Elucidation of phytochemicals and antioxidants properties of *Sasa quelpaertensis*

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ABSTRACT

*Sasa quelpaertensis* Nakai extract (SQE) has long been used as a traditional medicine and health drink. The present study aims to examine the properties and antioxidant activity of the phytochemicals isolated from *S. quelpaertensis*. Therefore, the SQE was fractionated with n-hexane, chloroform (CHCl₃), ethyl acetate (EtOAc), and n-butanol (BuOH). Phytochemicals present in the solvent fraction were also isolated using a high-performance liquid chromatography (HPLC) system. In addition, their DPPH radical scavenging ability and NO production inhibitory effect were compared. The results showed that phytochemicals *p*-hydroxybenzaldehyde (1), salicylic acid (2), syringaldehyde (3), methyl *cis*-p-hydroxycinnamate (4), methyl *trans*-p-hydroxyccinnamate (5), *p*-coumaric acid (6), 2,3-dihydroxypropyl 9Z,12Z-octadecadienoate (7), (+)-(6S,7αS)-epilolide (8), (-)-(6 R,7αS)-loliolide (9), naringenin (10), 3-O-p-coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-O-β-glucopyranosylpropanol (11), tricine (12), and tricine 7-O-b-D-glucopyranoside (13) were isolated from CHCl₃, EtOAc, and BuOH fractions and then identified using nuclear magnetic resonance (NMR) spectrometry. This is an initial study on compounds 2, 3, 4, 5, 7, 8, and 9 obtained from *S. quelpaertensis*. Compounds 11 (IC₅₀ 120.3 μM) and 7 (IC₅₀ 43.62 μM) showed significant DPPH radical scavenging activity and anti-inflammatory activity, respectively. We believe these results may provide the basic data for developing effective antioxidants using the SQE.

INTRODUCTION

Reactive oxygen and nitrogen species (ROS and RNS, respectively) are derived from oxygen and nitrogen molecules through the biological system in the body and include free radicals such as superoxide anion radical (O₂⁻), hydroxyl radical (·OH), nitric oxide (NO−), and nitric dioxide (NO₂⁻), as well as nonradical molecules such as hydrogen peroxide (H₂O₂), singlet oxygen (O₂), nitrous acid (HNO₂), and dinitrogen tetroxide (N₂O₄). ROS and RNS are important intermediaries in the synthesis of critical signaling molecules that respond to abiotic stress and play an important role in the immune response against pathogens. However, when their concentration in a cell exceeds the required levels, they begin to adversely affect critical cellular structures such as proteins, lipids, and nucleic acids and can lead to oxidative and nitrosative stress. Oxidative and nitrosative stress is associated with various diseases such as cancer, inflammation, Alzheimer’s, atherosclerosis, and arteriosclerosis. Therefore, research to isolate antioxidant compounds from natural resources...
such as herbs, fruits, and vegetables has evoked great interest in developing methods to overcome radical-mediated deleterious effects in biological systems.\textsuperscript{[7,8]}

Phenolic compounds are representative antioxidants widely distributed in plant tissues. They are secondary metabolites synthesized through shikimic acid and phenylpropanoid pathways.\textsuperscript{[9]} Plant-based phenolic compounds such as flavonoids, lignins, alkaloids, terpenoids, carotenoids, and vitamins are promising potential antioxidants because of their excellent radical scavenging activity.\textsuperscript{[10,11]} However, their antioxidant activity is lower than that of their crude extracts at the same concentration because of the synergistic effect between the extract constituents.\textsuperscript{[12,13]} The mechanism responsible for this synergistic antioxidant activity has not been deciphered yet because of the complex properties of the extract constituents. Therefore, identification of the crude extract constituents is an important research topic because it will help understand the synergistic properties of the extract.\textsuperscript{[14]}

\textit{Sasa quelpaertensis} Nakai is a bamboo grass that grows only on Jeju Island of South Korea. It has long been used as a health drink and traditional folk remedy for various diseases in Asian countries such as Korea. A few recent studies have proposed the use of its leaves as a health food and functional materials for cosmetic industries because of their useful biological activities and abundant secondary metabolites.\textsuperscript{[15,16]} Sultana and Lee identified 15 compounds from \textit{S. quelpaertensis}, such as the phenylpropanoids, alkene glycosides, flavonoids, and organic acids, and reported their DPPH radical scavenging activities.\textsuperscript{[17]} In our previous report, we showed through HPLC chromatograms that this plant may contain abundant phenolic compounds.\textsuperscript{[18]} The present study aims to isolate new phenolic compounds from \textit{S. quelpaertensis} and evaluate their DPPH radical scavenging activities and NO-production-inhibition activities in lipopolysaccharide-activated RAW 264.7 macrophages.

Materials and methods

\textbf{Chemicals}

Ethanol, \textit{n}-hexane, chloroform, ethyl acetate, and \textit{n}-butanol were purchased from Sigma-Aldrich (HPLC grade, Steinheim, Germany). Methanol (HPLC grade) used to separate the compound was purchased from Fisher Scientific Korea (Seoul, Korea). Folin & Ciocalteu’s phenol reagent, ascorbic acid (>99% pure), butylated hydroxyanisole (BHA, >99% pure), gallic acid (GA, 97.5–102.5% pure), naringin (NG, HPLC grade), sodium carbonate, dimethyl sulfoxide (DMSO, >99.7% pure), sodium hydroxide (>98.0% pure), diethylene glycol, 2,2-diphenyl-1-picrylhydrazyl (DPPH, >98.0% pure), and lipopolysaccharides (LPS, <3% protein) were obtained from Sigma-Aldrich (Steinheim, Germany). Dulbecco’s Modified Eagle’s Medium (DMEM), fetal bovine serum (FBS), and penicillin-streptomycin were obtained from Gibco BRL (Grand Island, NY, USA). Dimethyl Sulfoxide-D$_6$ (DMSO, >99.5% pure) and methanol-D4 (CD$_3$OD, >99.8% pure) were purchased from Cambridge Isotope Laboratories, Inc. (Tewksbury, MA, USA).

\textbf{Plant materials, extraction, and fractionation}

The leaves of \textit{S. quelpaertensis} were collected in October 2018 from Mt. Halla in Jeju Island, South Korea. It was authenticated and compared to a voucher specimen provided by Dr. Hyun Cheol Kim of the Hallasan Research Institute, Jeju Special Self-Governing Province (No. 20120524 HA006630). The leaves were cleaned under running water and dried using a hot air drier for 48 h at 60°C. The dried leaves were pulverized to powder less than 200 mesh using a pulverizer (HCM-190, Hansung Pulverizing Machinery Co., LTD, Gwangju-si, Korea). The powder was extracted with 70% ethanol for 48 h at room temperature. The extract filtered through a filter paper was dried in a rotary evaporator (Rotavapor R-100, Büchi, Labortechnik AG, Flawil, Switzerland) and a freeze dryer (IshinBioBase Co., Ltd., Gyeonggi, Korea) at 40°C. The \textit{S. quelpaertensis} extract (SQE) was dissolved using distilled water and then partitioned with \textit{n}-hexane, chloroform, ethyl acetate, and \textit{n}-butanol. The following partition procedures were used. First, 70% ethanol crude extract (100 g) was dissolved in
1.0 L distilled water, followed by the addition of 1.0 L n-hexane, chloroform, ethyl acetate, and n-butanol, three times. Each partitioned fraction was filtered through a filter paper and then concentrated using a rotary evaporator (Rotavapor R-100, Büchi, Labortechnik AG, Flawil, Switzerland) and a freeze dryer (IshinBioBase Co., Ltd., Gyeonggi, Korea) at 40°C. Each sample was stored in a refrigerator at −70°C until use.

**Isolation and identification of phenolic compounds**

An automated HPLC system (2695 Alliance system; Waters, Milford, MA, USA) equipped with a photodiode array (PDA) detector and a fraction collector was used to purify the phenolic compounds. Each phenolic compound was isolated using a Symmetryprep-columns C18 column (7.8 × 300 mm ID. 7 μm; Waters, Milford, MA, USA). The column oven was maintained at 40°C. The mobile phase consisted of MeOH (A) and water (B). The gradient elution program was as follows: 0.0–60.0 min linear from 20% to 100% of A, quick decrease to 20% A for 2 min, and isocratic for 10 min. The isolated compound was monitored by recording the UV spectra of the irradiated samples between 200 and 600 nm. The CHCl₃, EtOAc, and BuOH fractions were repeatedly injected in 100 μL at a concentration of 100 mg/mL. Their repeatedly injected fractions were obtained phytochemicals through a fraction collector. After monitoring the HPLC chromatogram, the fractions of the same compound were combined. The isolated compounds were analyzed using a JEOL FT/NMR 400 spectrometer (JEOL Ltd., Tokyo, Japan). Each isolated compound was recorded at 400 and 100 MHz by referring to the solvent signals (CD₃OD) and was identified by comparing with the reference data.

**Compound (1), p-Hydroxy benzaldehyde:** ¹H-NMR (CD₃OD, 400 MHz) δ: 9.86 (1 H, s, H-7), 7.8 (2 H, dd, J = 8.5, 2.8 Hz, H-2, 6), 6.9 (2 H, dd, J = 8.5, 2.8 Hz, H-3, 5), 13C NMR (CD₃OD, 100 MHz) δ: 190.9 (C-7), 163.3 (C-4), 132.1 (C-2, 6), 128.4 (C-1), 115.8 (C-3, 5).

**Compound (2), Salicylic acid:** ¹H-NMR (CD₃OD, 400 MHz) δ: 7.67 (1 H, dd, J = 15.8, 2.3 Hz, H-5), 7.13 (1 H, dd, J = 9.0, 2.3 Hz, H-7), 6.60 (2 H, dd, J = 15.8, 3.2 Hz, H-4, 6), 13C-NMR (CD₃OD, 100 MHz) δ: 171.7 (C-7), 162.8 (C-2), 131.3 (C-4), 129.9 (C-6), 120.4 (C-5), 115.9 (C-1), 115.6 (C-3).

**Compound (3), Syringaldehyde:** ¹H-NMR (CD₃OD, 400 MHz) δ: 9.77 (1 H, s, H-7), 7.25 (2 H, dd, J = 2.2 Hz, H-2, 6), 3.95 (6 H, s 3, 5-O Me); ¹³C NMR (CD₃OD, 100 MHz) δ: 192.9 (C-7), 149.7 (C-3, C-5), 143.8 (C-4), 129.2 (C-1), 108.3 (C-2, 6), 56.4 (3, 5-O Me).

**Compound (4), Methyl cis-p-hydroxy cinnamate:** ¹H-NMR (CD₃OD, 400 MHz) δ: 7.63 (2 H, d, J = 7.6 Hz, H-2, 6), 6.84 (1 H, d, J = 12.6 Hz, H-7), 6.76 (2 H, d, J = 7.6 Hz, H-3, 5), 5.87 (1 H, d, J = 12.6 Hz, H-8), 3.07 (3 H, s, 9-O Me); ¹³C NMR (CD₃OD, 100 MHz) δ: 168.7 (C-9), 160.1 (C-4), 145.0 (C-7), 133.6 (C-2, 6), 127.6 (C-1), 116.3 (C-8), 115.8 (C-3, 5), 51.9 (9-O Me).

**Compound (5), Methyl trans-p-hydroxycinnamate:** ¹H-NMR (CD₃OD, 400 MHz) δ: 7.58 (1 H, dd, J = 15.8, 3.2 Hz, H-2, 6), 7.43 (2 H, dd, J = 9.0, 2.3 Hz, H-2, 6), 6.79 (2 H, dd, J = 9.0, 2.3 Hz, H-3, 5), 6.28 (1 H, d, J = 15.8, 3.2 Hz, H-8), 3.36 (3 H, s, 9-O Me); ¹³C NMR (CD₃OD, 100 MHz) δ: 169.3 (C-9), 160.3 (C-4), 146.5 (C-7), 131.1 (C-2, 6), 127.1 (C-1), 116.8 (C-3, 5), 114.9 (C-8), 51.9 (9-O Me).

**Compound (6), p-Coumaric acid:** ¹H-NMR (CD₃OD, 400 MHz) δ: 7.62 (1 H, dd, J = 15.8, 5.0 Hz, H-8), 7.46 (2 H, dd, J = 8.5, 3.0 Hz, H-2, 6), 6.81 (2 H, dd, J = 8.5, 3.0 Hz, H-3, 5), 6.32 (1 H, J = 15.8, 5.0 Hz, H-7); ¹³C-NMR (CD₃OD, 100 MHz) δ: 172.1 (C-9), 161.1 (C-4), 146.2 (C-7), 131.1 (C-2, 6), 127.2 (C-1), 116.8 (C-3, 5), 115.9 (C-8).

**Compound (7), 2,3-Dihydroxypropyl 9Z,12Z-octadecadienoate:** ¹H-NMR (CD₃OD, 400 MHz) δ: 5.30 (4 H, m, H-9, 10, 12, 13), 4.15 (2 H, dd, J = 11.24, 5.2 Hz, H-1), 3.83 (1 H, m, H-2'), 3.56 (2 H, m, H-3), 2.82 (2 H, t, H-2'), 2.37 (2 H, m, H-11), 2.08 (2 H, m, H-8), 1.62 (2 H, m, H-3), 1.33 (14 H, m, -CH₂-), 0.99 (3 H, t, H-18); ¹³C-NMR (CD₃OD, 100 MHz) δ: 175.4 (C-1'), 132.7 (C-10'), 131.0 (C-12'), 129.2 (C-9'), 127.8 (C-13'), 71.7 (C-2), 66.4 (C-1), 64.1 (C-3), 34.9 (C-2'), 30.7 (C-7'), 30.3 (C-6'), 30.3 (C-15'), 28.1 (C-3'), 26.4 (C-8'), 26.4 (C-11'), 26.4 (C-14'), 26.0 (C-16'), 21.8 (C-17'), 14.7 (C-18').

**Compound (8), (+)-(6S,7aS)-Epilolide:** ¹H-NMR (CD₃OD, 400 MHz) δ: 5.72 (1 H, s, H-3), 4.19 (1 H, m, H-6), 2.42 (1 H, d, J = 14.0 Hz, H-7), 1.99 (1 H, dd, J = 14.4, 3.4 Hz, H-5), 1.74 (3 H, s, H-8), 1.70
Total phenolic (TP) and total flavonoid (TF) content

The TP contents were measured using the Folin–Ciocalteu method with some modifications.[19] Briefly, 0.5 mL of the extract (1 mg/mL in distilled water) was mixed thoroughly with 0.5 mL of 2 N Folin–Ciocalteu reagent for 5 min followed by the addition of 2 mL of 7% (w/v) sodium carbonate. The mixture was left to react at room temperature for 90 min and then its absorbance was measured at 750 nm. The TP contents were calculated using the calibration curve and were expressed as mg of GA equivalent (GAE) per gram dry weight. The TF contents were measured using the Davis method.[20] The extract (0.2 mL), 0.5 mL of 1 N NaOH, and 4 mL of diethylene glycol were mixed in a 15-mL conical tube and allowed to stand at 37°C for 1 h. The absorbance values were measured at 420 nm. Standard curves were prepared using NG standard solutions (5, 10, 50, 100, and 500 µg/mL). The TF contents were expressed as the mg of NG equivalent (NGE) per gram dry weight.

DPPH radical scavenging assay

DPPH radical scavenging activities of the extract, solvent fraction, and isolated compounds were investigated using the method of Floegel et al.[21] with some modifications. First, 100-µL samples of
appropriate concentration (1.0 mg/mL) were dispensed in 96-well plates and mixed with 100 μL of 0.4 mM DPPH solution. The plates were then left to react in the dark for 10 min, and their absorbance values were measured at 517 nm. The radical scavenging activity was calculated as follows: DPPH radical scavenging rate (%) = \( (A_{\text{control}} - A_{\text{sample}})/A_{\text{control}} \times 100 \), where \( A_{\text{control}} \) is the absorbance of the control (DPPH + methanol) and \( A_{\text{sample}} \) is the absorbance of the sample (DPPH + sample). The antioxidant activity was expressed as the \( IC_{50} \) value, where \( IC_{50} \) refers to the amount of antioxidant needed to reduce the initial radical concentration by 50%. BHA and ascorbic acid were used as reference compounds.

**Cell culture**

The Raw264.7 murine macrophage cell line was obtained from the Korea Cell Line Bank (Seoul, South Korea). The cells were cultured in 1% penicillin/streptomycin (PS)/DMEM containing 10% FBS at 37°C in a 5% CO₂ incubator.

**MTT assay**

Cell viability and cytotoxicity were determined by the MTT cell viability assay. The cells were seeded at a density of \( 3 \times 10^3 \) cells/well into a 96-well flat-bottom cell culture plate. After 24 h of incubation, the extract was added to the cell culture plate and then after 1 h, it was cultured with LPS for 24 h. Mitochondrial enzyme activity, which is an indirect measure of the number of viable resiping cells, was determined using the MTT reagent after 24 h of treatment with the extracts. The absorbance was read using a microplate reader (BioTek Instruments Inc., Winooski, VT, USA) at 595 nm. The effect of extracts on the cell viability was evaluated as the relative absorbance compared with that of control cultures.

**Nitrite production assay**

The amount of nitrite produced was determined by a colorimetric assay. Briefly, 100 μL of the cell culture medium were mixed with an equal volume of Griess reagent (1% sulfanilamide and 0.1% naphthyl ethylene diamine in 5% phosphoric acid) and incubated for 10 min. The absorbance at 540 nm was recorded using a microplate reader (Bio-Tek Instruments Inc., Winooski, VT, USA). The nitrite concentration was determined by extrapolating the sodium nitrite standard curve.

**Statistical analysis**

All experiments were conducted in triplicate. The results are expressed as the mean±standard deviation (SD). Values in the same column were measured using one-way ANOVA with Duncan’s Post-Hoc test. Differences were considered statistically significant at \( p < .05 \). Associations between phenolic contents and radical scavenging activities were assessed using the Spearman rank correlation coefficient. All statistical analyses were performed using the SPSS software (ver. 18.0; SPSS Inc., Chicago, IL, USA).

**Results and discussion**

**Phenolic contents and phytochemicals**

Solvent fractionation was sequentially performed with \( n \)-hexane, CHCl₃, EtOAc, and BuOH fractions to separate the phenolic compounds from *S. quelpaertensis* leaves. Their total phenol and flavonoid contents were then measured. As shown in Table 1, the EtOAc fraction (95.0 mg GAE/g) showed the highest phenol content, followed by the BuOH (83.4 mg GAE/g), CHCl₃ (48.2 mg GAE/g), SQE
Table 1. Total phenol contents, total flavonoid contents, and DPPH radical scavenging activity of the extract and fractions obtained from *Sasa quelpaertensis*.

| Sample  | Total phenol contents (mg GAE/g) | Total flavonoid contents (mg NGE/g) | DPPH radical scavenging activity (IC_{50} μg/mL) |
|---------|----------------------------------|-------------------------------------|-----------------------------------------------|
| SQE     | 45.6 ± 1.6^a                      | 93.1 ± 5.0^b                        | 246.8 ± 12.3^b                                |
| n-Hexane| 13.9 ± 0.5^b                      | 90.5 ± 4.4^b                        | 613.6 ± 5.8^b                                 |
| CHCl₃   | 48.2 ± 0.5^b                      | 143.5 ± 1.1^b                       | 152.6 ± 1.6^c                                 |
| EtOAc   | 95.0 ± 1.0^d                      | 226.4 ± 6.2^c                       | 42.9 ± 2.9^d                                  |
| BuOH    | 83.4 ± 0.2 ± 2.3^c                | 262.4 ± 4.1^d                       | 60.5 ± 2.2^e                                  |
| BHA     | -                                | -                                   | 5.5 ± 0.4                                     |
| Ascorbic acid | -                        | -                                   | 50.1 ± 3.1                                    |

Each value is the mean±standard deviation of triplicate determinations. Values in the same column (within the same compound) with different superscript letters (a-e) are significantly different (p < 0.05) as measured by Duncan’s Post-Hoc test. The total phenol content was measured using gallic acid as a standard compound. IC_{50} values were calculated from regression lines using four different concentrations in triplicate experiments. GAE, gallic acid equivalent; NGE, naringenin equivalent; SQE, 70% ethanol extract of *S. quelpaertensis*; CHCl₃, chloroform fraction; EtOAc, ethyl acetate fraction; BuOH, n-butanol fraction.

(45.6 mg GAE/g), and n-hexane fractions (13.9 mg GAE/g). The BuOH fraction has the highest TF content (262.4 mg NGE/g), followed by the EtOAc (226.4 mg NGE/g), CHCl₃ (143.5 mg NGE/g), SQE (93.1 mg NGE/g), and n-hexane fractions (90.5 mg NGE/g). We isolated 13 phenolic compounds from CHCl₃, EtOAc, and BuOH fractions. Among these, six compounds – p-hydroxybenzaldehyde (1), p-coumaric acid (6), naringenin (10), 3-O-p-coumaroyl-1-(4-hydroxy-3,5-dimethoxyphenyl)-1-O-β-glucopyranosylpropanol (11), tricin (12), and tricin 7-O-b-D-glucopyranoside (13) – have been previously reported.\textsuperscript{[22–28]} The remaining seven – salicylic acid (2), syringaldehyde (3), methyl cis-p-hydroxycinnamate (4), methyl *trans*-p-hydroxycinnamate (5), 2,3-dihydroxypropyl 9Z,12Z-octadecadienoate (7), (−)-(6S,7aS)-epilolide (8), (−)-(6 R,7aS)-loliolide (9) – were first identified from the *Sasa* species (Figure 1).

These seven compounds were identified from \textsuperscript{1}H and \textsuperscript{13}C NMR spectra analyses and confirmed to be consistent with the data reported in the literature.\textsuperscript{[22–28]} The \textsuperscript{1}H-NMR spectrum of compound 2 displayed resonance signals owing to aromatic protons at δ 7.67 (dd, J = 15.8, 2.3 Hz), 7.13 (dd, J = 9.0, 2.3 Hz), and 6.60 (2 H, dd, J = 15.8, 3.2 Hz). In contrast, in the \textsuperscript{13}C-NMR spectrum, two displayed a prominent resonance signal at δ_C 171.7 (C-7) for carbonyl carbon, a chemical shift of oxygen-bonded

![Figure 1. Chemical structures of the phytochemicals isolated from *Sasa quelpaertensis*.](image)
carbon at δ_C 162.8 (C-2), and an aromatic carbon signal at δ_C 115.6 (C-3), 115.9 (C-1), 129.9 (C-6), and 131.3 (C-4).

The 1H-NMR spectrum of compound 3 displayed a characteristic resonance signal at δ 3.95 (6 H, s, 3, 5-OMe) for methoxy proton and a signal at δ 9.77 (1 H, s, H-7) for aldehyde proton. The 13C-NMR spectrum of compound 3 presented signals compatible with the 1,3,4,5-tetrasubstituted aromatic ring with chemical and magnetic equivalence. The aldehyde carbonyl was found at δ_C 192.9 (C-7), two methine carbons at δ_C 108.3 (C-2, 6), and a doublet signal for two methoxy carbons at δ_C 56.4 (3, 5-OMe).

The 1H-NMR spectrum of compound 4 presented a methoxy group at 3.07 (3 H, s, 9-Ome), two double bond signals of benzene at 7.64 (2 H, dd, J = 9.0, 2.3 Hz, H-2, 6) and 6.78 (2 H, dd, J = 9.0, 2.3 Hz, H-3, 5), and two cis-olefin signals at 6.84 (1 H, d, J = 12.6 Hz, H-7) and 5.87 (1 H, d, J = 12.6 Hz, H-8). The 13C-NMR spectrum of compound 3 showed a methoxy group at δ_C 51.9 (9-Ome) and a carbonyl group δ_C 168.7 (C-9). A couple of characteristic signals at δ_C 115.8 (C-3, 5) and 133.6 (C-2, 6) confirmed the AA′/BB′ form. The 1H and 13C-NMR spectra of compound 5 were similar to those of compound 4. However, the 1H-NMR spectrum of compound 5 has a coupling constant of 15.8 Hz at δ 7.58 (1 H, dd, J = 15.8, 3.2 Hz, H-7) and δ 6.28 (1 H, d, J = 15.8, 3.2 Hz, H-8) for the trans-olefin type composition.

The 1H-NMR spectrum of compound 7 presented signals of two pairs of coupled protons, which should be assigned to two methylene groups (δ 5.30) and an aliphatic chain (δ 0.99, 1.33, 1.62, 2.08, 2.37, 2.82) attached to a chiral carbon structure at δ 3.56 (2 H, m, H-3), 3.83 (1 H, m, H-2′), and 4.13 (2 H, dd, J = 11.24, 5.2 Hz, H-1). The 13C-NMR spectrum of compound 7 displayed a signal at 175.4 (C-1′) for the ester carbonyl group and four olefinic carbons at δ_C 127.8 (C-13′), 129.2 (C-9′), 131.0 (C-12′), and 132.7 (C-10′). Additionally, 12 methylene carbons (δ_C 21.8–34.9) and a methyl carbon (δ_C 14.7) were confirmed from the 13C-NMR spectrum.

The NMR spectra of compounds 8 and 9 showed that these two compounds have an enantiomeric structure. Their 1H-NMR spectrum presented methyl signals at δ 1.25 (3 H, s, H-10), 1.44 (3 H, s, H-9), and 1.74 (3 H, s, H-8) and δ 1.30 (6 H, s, H-9, 10, 5), and 1.65 (3 H, s, H-8), respectively. Compound 8 showed a carbinol and two methylene signals at δ 1.53 (1 H, dd, J = 14.4, 3.4 Hz, H-5), 1.70 (1 H, d, J = 14.0 Hz, H-7), 1.99 (1 H, dd, J = 14.4, 3.4 Hz, H-5), and 2.42 (1 H, d, J = 14.0 Hz, H-7). A proton peak involved in one double bond was confirmed at δ 5.72 (1 H, s, H-3). The 13C-NMR spectrum of compound 8 showed an α,β-unsaturated-γ-lactone group at δ_C 113.7 (C-3), 174.0 (C-2), and 185.9 (C-3a) and two oxygenated carbons signal at δ_C 65.2 (C-6) and 88.5 (C-7a). Therefore, it was estimated that compound 8 is a bicyclic structure composed of an α,β-unsaturated-γ-lactone group, a carbinol carbon, two methylene carbons, and three tertiary methyl groups. The signal patterns of compound 9 were also similar to those of compound 8. It was presented that the 13C-NMR spectrum is composed of groups, including an α,β-unsaturated-γ-lactone group, carbinol carbon, two methylene carbons, and three tertiary methyl groups. Therefore, compound 9 was identified as (−) (6 R,7aS)-lolilide and was confirmed enantiomer of compound 8 by comparison with literature.[28]

Antioxidant activities of the fractions and phytochemicals

Both SQE and all solvent fractions were evaluated for antioxidant activities using DPPH free radical scavenging assay (Table 1). The EtOAc fraction showed the strongest DPPH free radical scavenging activity (IC₅₀ = 42.9 µg/mL), followed by BuOH (IC₅₀ = 60.5 µg/mL), CHCl₃ (IC₅₀ = 152.6 µg/mL), SQE (IC₅₀ = 246.8 µg/mL), and n-hexane (IC₅₀ = 613.6 µg/mL). Previous studies have shown a high correlation between the phenolic compounds and the antioxidant capacity.[29,30] In this study, the relationship between the phenolic compounds of the extract and radical scavenging reactions (IC₅₀) is evaluated through Spearman rank correlation analysis, and a correlation coefficient value of −0.916 was obtained, which confirmed the significance at the 0.05 level. These results showed that phenolic compounds had a strong and close correlation with the DPPH free radical scavenging activity and that phenolic compounds are major contributors to the antioxidant capacity of S. quelpaertensis. Thus, the antioxidant activities of the
isolated 13 phenols can also be used to investigate the DPPH free radical scavenging assay. Compounds 11 and 13 showed DPPH free radical scavenging activity in a concentration-dependent manner. BHA, which was used as a control group to evaluate the antioxidant effects of the SQE, showed 66.3% radical scavenging activity at 100 μM, while compounds 11 and 13 showed 43.7% and 16.6% radical scavenging activity, respectively (Figure 2). These results show that compound 11 is a potential antioxidant for BHA.

Nitrogen oxides can induce an oxidative reaction in the human body and may be responsible for diseases such as cancer, arthritis, and neurodegenerative disorders. Therefore, NO-production-inhibitory activity of the fractions was evaluated in LPS-stimulated RAW 264.7 macrophage cells. The SQE significantly inhibited NO production at concentrations above 62.5 mg/mL. No cytotoxicity was observed at any concentration level (Figure 3a). All fractions also inhibited NO production in a concentration-dependent manner (Figure 3b–e). The CHCl₃ fraction showed the most potent NO-inhibition activity, followed by ethyl acetate, n-hexane, SQE, and n-butanol fractions. However, n-hexane and chloroform fractions showed cytotoxicity at a concentration of 250 ng/mL. Correlation analysis between each sample and inhibition of NO production was performed by Spearman rank correlation analysis. Unlike the high correlation between the antioxidant activity and the phenolic compounds, a low correlation of −0.641 (p < .05) was observed for the inhibitory activity of NO production on LPS-stimulated RAW 264.7 cells. For an in-depth evaluation of these results, more studies should be performed on the complex mechanism between phenol components and NO production in cells. The inhibitory effect of NO production in LPS-stimulated cells was confirmed for the 13 compounds isolated from S. quelpaertensis (Figure 4). Compounds 4, 7, 11, and 13 showed NO-production-inhibitory effect under experimental conditions. Compound 7 not only reduced NO production in a concentration-dependent manner but also showed the strongest NO-production-inhibitory effect in LPS-stimulated RAW 264.7 macrophages. The isolated compounds did not show any cytotoxicity at all concentrations tested in the MTT assay.

**Conclusion**

This study provides good evidence that extracts and fractions, as well as the phenolic compounds isolated from S. quelpaertensis, can be used as antioxidants, and further suggested two available DPPH free radical inhibitors (compound 11 and 13) and four NO-production inhibitors (compound 4, 7, 11, 13). This is the first report on the NO-inhibition activity of 2,3-dihydroxypropyl 9Z,12Z-
octadecadienoate derived from *S. quelpaertensis*. These results can be used as the basic data for developing effective antioxidant components from *S. quelpaertensis*. However, further studies are needed to investigate the chemical mechanism and synergistic effects of the relation between the antioxidant activity and phenolic compounds.

**Abbreviations**

*Sasa quelpaertensis* extract

SQE

Chloroform

CHCl₃
Figure 4. Effect of isolated four compounds (compounds 4, 7, 11, and 13) on NO production in LPS-stimulated RAW 264.7 cells. Cells were incubated with the indicated concentrations (0, 6.25, 12.5, 25, 50, and 100 μM) of each compound for 1 h, and then incubated with LPS (100 ng/mL) for 24 h. The data represent the mean±standard deviation of determination values. (a) compound 4; (b) compound 7; (c) compound 11; (d) compound 13.

Ethyl acetate
EtOAc
n-Butanol
BuOH

High-performance liquid chromatography
HPLC

Nitric oxide
NO

Nuclear magnetic resonance
NMR

2,2-Diphenyl-1-picrylhydrazyl
DPPH

Butylated hydroxyanisole
BHA

Total phenolic
TP

Total flavonoid
TF
Gallic acid equivalent
GAE

Naringin equivalent
NGE

**Disclosure statement**

The authors declare that they have no conflict of interest.

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**References**

[1] Son, S. M. Reactive Oxygen and Nitrogen Species in Pathogenesis of Vascular Complications of Diabetes. *Diabet. Metabol. J.* 2012, 36, 190–198. DOI: 10.4093/dmj.2012.36.3.190.

[2] Del Rio, L. A. ROS and RNS in Plant Physiology: An Overview. *J. Exp. Bot.* 2015, 66, 2287–2837. DOI: 10.1093/jxb/jrv251.

[3] Di Meo, S.; Reed, T. T.; Venditti, P.; Victor, V. M. Role of ROS and RNS Sources in Physiological and Pathological Conditions. *Oxid. Med. Cell. Longev.* 2016, Article 1245049. DOI: 10.1155/2016/1245049.

[4] Yan, L. J. Positive Oxidative Stress in Aging and Aging-related Disease Tolerance. *Redox Biol.* 2014, 2, 165–169. DOI: 10.1016/j.redox.2013.11.001.

[5] Mozos, I.; Luca, C. T. Crosstalk between Oxidative and Nitrosative Stress and Arterial Stiffness. *Curr. Vasc. Pharmacol.* 2017, 15, 446–456. DOI: 10.2174/1570161115666170201115428.

[6] Butterfield, D. A.; Boyd-Kimball, D. Mitochondrial Oxidative and Nitrosative Stress and Alzheimer Disease. *Antioxidants.* 2020, 9, 818. DOI: 10.3390/antiox9090818.

[7] Lourence, S. C.; Moldao-Martins, M.; Alves, V. D. Antioxidants of Natural Plant Origins: From Sources to Food Industry Applications. *Molecules.* 2019, 24, 4132. DOI: 10.3390/molecules24224132.

[8] Xu, D. P.; Li, Y.; Meng, X.; Zhou, T.; Zhou, Y.; Zheng, J.; Zhang, J. J.; Li, H. B. Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *Int. J. Mol. Sci.* 2017, 18, 96. DOI: 10.3390/ijms18010096.

[9] de la Rosa, L. A.; Moreno-Escamilla, J. O.; Rodrigo-Garcia, J.; Alvarez-Parrilla, E. Phenolic Compounds Chapter 12. Postharvest Physiology and Biochemistry of Fruits and Vegetables; Yahia, E. M., Eds.; Woodhead Publishing, 2019, 253–271. doi:10.1016/B978-0-12-813278-4.00012-9.

[10] Kasote, D. M.; Katypare, S. S.; Hegde, M. V.; Bae, H. Significance of Antioxidant Potential of Plants and Its Relevance to Therapeutic Applications. *Int. J. Bio. Sci.* 2015, 11, 982–991. DOI: 10.7150/ijbs.12096.

[11] Fiedor, J.; Burda, K. Potential Role of Carotenoids as Antioxidants in Human Health and Disease. *Nutrients.* 2014, 6, 466–488. DOI: 10.3390/nu6020466.

[12] Wagner, H.; Ulrich-Merzenich, G. Synergy Research: Approaching a New Generation of Phytopharmaceuticals. *Phytotherapy.* 2009, 16, 97–110. DOI: 10.1016/j.phymed.2008.12.018.

[13] Zhang, Q. W.; Lin, L.; Ye, W. Techniques for Extraction and Isolation of Natural Products: A Comprehensive Review. *Chin. Med.* 2018, 13, 20. DOI: 10.1186/s13020-018-0177-x.

[14] Sonam, K. S.; Guleria, S. Synergistic Antioxidant Activity of Natural Products. *Ann. Pharmacol. Pharmaceutic.* 2017, 2, Article 1086.

[15] Jang, M. G.; Ko, H. C.; Kim, S. J. Effects of P-coumaric Acid on Micro RNA Expression Profiles in SNU-16 Human Gastric Cancer Cells. *Genes Genomics.* 2020, 42, 817–825. DOI: 10.1007/s13258-020-00944-6.

[16] An, S. M.; Koh, J. S.; Boo, Y. C. p-Coumaric Acid Not Only Inhibits Human Tyrosinase Activity in Vitro but Also Melanogenesis in Cells Exposed to UVB. *Phytotherapy Res.* 2010, 24, 1175–1180. DOI: 10.1002/ptr.3095.
[17] Sultana, N.; Lee, N. H. New Phenylpropanoids from Sasa Quelpaertensis Nakai with Tyrosinase Inhibition Activities. *Bull. Korean Chem. Soc.* 2009, 30, 1729–1732. DOI: 10.5012/bkcs.2009.30.8.1729.

[18] Ko, H. C.; Lee, J. Y.; Jang, M. G.; Song, H.; Kim, S. J. Seasonal Variations in the Phenolic Compounds and Antioxidant Activity of Sasa Quelpaertensis. *Ind. Crops Prod.* 2018, 122, 506–512. DOI: 10.1016/j.indcrop.2018.06.031.

[19] Javanmardi, J.; Stushnoff, C.; Locke, E.; Vivanco, J. M. Antioxidant Activity and Total Phenolic Content of Iranian Ocimum Accessions. *Food Chem.* 2003, 83, 547–550. DOI: 10.1016/S0308-8146(03)00151-1.

[20] Birasuren, B.; Kim, N. Y.; Jeon, H. L.; Kim, M. R. Evaluation of the Antioxidant Capacity and Phenolic Content of Agriophyllum Pungens Seed Extracts from Mongolia. *Prevent. Nutrit. Food Sci.* 2013, 18, 188–195. DOI: 10.3746/pnf.2013.18.3.188.

[21] Floegel, A.; Kim, D. O.; Chung, S. J.; Koo, S. I.; Chun, O. K. Comparison of ABTS/DPPH Assays to Measure Antioxidant Capacity in Popular Antioxidant-rich US Foods. *J. Food Compost. Anal.* 2011, 24, 1043–1048. DOI: 10.1016/j.jfca.2011.01.008.

[22] Shing, Y. G.; Yoon, S. H. New One-pot Synthesis of 4-hydroxybenzaldehyde Derivatives and Picric Acid from 4-hydroxyphenylglycine with HNO_3/H_2O. *Bull. Korean Chem. Soc.* 2009, 30, 2819–2822. DOI: 10.5012/bkcs.2009.30.11.2819.

[23] Duan, W.; Pan, S.; Yu, Z.; Wang, X.; Li, J.; Zhang, Y. Studies on Chemical Constituents of Twigs of Trichosanthes Kirilowii Maxim. *Asian J. Chem.* 2015, 8, 2756–2758. DOI: 10.14233/ajchem.2015.18021.

[24] Panyo, J.; Matsumani, K.; Panichayupakaranant, P. Bioassay-guided Isolation and Evaluation of Antimicrobial Compounds from Ixora Megalophylly against Some Oral Pathogens. *Pharm. Biol.* 2016, 54, 1522–1527. DOI: 10.3109/13880209.2015.1107106.

[25] Kwon, Y. S.; Kim, C. M. Antioxidant Constituents from the Stem of Sorghum Bicolor. *Arch. Pharmacal Res.* 2003, 26, 535–539. DOI: 10.1007/BF02976877.

[26] Sultana, N.; Lee, N. H. A New Alkene Glycoside from Sasa Quelpaertensis Nakai. *Bull. Korean Chem. Soc.* 2010, 31, 1088–1090. DOI: 10.5012/bkcs.2010.31.04.1088.

[27] Chung, W.; Goo, Y. M.; Na, D. S.; Kim, K. J. A Phospholipase A2 Inhibitor from Arisaema Amurense Max. Var. Serratum Nakai. *Rapid Commun. Chem.* 1995, 18, 293–294. DOI: 10.1007/BF02976416.

[28] Park, K. E.; Kim, Y. A.; Jung, H. A.; Lee, H. J.; Ahn, J. W.; Lee, B. I.; Seo, Y. Three Norisoprenoids from the Brown Alga Sargassum Thunbergii. *J. Korean Chem. Soc.* 2004, 48, 394–398. DOI: 10.5012/jkcs.2004.48.4.394.

[29] Huang, W. Y.; Zhang, H. C.; Liu, W.; Li, C. Y. Survey of Antioxidant Capacity and Phenolic Composition of Blueberry, Blackberry, and Strawberry in Nanjing. *J. Zhejiang Univ. Sci. B.* 2012, 13, 94–102. DOI: 10.1631/jzus.B1100137.

[30] Zhao, H.; Zhang, H. S.; Yang, S. F. Phenolic Compounds and Its Antioxidant Activities in Ethanolic Extracts from Seven Cultivars of Chinese Jujube. *Food Sci. Hum. Wellness.* 2014, 3, 183–190. DOI: 10.1016/j.fshw.2014.12.005.

[31] Phaniendra, A.; Jestadi, D. B.; Periyasamy, L. Free Radicals: Properties, Sources, Targets, and Their Implication in Various Diseases. *Indian J. Clin. Biochem.* 2015, 30, 11–26. DOI: 10.1007/s12291-014-0446-0.

[32] Castaneda, O. A.; Lee, S. C.; Ho, C. T.; Huang, T. C. Macrophages in Oxidative Stress and Models to Evaluate the Antioxidant Function of Dietary Natural Compounds. *J. Food Drug Anal.* 2017, 25, 111–118. DOI: 10.1016/j.jfda.2016.11.006.