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Characterization of flavonoid and quinic acid derivatives from *Calystegia soldanella* with antioxidant activities

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Abstract

Calystegia soldanella is a species broadly used in an edible source and traditional herb in South Korea and China. It has diverse bioactivities, such as dropsy, antipyretic, and diuretic effect. In this study, we investigated the main components with antioxidant activities from the 70% EtOH extract of *C. soldanella*. The structural determination of compounds was achieved by nuclear magnetic resonance and HR-ESI-MS spectroscopic data. The main components from *C. soldanella* were identified as flavonoids and quinic acid derivatives. Furthermore, the 70% EtOH extract, partitioned layers, and compounds (1–8) of *C. soldanella* were evaluated for their antioxidant effects. The most active compound 8 (isochlorogenic acid C) exhibited the IC_{50} values of 12.4 ± 0.1 (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical) and 37.9 ± 0.5 μM (2,2-diphenyl-1-picrylhydrazyl radical), respectively. Two active compounds (7 and 8) displayed higher activities than ascorbic acid (positive control). In addition, we also confirmed the antioxidant effects on the intracellular reactive oxygen species production of compound 8. Therefore, the present study suggests extract of *C. soldanella* is a promising natural antioxidative candidate.

Keywords: *Calystegia soldanella*, Anti-oxidants, Flavonoid, Quinic acid

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Introduction

Oxidative stress is related with the cause of several diseases and decreasing oxidative stress is also essential role to maintain human health (Pizzino et al., 2017). It is normally caused by the accumulation of reactive oxygen species (ROS) including superoxide, hydrogen peroxide, and free radicals. In particular, free radicals are very reactive and unstable (Dong et al., 2022; Halliwell, 2022; Yang & Lian, 2020). These radicals can trigger structural instability to receive and donate electrons in normal molecules. The accumulation of free radicals induces a disparity between the ROS and anti-oxidant level. They damage the cellular macromolecules, lipids, gene expression pathways, and nucleic acid (Schieber et al., 2014; Simioni et al., 2018; Tryfidou et al., 2020). Consequently, several harmful effects according to accumulation of ROS level are highly related to various diseases including diabetes, asthma, atherosclerosis, pulmonary diseases, neurodegenerative diseases, and caner (Batty et al., 2022; Kim et al., 2015; Nakamura & Takada, 2021; Qu et al., 2017; Taniguchi et al., 2021; Volpe et al., 2018). Antioxidants, which are known free radical scavengers, are important constituents to protect the oxidation. It plays critical role in removing or altering the oxidative stress related to diverse free radicals. Halophytes are known to produce the various biological secondary metabolites due to salt-tolerance condition (Patel et al., 2022; Ravi et al., 2020). Therefore, halophytes are rich sources of natural antioxidant. The halophytes are comprised with about 72 species in South Korea (Lee et al., 2019). *Calystegia soldanella* is a perennial plant which generally grows with a procumbent stem (50–100 cm long) and reniform-shaped leaves. They are distributed with temperate regions (South Korea, Japan, and Europe). The root of *C. soldanella* have been treated as edible and medicinal herbs to cure the dropsy, antipyretic, and diuretic effects (Eom et al., 2021; Lee et al., 2017). In previous papers, the chemical constituents of *C. soldanella* have been known to possess diverse type chemical skeletons (resin glycosides, flavonoids, caffeic ester, and courmaric acid esters) and diverse bioactivities including anti-diabetic, anti-cancer, anti-viral, and anti-inflammatory activities (Lee et al., 2014; Murai et al., 2015; Ono et al., 2014; Tori et al., 2010). In the current study, we reported the chemical characterization of flavonoid and quinic acid derivatives (1–8) from the aerial parts of *C. soldanella* with antioxidant activities.

Materials and Methods

General experimental section

The nuclear magnetic resonance (NMR) spectra were generated using Varian VNMRS 500 MHz FT-NMR spectrometer (Varian, Palo Alto, CA, USA). The high-performance liquid chromatography (HPLC; Waters, Miliford, MA, USA) with a PDA detector and C_{18} column (Cosmosil, Kyoto, Japan) was analyzed using extract and isolated compounds. MPLC (Biotage, Uppsala, Sweden) and preparative-HPLC (Shimadzu, Kyoto, Japan) were performed to isolation and purification. The HPLC of elution solvents were used for the HPLC grade (ACN, Water, MeOH, J. T. Baker®, Phillipsburg, NJ, USA) and Mass elution solvents were used by mass grade (ACN, MeOH, Supelco, Bellefonte, PA, USA). The HR-ESI-MS spectra were measured on a SCIEX X500R Q-TOF LC-MS/MS spectrometer (SCIEX, Framingham, MA, USA).

Plant material

Aerial parts of *C. soldanella* were collected from Ujeong-eup, Hwaseong-si, Gyeonggi-do, Korea in September 2019. A voucher specimen (NP-0682) was kept in the National Biodiversity institute of Korea.

Extraction and isolation

The aerial parts of *C. soldanella* (150 g) were cut and extracted three times with 70% EtOH at room temperature for 8 h. The 70% EtOH extract was successively partitioned with n-hexane, chloroform, ethyl acetate, and n-butanol. The most active portion (n-BuOH-soluble layer) was chromatographed by MPLC eluting with an increasing MeOH in water to give seven fractions (S1–S7). The mixed fraction was further followed by Sephadex LH-20 column chromatography (eluent: MeOH) to yield two compounds 5 (27.6 mg), 6 (2.7 mg), and nine subfractions (S1–S9). The subfraction 5 was finally isolated using preparative-HPLC with increasing solvents (2:8/MeOH:Water → 5:5/MeOH:Water) to obtain four compounds 1 (9.6 mg), 2 (1.2 mg), 3 (2.9 mg), and 4 (14.5 mg). The subfraction 8 was further divided with preparative-HPLC eluted with increasing solvents (3:7/MeOH:Water → 65:35/MeOH:Water) in water to obtain two compounds 7 (5.0 mg) and 8 (3.7 mg).

Chlorogenic acid (1): HR-ESI-MS (negative) *m/z* 353.0878 $[M-H]^-$, calcd for $C_{16}H_{17}O_9$ 353.0873; ^1H NMR (500 MHz, CD_3OD): δ7.56 (1H, d, *J* = 15.9 Hz, H-7'), 7.05 (1H, d, *J* = 1.8 Hz, H-2'), 6.95 (1H, dd, *J* = 8.2, 1.8 Hz, H-6'), 6.77 (1H, d, *J* = 8.2 Hz, H-5'), 6.27 (1H, d, *J* = 15.9 Hz, H-8'), 5.34 (1H, m,

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H-3), 4.17 (1H, m, H-5), 3.73 (1H, m, H-4), 2.20/2.06 (2H, m, H-2), 2.18/2.05 (2H, m, H-6); ^{13}C NMR (125 MHz, CD_3OD): δ177.5 (C-7), 168.8 (C-9'), 149.7 (C-4'), 147.2 (C-7'), 146.9 (C-3'), 127.9 (C-1'), 123.1 (C-6'), 116.5 (C-5'), 115.3 (C-8'), 115.2 (C-2'), 76.4 (C-1), 73.7 (C-4), 72.1 (C-3), 71.6 (C-5), 39.0 (C-6), 38.4 (C-2) (Supplementary Figs. S1-S3).

Cryptochlorogenic acid (2): HR-ESI-MS (negative) *m/z* 353.0880 $[M-H]^-$, calcd for $C_{16}H_{17}O_9$ 353.0873; ^{1}H NMR (500 MHz, CD_3OD): δ7.63 (1H, d, *J* = 15.9 Hz, H-7'), 7.07 (1H, d, *J* =1.7 Hz, H-2'), 6.97 (1H, dd, *J* = 8.1, 1.7 Hz, H-6'), 6.78 (1H, d, *J* = 8.1 Hz, H-5'), 6.37 (1H, d, *J* = 15.9 Hz, H-8'), 4.86 (1H, m, overlap, H-3), 4.30 (1H, m, H-4), 4.21 (1H, m, H-2), 2.14-2.02 (4H, m, H-1 and 5); ^{13}C NMR (125 MHz, CD_3OD): δ177.4 (C-7), 169.0 (C-9'), 149.7 (C-4'), 147.2 (C-7'), 146.9 (C-3'), 128.0 (C-1'), 123.1 (C-6'), 116.6 (C-5'), 115.5 (C-8'), 115.2 (C-2'), 78.9 (C-3), 75.9 (C-6), 69.3 (C-4), 66.4 (C-2), 39.2 (C-5), 38.7 (C-1) (Supplementary Figs. S4-S7).

5-O-(*E*)-*p*-coumaroylquinic acid (3): HR-ESI-MS (negative) *m/z* 337.0924 $[M-H]^-$, calcd for $C_{16}H_{17}O_8$ 337.0923; ^{1}H NMR (500 MHz, CD_3OD): δ7.63 (1H, d, *J* = 15.9 Hz, H-7'), 7.46 (2H, d, *J* = 8.5 Hz, H-2' and H-6'), 6.80 (2H, d, *J* = 8.5 Hz, H-3' and H-5'), 6.34 (1H, d, *J* = 15.9 Hz, H-8'), 5.34 (1H, m, H-3), 4.16 (1H, m, H-5), 3.72 (1H, dd, *J* = 8.5, 2.6 Hz), 2.22-2.02 (4H, m, H-2 and H-6); ^{13}C NMR (125 MHz, CD_3OD): δ179.2 (C-7) 168.8 (C-9'), 161.4 (C-4'), 146.7 (C-7'), 131.3 (C-2' and 6'), 127.3 (C-1'), 116.9 (C-3' and 5'), 115.5 (C-8'), 75.8 (C-1), 73.9 (C-4), 72.3 (C-3), 71.9 (C-5), 39.3 (C-2), 38.5 (C-6) (Supplementary Figs. S8-S11).

Rutin (4): HR-ESI-MS (negative) *m/z* 609.1449 $[M-H]^-$, calcd for $C_{27}H_{29}O_{16}$ 609.1456; ^{1}H NMR (500 MHz, CD_3OD): δ7.67 (1H, d, *J* = 1.9 Hz, H-2'), 7.63 (1H, dd, *J* = 8.4, 2.0 Hz, H-6'), 6.87 (1H, d, *J* = 8.4 Hz, H-5'), 6.38 (1H, d, *J* = 1.8 Hz, H-8), 6.20 (1H, d, *J* = 1.8 Hz, H-6), 5.11 (1H, d, *J* = 7.6 Hz, H-1"), 4.52 (1H, d, *J* = 1.0 Hz, H-1'"), 3.81/3.39 (2H, m, H-5"), 3.64 (1H, m, H-2'"), 3.55 (1H, m, H-3'"), 3.47 (1H, m, H-2"), 3.45 (1H, m, H-5'"), 3.44 (1H, m, H-3"), 3.33 (1H, m, H-5"), 3.30 (1H, m, H-4'"), 3.29 (1H, m, H-4"), 1.12 (3H, d, *J* = 6.2 Hz, H-6'"); ^{13}C NMR (125 MHz, CD_3OD): δ179.5 (C-4), 166.1 (C-7), 163.0 (C-5), 159.4 (C-9), 158.6 (C-2), 149.9 (C-4'), 145.9 (C-3'), 135.7 (C-3), 123.6 (C-6'), 123.2 (C-1'), 117.8 (C-2'), 116.1 (C-5'), 105.7 (C-10), 104.8 (C-1"), 102.5 (C-1'"), 100.0 (C-6), 94.9 (C-8), 78.2 (C-3"), 77.3 (C-5"), 75.8 (C-2"), 74.0 (C-4'"), 72.3 (H-3'"), 72.2 (H-2'"), 71.5 (H-4"), 69.8 (H-5'"), 68.6 (H-6"), 18.0 (H-6'") (Supplementary Figs. S12-S14).

Isoquercetin (5): HR-ESI-MS (negative) *m/z* 463.0873 $[M-H]^-$, calcd for $C_{21}H_{19}O_{12}$ 463.0877; ^{1}H NMR (500 MHz, CD_3OD): δ7.71 (1H, brs, H-2'), 7.57 (1H, d, *J* = 7.3 Hz, H-6'), 6.86 (1H, d, *J* = 7.3 Hz, H-5'), 6.36 (1H, brs, H-8), 6.17 (1H, brs, H-6), 5.25 (1H, d, *J* = 6.6 Hz), 3.72/3.59 (2H, m, H-6"), 3.46 (1H, m, H-2"), 3.44 (1H, m, H-3"), 3.35 (1H, m, H-4"), 3.25 (1H, m, H-5"); ^{13}C NMR (125 MHz, CD_3OD): δ179.5 (C-4), 166.1 (C-7), 163.0 (C-5), 159.0 (C-2), 158.5 (C-9), 149.5 (C-4'), 145.9 (C-3'), 135.7 (C-3), 123.2 (C-6'), 123.1 (C-1'), 117.6 (C-2'), 116.0 (C-5'), 105.7 (C-10), 104.4 (C-1"), 100.0 (C-6), 94.8 (C-8), 78.4 (C-5"), 78.1 (C-3"), 75.8 (C-2"), 71.5 (C-4"), 62.6 (C-6") (Supplementary Figs. S15-S17).

Nicotiflorin (6): HR-ESI-MS (negative) *m/z* 593.1501 $[M-H]^-$, calcd for $C_{27}H_{29}O15$ 593.1507; ^{1}H NMR (500 MHz, CD_3OD): δ8.07 (2H, d, *J* = 8.7 Hz, H-2' and H-6'), 6.89 (2H, d, *J* = 8.7 Hz, H-3' and H-5'), 6.40 (1H, brs, H-8), 6.21 (1H, brs, H-6), 5.13 (1H, d, *J* = 7.4 Hz, H-1"), 4.51 (1H, brs, H-1'"), 3.81/3.38 (2H, m, H-6"), 3.64 (1H, m, H-2'"), 3.52 (1H, m, H-3'"), 3.45 (1H, m, H-5'"), 3.44 (1H, m, H-2"), 3.42 (1H, m, H-3"), 3.33 (1H, m, H-5"), 3.29 (1H, m, H-4'"), 3.27 (1H, m, H-4"), 1.12 (3H, d, *J* = 6.2 Hz); ^{13}C NMR (125 MHz, CD_3OD): δ179.5 (C-4), 166.2 (C-7), 163.1 (C-5), 161.6 (C-4'), 159.5 (C-9), 158.6 (C-2), 135.6 (C-3), 132.5 (C-2' and C-6'), 122.8 (C-1'), 116.2 (C-3' and C-5'), 105.7 (C-10), 104.7 (C-1"), 102.5 (C-1'"), 100.1 (C-6), 95.0 (C-8), 78.2 (C-3"), 77.3 (C-5"), 75.9 (C-2"), 74.0 (C-4'"), 72.4 (C-3'"), 72.2 (C-2'"), 71.5 (C-4"), 69.8 (C-5'"), 68.7 (C-6"), 18.0 (C-6'") (Supplementary Figs. S18-S20).

Isochlorogenic acid A (7): HR-ESI-MS (negative) *m/z* 515.1188 $[M-H]^-$, calcd for $C_{25}H_{23}O_{12}$ 515.1190; ^{1}H NMR (500 MHz, CD_3OD): δ7.62 (1H, d, *J* = 16.0 Hz, H-3'), 7.58 (1H, d, *J* = 16.0 Hz, H-3"), 7.07 (2H, s, H-9' and H-9"), 6.96 (2H, m, H-5' and H-5"), 6.78 (2H, d, *J* = 8.0 Hz, H-6' and H-6"), 6.36 (1H, d, *J* = 16.0 Hz, H-2'), 6.27 (1H, d, *J* = 16.0 Hz, H-2"), 5.42 (1H, m, H-3), 5.40 (1H, m, H-5), 3.97 (1H, m, H-4), 2.32/2.15 (2H, m, H-2), 2.23/2.19 (2H, m, H-6); ^{13}C NMR (125 MHz, CD_3OD): δ177.8 (C-7), 169.0 (C-1'), 168.5 (C-1"), 149.7 (C-7'), 149.6 (C-7"), 147.3 (C-3'), 147.1 (C-3"), 146.9 (C-8' and C-8"), 128.0 (C-4'), 127.9 (C-4"), 123.2 (C-5'), 123.1 (C-5"), 116.5 (C-6' and C-6"), 115.7 (C-2'), 115.3 (C-2"), 115.2 (C-9' and C-9"), 74.9 (C-1), 72.8 (C-3), 72.2 (C-5), 70.9 (C-4), 38.0 (C-2), 36.2 (C-6) (Supplementary Figs. S21-S23).

Isochlorogenic acid C (8): HR-ESI-MS (negative) *m/z* 515.1184 $[M-H]^-$, calcd for $C_{25}H_{23}O_{12}$ 515.1190; ^{1}H NMR (500 MHz, CD_3OD): δ7.60 (1H, d, *J* = 15.8 Hz, H-3'), 7.51 (1H, d, *J* = 15.8 Hz, H-3"), 7.01 (2H, d, *J* = 1.8 Hz, H-9' and H-9"), 6.91 (2H, brt, H-5' and H-5"), 6.74 (2H, d, *J* = 1.8 Hz, H-6' and H-6"), 6.29 (1H, d, *J* = 15.8 Hz, H-2'), 6.19 (1H, d, *J* = 15.8 Hz, H-2"), 5.62 (1H, m, H-5), 5.12 (1H, dd, *J* = 9.0, 2.8 Hz, H-4), 4.37 (1H, m, H-3),

2.22-2.05 (4H, m, H-2 and H-6); ^{13}C NMR (125 MHz, CD_3OD): δ177.3 (C-7), 168.6 (C-1'), 168.3 (C-1"), 149.8 (C-7' and C-7"), 147.8 (C-3'), 147.7 (C-3"), 146.9 (C-8' and C-8"), 127.8 (C-4'), 127.7 (C-4"), 123.2 (C-5' and C-5"), 116.5 (C-6' and C-6"), 115.2 (C-9' and C-9"), 114.8 (C-2' and C-2"), 76.4 (C-1), 76.0 (C-4), 69.6 (C-3), 69.2 (C-5), 39.6 (C-6), 38.5(C-2) (Supplementary Figs. S24-S26).

HR-UPLC-ESI-Q-TOF mass spectrometry profiles

The separation of main peaks was carried out by UPLC-ESI-Q-TOF mass spectrometry using a BEH C_{18} column (I.D 1.7 μm, 2.1 × 100 mm). The injection amount (10 μL) of samples was injected and eluted with the gradient solvent conditions of line A (water in 0.1% formic acid) and line C (ACN) with a flow rate at 0.4 mL/min. The gradient solvent was performed using the following protocol: (0–2 min/90:10, A:C), (2–35 min/90:10, A:C → 0:100, A:C), (35–38 min/0:100, A:C), (38–38.5 min/90:10, A:C), and (38.5–45 min/90:10, A:C). The mass spectrometer was operated in negative mode using the mass ranging from 100 to 2,000 Da, spray voltage at −4,500 V, declustering potential at −80 V, and collision energy at −25.

Measurement of ABTS and DPPH radical scavenging activities

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities were determined according to slightly modified methods. Simply, ABTS solution (7 mM) including potassium persulfate (2.45 mM) was dissolved in water with dark condition. The sample (10 μL) and ABTS solution (190 μL) were mixed and the absorbance was recorded at 734 nm. The dissolved DPPH solution and the sample were mixed and performed at ultraviolet (UV) absorbance at 517 nm (Kim et al., 2022, 2020).

Measurement of total polyphenol content (TPC) and total flavonoid content (TFC)

The total polyphenol content (TPC) in *C. soldanella* was measured using the Folin-Denis colorimetric method according to some modifications. Simply, samples (20 μL) were mixed with Folin-Denis agent (100 μL) for 1 min at 40℃. The mixtures were contained to a Na_2CO_3 solution and reacted for 15 min at 40℃. The reaction solutions were performed by the microplate reader using UV absorbance at 765 nm. The total flavonoid content (TFC) was recorded using an aluminum chloride colorimetric assay with slight modifications. The calibration curve was prepared by stock solution (5 mg/mL) and then diluted to concentrations ranging from 31.25–500 μg/mL. Briefly, 50 μL of the diluted samples were separately mixed with 150 μL (MeOH), 10 μL (10% aluminum chloride), 10 μL (1 M potassium acetate), and 280 μL (distilled water). After mixing, the solutions were incubated at room temperature for 30 min and measured at 415 nm (Hassan & Al Aqil, 2013; Sant'Ana et al., 2014).

Measurement of intracellular ROS

Intracellular ROS formation was investigated using a 2'-7'-dichlorodihydrofluorescein diacetate (DCFH-DA) assay. Briefly, Raw264.7 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM). Cells (3×10^5 cells/mL) were conducted with compound 8 for 1 h and then lipopolysaccharide (LPS; 1 μg/mL) was treated each well plate for 24 h at 37℃. Then, the cells were washed and added serum-free DMEM including DCFH-DA (10 μM) in incubation (37℃ and 30 min). The mixed solutions were washed and recorded using a flow cytometer (BD Bioscience, San Jose, CA, USA) (Oh et al., 2023).

Statistical analysis

All statistical analyses were conducted using IBM SPSS software (Version 22.0, IBM, Armonk, NY, USA). The differences were considered as statistically significant at $p < 0.05$.

Results and Discussions

Chemical characterization

The investigation of anti-oxidative metabolites from 70% EtOH extract of *C. soldanella* resulted in the isolation of eight compounds (1–8) including three flavonoid derivatives: rutin (4), isoquercertin (5), and nicotiflorin (6) and five quinic acid derivatives: chlorogenic acid (1), crytochlorogenic acid (2), 5-O-(*E*)-*p*-coumaroylquinic acid (3), isochlorogenic acid A (7), and isochlorogenic acid C (8), respectively (Fig. 1). The chemical structures were determined by spectroscopic analysis (HR-ESI-MS and NMR spectra) with comparison of reported in the literature (Ganbaatar et al., 2019; Lee et al., 2010; Ma et al., 2022; Pan et al., 2019; Tao et al., 2012).

HR-ESI-Q-TOF mass profiles

To confirm main components, the 70% EtOH extract (sample concentration: 1 mg/mL) of *C. soldanella* was analyzed using HR-ESI-Q-TOF mass spectrometry. The peaks (1–8) showed molecular ions based on negative mode of masses consistent with those of isolated compounds (Precursor ions: *m/z* 1: 353.0878; 2: 353.0880; 3: 337.0924; 4: 609.1449; 5: 463.0873; 6:

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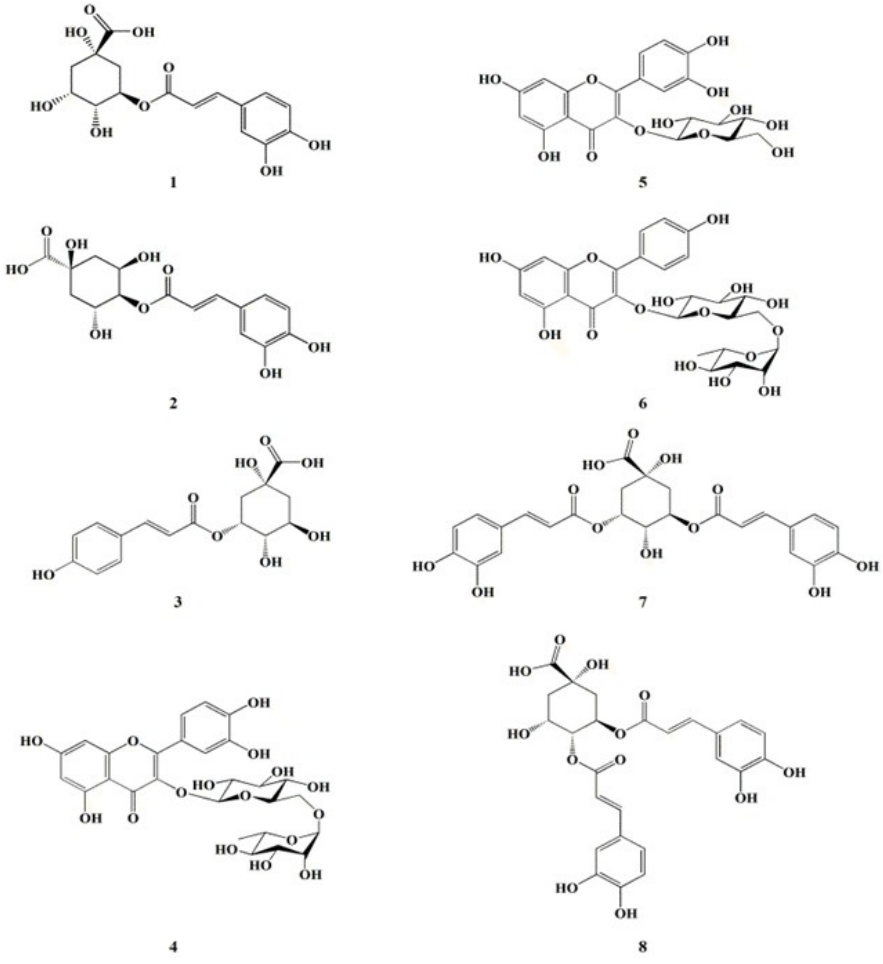

Fig. 1. Chemical structures of 1 to 8. (1) chlorogenic acid; (2) cryptochlorogenic acid; (3) 5-O-(*E*)-*p*-coumaroylquinic acid; (4) rutin; (5) isoquercetin; (6) nicotiflorin; (7) isochlorogenic acid A; (8) isochlorogenic acid C.

593.1501; 7: 515.1188; 8: 515.1184 $[M-H]^-$). The retention time, molecular formula, error, and characterization of peaks (1–8) are shown in (Table 1; Fig. 2).

TPC, TFC, and antioxidant activities

Chemical antioxidant assays (ABTS and DPPH radicals scavenging assays, TPC, and TFC protocols) are broadly used for the primary screening of antioxidant activities. Furthermore, these assays are simple and inexpensive. Polyphenols and flavonoids are divided into various moieties related with attachment of phenol rings. These derivatives exhibit potent antioxidant activities. The results are shown (Table 2). The extract of *C. soldanella* in TPC and TFC revealed that the values are 13.7 ± 0.9 mg GAE/g and 16.2 ± 0.3 mg QE/g, respectively. Furthermore, the amounts of partitioned portions (n-hexane, EtOAc, and n-BuOH- soluble layers) were varied, ranging from 28.6 ± 0.2 to 30.6 ± 0.5 mg GAE/g and from 44.5 ± 3.1 to 72.4 ± 2.9 mg QE/g in TPC and TFC assays. The remaining water-soluble portion exhibited lower values (8.1 ± 0.2 mg GAE/g in TPC, and 10.3 ± 0.1 mg QE/g in TFC) compared with partitioned portions. In addition, the 70% EtOH extract of *C. soldanella* were evaluated for its scavenging activity against ABTS and DPPH radicals. The 70% EtOH extract of *C. soldanella* displayed moderate anti-oxidant activities with IC_{50} values of 167.5 ± 9.4 (ABTS radical) and 379.8 ± 9.8 μg/mL (DPPH radical), respectively. The most active portion (n-BuOH-soluble layer) in ABTS and DPPH radical scavenging assay showed the IC_{50} values of 69.1 ± 10.0 and 120.6 ± 4.9 μg/mL, respectively. However, other separated portions (n-Hexane, EtOAc, and water-soluble portions) showed no antioxidant activity in the concentrate with an IC_{50}

Table 1. Identification of eight compounds (1–8) from 70% EtOH extract of *Calystegia soldanella*

Compounds	Retention time (min)	*m/z*	Molecular formula	Error (mmu)	Identification
1	2.8	$[M-H]^-$ = 353.0878	$C_{16}H_{18}O_9$	+0.5	Chlorogenic acid
2	3.3	$[M-H]^-$ = 353.0880	$C_{16}H_{18}O_9$	+0.7	Cryptochlorogenic acid
3	4.5	$[M-H]^-$ = 337.0924	$C_{16}H_{18}O_8$	+0.1	5-0-(*E*)-*p*-courmaroylquinic acid
4	6.6	$[M-H]^-$ = 609.1449	$C_{27}H_{30}O_{16}$	–0.7	Rutin
5	6.9	$[M-H]^-$ = 463.0873	$C_{21}H_{20}O_{12}$	–0.4	Isoquercetin
6	7.4	$[M-H]^-$ = 593.1501	$C_{27}H_{30}O_{15}$	–0.6	Nicotiflorin
7	7.8	$[M-H]^-$ = 515.1188	$C_{25}H_{24}O_{12}$	–0.2	Isochlorogenic acid A
8	8.4	$[M-H]^-$ = 515.1184	$C_{25}H_{24}O_{12}$	–0.6	Isochlorogenic acid C

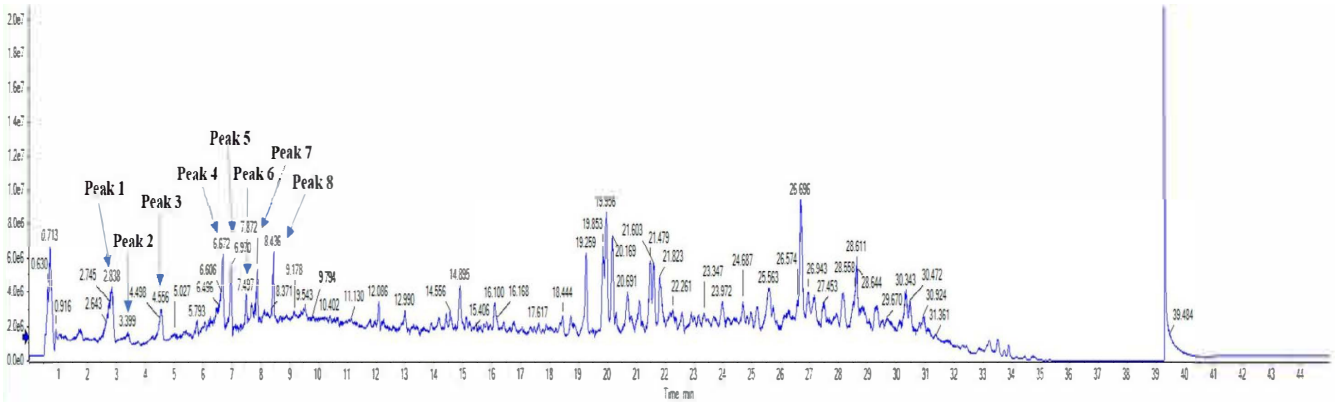

Fig. 2. HR-MS profile using negative mode of 70% EtOH extract of *Calystegia soldanella*.

Table 2. Total polyphenol content (TPC) and total flavonoid content (TFC) of the extracts and partitioned portions from *Calystegia soldanella*

	TPC (mg GAE/g)[1)]	TFC (mg QE/g)[1)]
70% EtOH	13.7 ± 0.9	16.2 ± 0.3
Partition		
n-Hexane-soluble layer	30.6 ± 0.2	44.5 ± 3.1
EtOAc-soluble layer	28.6 ± 0.2	56.1 ± 2.9
n-BuOH-soluble layer	30.5 ± 0.5	72.4 ± 2.9
Water	8.1 ± 0.2	10.3 ± 0.1

[1)] Expressed as milligram of gallic acid equivalent (GAE) and quercetin (QE).

Table 3. Antioxidant (ABTS and DPPH radicals) activities from the partitioned portions and compounds from 70% EtOH extract of *Calystegia soldanella*

	ABTS	DPPH
70% EtOH	167.5 ± 9.4 μg/mL	379.8 ± 9.8 μg/mL
Partition		
n-Hexane-soluble layer	> 200 μg/mL	> 200 μg/mL
EtOAc-soluble layer	> 200 μg/mL	> 200 μg/mL
n-BuOH-soluble layer	69.1 ± 10.0 μg/mL	120.6 ± 4.9 μg/mL
Water	166.6 ± 4.2 μg/mL	> 200 μg/mL
Compounds		
1	33.2 ± 1.1 μM	82.5 ± 4.9 μM
2	37.7 ± 0.3 μM	146.8 ± 5.2 μM
3	> 200 μM	> 200 μM
4	31.9 ± 2.4 μM	52.3 ± 3.9 μM
5	27.4 ± 0.6 μM	47.0 ± 1.9 μM
6	> 200 μM	> 200 μM
7	19.7 ± 0.3 μM	70.0 ± 5.7 μM
8	12.4 ± 0.1 μM	37.9 ± 0.5 μM
Ascorbic acid	24.4 ± 0.4 μM	74.4 ± 0.7 μM

value of 150 μg/mL (Table 3). Therefore, the main components of the n-BuOH-soluble layer of *C. soldanella* were isolated and purified to identify the active anti-oxidative compounds.

Antioxidant activities of main components (1–8) isolated from *C. soldanella*

To search anti-oxidative compounds from the n-BuOH-soluble

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portion of *C. soldanella*, Compounds (1–8) were isolated through purification and isolation, which is followed by column chromatography techniques. The anti-oxidant activities of compounds (1–8) showed IC_{50} values ranging from 12.4 μM to > 200 μM and 37.9 μM to > 200 μM, respectively. The most active compound 8 (isochlorogenic acid C) exhibited the IC_{50} values of 12.4 ± 0.1 μM (ABTS radical) and 37.9 ± 0.5 μM (DPPH radical). Compounds 7 and 8 displayed higher activities than ascorbic acid (IC_{50} values: 24.4 ± 0.4 μM/ABTS radical and 74.4 ± 0.7 μM/DPPH radical). Furthermore, compound 5 (isoquercetin) also showed potent antioxidant activities (ABTS and DPPH radicals) with IC_{50} values of 27.4 ± 0.6 and 47.0 ± 1.9 μM, successively (Table 3). In particular, we confirmed that two main classes were flavonoid and quinic acid derivatives from the extract of *C. soldanella*. Increasingly, compound 5 showed higher antioxidant effect than compound 6, indicating that is contributed to the existence of the hydroxyl unit at C ring in the flavone moiety. Furthermore, the attachment of an additional sugar moiety at glucose unit in isoquercetin slightly decreased the antioxidative activity compared with that of compound 5. Therefore, the attachment of the hydroxyl unit at C ring in flavone moiety plays a role for antioxidant activity. In addition, the presence of coumaroyl moiety in compound 3 considerably decreased its antioxidant activity compared with caffeoyl moiety in compounds 1 and 2. Furthermore, the additional attachment of a caffeoyl moiety (compounds 7 and 8) at quinic acid moiety significantly enhance the antioxidant activity than compounds 1 and 2. Therefore, these results considered that the existence of a caffeoyl moiety and the attachment between quinic acid and an additional caffeoyl moiety play important role for the antioxidant activity.

Intracellular ROS production by compound 8 in LPS-stimulated RAW 264.7 cells

DCFH-DA is one of the most commonly used dyes for the direct detection of intracellular ROS levels. Therefore, we used flow cytometric analysis to measure the effect of compound 8 on the intracellular ROS levels in LPS-stimulated RAW 246.7 cells. As shown in Fig. 3, compound 8 did not show the cytotoxicity at the concentration of 50, 100, and 200 μM. The results displayed that the LPS-treated group shifted rightward compared with LPS non-treated group. However, the compound 8 and ascorbic acid treated groups were shifted at leftward compared with the LPS-treated group. In particular, compound 8 decreased intracellular ROS levels at the concentrate of 50, 100, and 200 μM. Additionally, we presented DCF-DA fluorescence

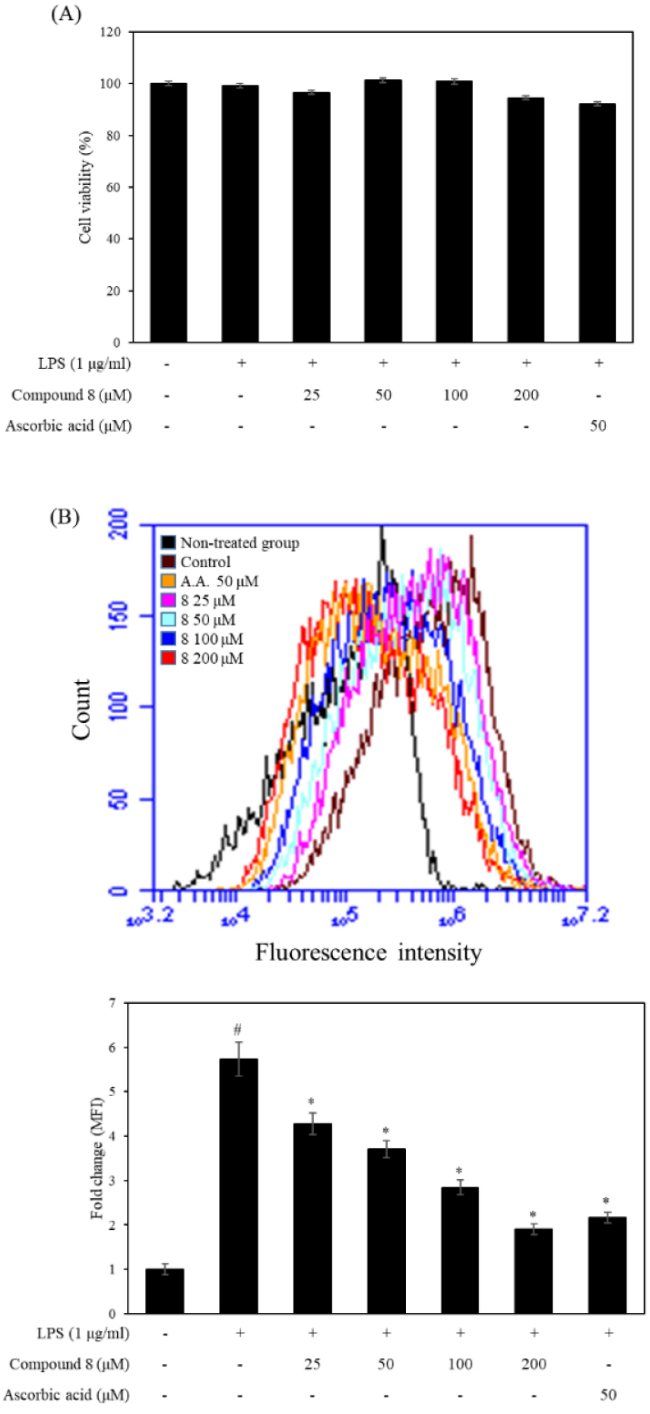

Fig. 3. Cytotoxicity of 8 at the concentration of 25-200 uM (A); Effect of 8 on intracellular reactive oxygen species production in lipopolysaccharide-treated (LPS-treated) RAW264.7 (B). # $p < 0.05$ vs. the non-treated group; * $p < 0.05$ vs. the sample treated group.

as fold changes in mean fluorescence intensity (MFI) compared to the non-treated group. The compound 8–treated groups significantly decreased MFI compared to the non-treated group, Therefore, compound 8 showed the antioxidant effects against intracellular ROS production.

Conclusion

In the current study, we demonstrated the main components with their antioxidative activities from 70% EtOH extract of *C. soldanella*. We found that the n-BuOH-soluble layer have potent anti-oxidant effects against TPC, TFC, ABT, and DPPH radicals scavenging assays. Compounds (1–8) were acquired from the n-BuOH-soluble layer of *C. soldanella* through isolation process and identified as chlorogenic acid (1), cryptochlorogenic acid (2), 5-O-(E)-*p*-coumaroylquinic acid (3), rutin (4), isoquercetin (5), nicotiflorin (6), isochlorogenic acid A (7), and isochlorogenic acid C (8). The isolated compounds (1–8) were detected by HR-ESI-MS spectrum of the 70% EtOH extract of *C. soldanella*. Furthermore, two main classes including flavonoid derivatives and quinic acid derivatives from the 70% EtOH extracts of *C. soldanella* were characterized and evaluated for their antioxidant activities. The most active compound 8 exhibited the IC_{50} values of 12.4 ± 0.1 and 37.9 ± 0.5 μM in the ABTS and DPPH radical scavenging assays. Compound 5 (isoquercetin) also showed strong antioxidant activities with IC_{50} values of 27.4 ± 0.6 (ABTS radical) and 47.0 ± 1.9 μM (DPPH radical). Furthermore, the presence of a hydroxyl unit at C ring in flavone moiety indicated a role in the structure-activity relationship. The presence of a caffeoyl moiety and the attachment between quinic acid and an additional caffeoyl moiety considerably enhanced the antioxidant activity. We also evaluated for inhibitory activity on intracellular ROS production and confirmed the anti-oxidant effects of compound 8. These results can provide valuable information as natural antioxidants from *C. soldanella*.

Supplementary materials

Supplementary materials are only available online from: https://doi.org/10.47853/FAS.2024.e61.

Competing interests

No potential conflict of interest relevant to this article was reported.

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Not applicable.

Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

Not applicable.

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References

Batty M, Bennett MR, Yu E. The role of oxidative stress in atherosclerosis. Cells. 2022;11:3843.

Dong C, Fang W, Yi Q, Zhang J. A comprehensive review on reactive oxygen species (ROS) in advanced oxidation processes (AOPs). Chemosphere. 2022;308:136205.

Eom T, Kim IH, Kim HJ, Choi YH, Nam TJ. *Calystegia soldanella* extract exerts anti-oxidative and anti-inflammatory effects via the regulation of the NF-κB/Nrf-2 pathways in mouse macrophages. Antioxidants. 2021;10:1639.

Ganbaatar C, Gruner M, Mishig D, Duger R, Schmidt AW, Knölker HJ. Flavonoid glycosides from the aerial parts of *Polygonatum odoratum* (Mill.) Druce growing in Mongolia. Open Nat Prod J. 2015;8:1-7.

Halliwell B. Reactive oxygen species (ROS), oxygen radicals and antioxidants: where are we now, where is the field going

and where should we go? Biochem Biophys Res Commun. 2022;633:17-9.

Hassan SM, Al Aqil AA, Attimarad M. Determination of crude saponin and total flavonoids content in guar meal. Adv Med Plant Res. 2013;1:24-8.

Kim GH, Kim JE, Rhie SJ, Yoon S. The role of oxidative stress in neurodegenerative diseases. Exp Neurobiol. 2015;24:325-40.

Kim JY, Lee JM, Kim HS, Ki DW, Yim MJ, Ko SC, et al. A new butenolide derivative from the brown alga *Sargassum micracanthum*. Nat Prod Commun. 2022;17:1-4.

Kim JY, Son E, Kim DS. One new veratramine-type alkaloid from *Veratrum maackii* var. *japonicum* and antioxidative activities of isolated compounds. Nat Prod Commun. 2020;15:1-5.

Lee HJ, Lee JY, Kim SM, Nho CW, Jung SH, Song DG, et al. Inhibitory effects of dicaffeoylquinic acids from *Artemisia dubia* on aldo-keto reductase family 1B10. J Korean Soc Appl Biol Chem. 2010;53:826-30.

Lee JI, Kim IH, Choi YH, Kim EY, Nam TJ. PTP1B inhibitory effect of alkyl *p*-coumarates from *Calystegia soldanella*. Nat Prod Commun. 2014;9:1585-8.

Lee JI, Kim IH, Nam TJ. Crude extract and solvent fractions of *Calystegia soldanella* induce G1 and S phase arrest of the cell cycle in HepG2 cells. Int J Oncol. 2017;50:414-20.

Lee M, Kim S, Jung H. Distribution patterns of halophytes in the coastal area in Korea. Sea J Korean Soc Oceanogr. 2019;24:139-59.

Ma T, Sun Y, Wang L, Wang J, Wu B, Yan T, et al. An investigation of the anti-depressive properties of phenylpropanoids and flavonoids in *Hemerocallis citrina* Baroni. Molecules. 2022;27:5809.

Murai Y, Setoguchi H, Ono E, Iwashina T. Flavonoids and their qualitative variation in *Calystegia soldanella* and related species (Convolvulaceae). Nat Prod Commun. 2015;10:429-32.

Nakamura H, Takada K. Reactive oxygen species in cancer: current findings and future directions. Cancer Sci. 2021;112:3945-52.

Oh GW, Ko SC, Lee JM, Yim MJ, Kim KW, Kim JY, et al. Tyrosinase inhibitory and antioxidant potential of eckmaxol isolated from the brown seaweed *Ecklonia maxima*. S Afr J Bot. 2023;157:648-55.

Ono M, Takigawa A, Kanemaru Y, Kawakami G, Kabata K, Okawa M, et al. Calysolins V–IX, resin glycosides from *Calystegia soldanella* and their antiviral activity toward herpes. Chem Pharm Bull. 2014;62:97-105.

Pan RY, Ma J, Kong XX, Wang XF, Li SS, Qi XL, et al. Sodium rutin ameliorates Alzheimer's disease–like pathology by enhancing microglial amyloid-β clearance. Sci Adv. 2019;5:eaau6328.

Patel MK, Pandey S, Tanna B, Alkan N, Mishra A. Comparative metabolomics unveils the role of metabolites and metabolic pathways in the adaptive mechanisms of shrubby halophytes. Environ Exp Bot. 2022;202:105030.

Pizzino G, Irrera N, Cucinotta M, Pallio G, Mannino F, Arcoraci V, et al. Oxidative stress: harms and benefits for human health. Oxid Med Cell Longev. 2017;2017:8416763.

Qu J, Li Y, Zhong W, Gao P, Hu C. Recent developments in the role of reactive oxygen species in allergic asthma. J Thorac Dis. 2017;9:E32-43.

Ravi S, Young T, Macinnis-Ng C, Nyugen TV, Duxbury M, Alfaro AC, et al. Untargeted metabolomics in halophytes: the role of different metabolites in New Zealand mangroves under multi-factorial abiotic stress conditions. Environ Exp Bot. 2020;173:103993.

Sant'Ana LD, Ferreira ABB, Lorenzon MCA, Louro Berbara RL, Castro RN. Correlation of total phenolic and flavonoid contents of Brazilian honeys with colour and antioxidant capacity. Int J Food Prop. 2014;17:65-76.

Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol. 2014;24:453-62.

Simioni C, Zauli G, Martelli AM, Vitale M, Sacchetti G, Gonelli A, et al. Oxidative stress: role of physical exercise and antioxidant nutraceuticals in adulthood and aging. Oncotarget. 2018;9:17181-98.

Taniguchi A, Tsuge M, Miyahara N, Tsukahara H. Reactive oxygen species and antioxidative defense in chronic obstructive pulmonary disease. Antioxidants. 2021;10:1537.

Tao C, Ahn DR, Xing MM, Lee EB, Kim DK. Phenolic components from the aerial parts of *Bromus japonicus* Thunb. Korean J Pharmacogn. 2012;43:213-6.

Tori M, Ohara Y, Nakashima K, Sono M. Caffeic and coumaric acid esters from *Calystegia soldanella*. Fitoterapia. 2000;71:353-9.

Tryfidou DV, McClean C, Nikolaidis MG, Davison GW. DNA damage following acute aerobic exercise: a systematic review and meta-analysis. Sports Med. 2020;50:103-27.

Volpe CMO, Villar-Delfino PH, Dos Anjos PMF, Nogueira-Machado JA. Cellular death, reactive oxygen species (ROS) and diabetic complications. Cell Death Dis. 2018;9:119.

Yang S, Lian G. ROS and diseases: role in metabolism and energy supply. Mol Cell Biochem. 2020;467:1-12.