \text{aeruginosa} \) at 0.40% but \( B. \) \( \text{subtilis} \) at 0.20%. In particular, ellagic acid showed high anti-microorganism against \( B. \) \( \text{subtilis} \), \( P. \) \( \text{acnes} \), \( S. \) \( \text{aureus} \), \( E. \) \( \text{coli} \), and \( P. \) \( \text{aeruginosa} \) at 0.0062, 0.0625, 0.0015, 0.0015 and 0.0015%, respectively. Ellagic acid indicated lower MBC values than other compounds, furthermore it showed 3.2- to 266-fold higher anti-bacterial activities than methyl paraben against the bacteria spectrums.

**Discussion**

In this study, 50% ethanol fraction, ethyl acetate fraction of the RCM root are prepared and also the ethyl acetate fraction is deglycosylated (aglycone fraction) for enhancing antibacterial activity. The extract and fractions were investigated on antibacterial activities against \( P. \) \( \text{acnes} \), \( S. \) \( \text{aureus} \), \( E. \) \( \text{coli} \), and \( P. \) \( \text{aeruginosa} \).特に, ellagic acid acetylpentose showed antibacterial activities against \( P. \) \( \text{acnes} \), \( S. \) \( \text{aureus} \), \( E. \) \( \text{coli} \), and \( P. \) \( \text{aeruginosa} \). The ethyl acetate fraction showed more twice higher MICs than the 50% ethanol fraction against \( S. \) \( \text{aureus} \), \( E. \) \( \text{coli} \), and \( P. \) \( \text{acnes} \). The ethyl acetate

### Table 4. Peak number, UV spectra, retention time, m/z \([\text{M-H}]^-\) ion, \( \text{MS}^n \) fragments of compounds obtained by LC-ESI-MS analysis of \( R. \) \( \text{coreanus} \) Miquel root.

<table>
<thead>
<tr>
<th>Peak</th>
<th>( t_R ) (min)</th>
<th>MW</th>
<th>UV (nm)</th>
<th>([\text{M-H}]^-) (m/z)</th>
<th>([\text{M-H}]^+) (m/z)</th>
<th>Identified names</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>866</td>
<td>243, 276</td>
<td>865</td>
<td>867</td>
<td>Procyanidin C</td>
<td>[20, 21]</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>563</td>
<td>240, 279</td>
<td>562</td>
<td>564</td>
<td>Propelagonidin dimer</td>
<td>[22, 23]</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>302</td>
<td>252, 360</td>
<td>301</td>
<td>303</td>
<td>Ellagic acid</td>
<td>[24, 25]</td>
</tr>
<tr>
<td>4</td>
<td>35</td>
<td>490</td>
<td>252, 360</td>
<td>489</td>
<td>491</td>
<td>Methyl ellagic acid acetylpentose</td>
<td>[26]</td>
</tr>
</tbody>
</table>

\( t_R \) Retention time.

### Table 5. MIC and MBC of the compounds identified from \( R. \) \( \text{coreanus} \) Miquel root against various bacteria.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC/MBC (% w/v)</th>
<th>Procyanidin C</th>
<th>Propelagonidin dimer</th>
<th>Ellagic acid</th>
<th>Methyl ellagic acid acetylpentose</th>
<th>Methyl paraben</th>
</tr>
</thead>
<tbody>
<tr>
<td>( B. ) ( \text{subtilis} )</td>
<td>0.20/0.40</td>
<td>0.40/0.80</td>
<td>0.0062/0.0125</td>
<td>0.20/0.40</td>
<td>0.20/0.80</td>
<td>0.20/0.80</td>
</tr>
<tr>
<td>( P. ) ( \text{acnes} )</td>
<td>0.10/0.40</td>
<td>0.20/0.20</td>
<td>0.0625/0.2500</td>
<td>0.40/0.40</td>
<td>0.20/0.40</td>
<td>0.20/0.40</td>
</tr>
<tr>
<td>( S. ) ( \text{aureus} )</td>
<td>0.10/0.20</td>
<td>0.20/0.40</td>
<td>0.0015/0.0062</td>
<td>0.40/0.80</td>
<td>0.20/0.40</td>
<td>0.10/0.40</td>
</tr>
<tr>
<td>( E. ) ( \text{coli} )</td>
<td>0.10/0.20</td>
<td>0.20/0.80</td>
<td>0.0015/0.0062</td>
<td>0.40/0.80</td>
<td>0.20/0.40</td>
<td>0.10/0.40</td>
</tr>
<tr>
<td>( P. ) ( \text{aeruginosa} )</td>
<td>0.10/0.20</td>
<td>0.20/0.40</td>
<td>0.0015/0.0062</td>
<td>0.40/0.80</td>
<td>0.20/0.40</td>
<td>0.10/0.40</td>
</tr>
</tbody>
</table>

*Negative control (DMSO) indicated no anti-bacterial activity.*
fraction possessed MBCs similar to that of methyl paraben. Ethyl acetate was also not cytotoxic and showed thermal stability after incubation at high temperatures (60–121°C) but not aglycone fraction. Four components (procyanidin C, propelargonidin dimer, ellagic acid, and methyl ellagic acid acetyl pentose) were identified from the ethyl acetate fraction. The compounds showed high antibacterial activities against skin pathogens. In particular, ellagic acid showed 3.2- to 266-fold higher antimicrobial activities than methyl paraben against all bacterial spectra. The antibacterial activity of ellagic acid was contributed by functional group as catechol, which is already reported to have greater activity against Gram-positive than Gram-negative bacteria [28] through the perturbation in lipid bilayers, by directly penetration and induction of damage to disrupt the barrier function [29]. For this reasons, ellagic acid has various antibacterial activities. 1) Ellagic acid attenuates the function of many proton pumps, proteins, and receptors on the cell membrane. 2) It has an intramolecular catechol structure it can impair homeostasis or calcium signaling by chelating calcium and, thereby, disrupting the metabolism of the microorganism with resultant death. 3) It has antioxidative effect by phenolic structure. Antioxidative effect can quench the electrons from free radicals and disrupt the flow of the electrons at the level of cytochromes and inhibit the growth of bacteria by disrupting oxidative phosphorylation. 4) It is made by partial hydrophobicity, which become stack or embed on the membrane surface. It induce a change in the membrane structure by creating a hydrophobic environment around the cell. In addition, this may inactivation proteins by inducing protein unfolding. A rigid membrane destabilizes the cell by weakening membrane integrity, which may disrupt critical transport processes and induce the collapse of the bacterial membrane [30, 31]. Thus, ellagic acid may contribute the antibacterial activities of the ethyl acetate fraction of RCM root.

In conclusion, RCM root suggest that it is applicable to natural preservatives in cosmetics by high antimicrobial activities, no cytotoxicity and thermal stability. In further study, these preliminary results need to be improved by other works with in vivo study (pharmacokinetic, toxicity and etc.) in animal or human model for application of such compounds as natural preservatives in cosmetics.
Antimicrobial Activity of Rubus coreanus Miquel Root