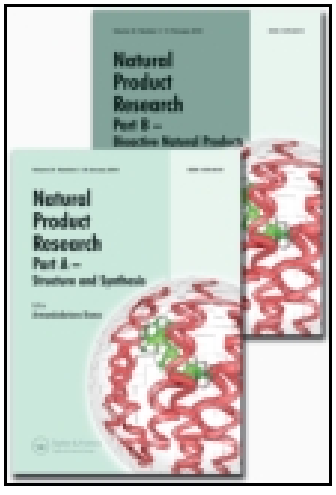

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A novel phenolic acid from the fruits of Rosa soulieana

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A novel phenolic acid from the fruits of *Rosa soulieana*

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From the *n*-BuOH-soluble fraction of a MeOH extract of the fruits of *Rosa soulieana*, one new phenolic glucoside (**1**) was isolated along with five known compounds, comprising two lignin glycosides, two flavonoid glycosides and a phenolic glycoside. The chemical structure of the new compound was elucidated by extensive spectroscopic analyses, including ESI-MS, UV, IR, ^{1}H and ^{13}C NMR, DEPT and 2D NMR (HSQC and HMBC). All the isolated compounds were evaluated for their antioxidant activity by using ABTS (2,2′-azino-bis(3-ethylbenzoline-6-sulfonic acid)) assay. Among these compounds, **1**, **3** and **6** exhibited strong scavenging activity in $ABTS^{\cdot+}$ (SC_{50} = 102.10, 193.85, 65.38 μmol/L, respectively) compared with the positive control L-ascorbic acid (*Vc*) (SC_{50} = 117.16 μmol/L).

Keywords: *Rosa soulieana*; phenolic glucoside; antioxidative; ABTS

1. Introduction

Rosa soulieana belonging to the family of Rosaceae is widely distributed in the dry valley of Sichuan province, China (Zhou et al. 2009). Most of the plants of the genus *Rosa* are rich in triterpenoids, phenolics and essential oils with various biological activities (Liang et al. 1989; Fang et al. 1991; Velioglu & Mazza 1991; Okuda et al. 1992; Hashidoko 1996). In recent years, phenolic compounds have attracted more and more attention due to their ability to scavenge superfluous free radicals in the body which can cause oxidative damage to biomolecules and eventually lead to many chronic diseases such as cancer, atherosclerosis, diabetics, myocardial infarction, cardiovascular diseases, chronic inflammation and stroke (Uttara et al. 2009). Previous phytochemical investigation of the fruits of *R. soulieana* led to the isolation of several non-polar triterpenoids which can be used to treat cardiovascular disorders (Chen et al. 2000). We have reported the isolation and antioxidant activity of nine phenolic compounds from the flowers of *R. soulieana* (Yang et al. 2013). However, phenolics from the fruits of this plant have rarely been reported. Therefore, this study focused on further investigation on the chemical constituent of *R. soulieana* fruits in order to provide a scientific foundation for further use of this plant. Herein, we describe the isolation and structural elucidation of one new phenolic compound, together with five known compounds. Moreover, the antioxidant activity of the isolated phenolic compounds was evaluated by using the ABTS assay.

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2. Results and discussion

2.1. *Structural elucidation of the isolated compounds*

The *n*-BuOH part of methanol extract of *R. soulieana* was subjected to silica gel, Sephadex LH-20 column chromatography (CC) and preparative HPLC to yield compounds **1**–**6**. The structures of these compounds were elucidated on the basis of spectroscopic data (1D and 2D NMR, MS) and chemical evidences.

Compound **1** was obtained as a yellow gum. The molecular formula of **1** was determined as $C_{22}H_{22}O_{12}$ based on the pseudo-molecular ion at *m/z* $[M + Na]^+$ 501.1007 (calcd for $C_{15}H_{26}O_9Na$, 501.1003) in HR-ESI-MS, requiring 12 degrees of unsaturation. The UV spectrum revealed absorption at λ_{max} 200 nm (ϵ 13,734) and λ_{max} 290 nm (ϵ 7950) in MeOH. The IR spectrum indicated the presence of hydroxyl groups (3389 cm^{-1}), carbonyl groups (1688 cm^{-1}) and C—O—C bonds (1099 and 1075 cm^{-1}). In the ^{1}H NMR spectrum (Figure S1), the proton signals at δ_H 6.89 (2H, d, $J = 7.2$ Hz, H-2′, 6′) and δ_H 6.46 (2H, d, $J = 7.2$ Hz, H-3′, 5′) indicated an aromatic moiety in an A2B2 coupling system. While the signals at δ_H 5.94 (1H, s, H-5) and δ_H 5.83 (1H, s, H-3) suggested the presence of a 1,2,4,6-tetrasubstituted or 1,3,4,6-tetrasubstituted benzene ring. The ^{1}H NMR spectrum also revealed a typical anomeric proton at δ_H 4.76 (1H, d, H-1″) and two methylene protons at δ_H 2.97 (2H, s, H-7′). The ^{13}C NMR spectrum (Figure S2) presented 6 signals belonging to a hexose residue, 12 signals for 2 aromatic rings, 2 signals for 2 carboxyls and 2 signals for a carbonyl and a methylene. The hexose was determined to be D-glucose by TLC analysis and measuring the optical rotation of the acid hydrolysis solution of **1**. The coupling constant of the anomeric proton revealed a β configuration (Li et al. 2012). The HMBC (Figure S3) correlations between H-3 (δ_H 5.83) and C-4, C-7, C-8 suggested that the carboxyl groups were connected to C-2 and C-4, respectively (Figure 2). Furthermore, the HMBC correlations between H-3 (δ_H 5.83) and C-7, C-8, and the HMBC correlation between H-5 (δ_H 5.94) and C-8 provided further evidence that H-3 and H-5 are in *meta*-position. H-7′ displayed HMBC correlations with C-1′, C-6′ and C-8′, indicating that the carbonyl group was linked to C-1 and the methylene group was linked to C-1′. The glucose residue was connected to C-6 according to the HMBC correlation between H-1″ and C-6. Based on the above-mentioned evidences, compound **1** was identified and named as soulieana acid 1 (Figure 1). The known compounds were identified as (7*S*, 8*S*)-threo-7,9,9′-tetrehydroxy-3,3′-dimethoxy-8-*O*-4′-neolignan-4-*O*-β-D-glucopyrano-side (**2**) (Huo et al. 2008), dihydrodehydrodiconiferyl alcohol 4′-*O*-β-D-glucopyranoside (**3**) (Abe & Yamauchi 1986), miquelianin (**4**) (Parejo et al. 2004), kaempferol-3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnoside (**5**) (Ni et al. 2012) and methyl 3-*O*-β-glucopyranosyl-gallate (**6**) (Kuang et al. 1989; Ma et al. 2013) based on NMR data and MS analysis, as well as by comparing the spectral data with those reported.

2.2. *Antioxidant activity of the isolated compounds and the crude samples*

The experiment was to apply an antioxidant activity-guided screening procedure to the extracts of the fruits of *R. soulieana* similar to the former study of flower of *R. soulieana* (Yang et al. 2013). Furthermore, we also screened antioxidants from the crude methanol extract of the fruits of the *R. soulieana*, which exhibited potential antioxidant activity with 10.4% inhibition against ABTS at 50 μg/mL (Figure 2). The study demonstrated that the *n*-butanol-soluble (RB) extract contained the bioactive compounds responsible for the antioxidant activity. Bioactivity-guided fractionation of antioxidant constituents from RB extracts led to the isolation of compounds **1**–**6** with different levels of antioxidant activity at 50 μg/mL (Figure 1).

In this article, the antioxidant activity of the isolated phenolic compounds was evaluated by using the $ABTS^{\cdot+}$ radical-scavenging assay with L-ascorbic acid (*Vc*) as the positive control, and the results are shown in Table 1.

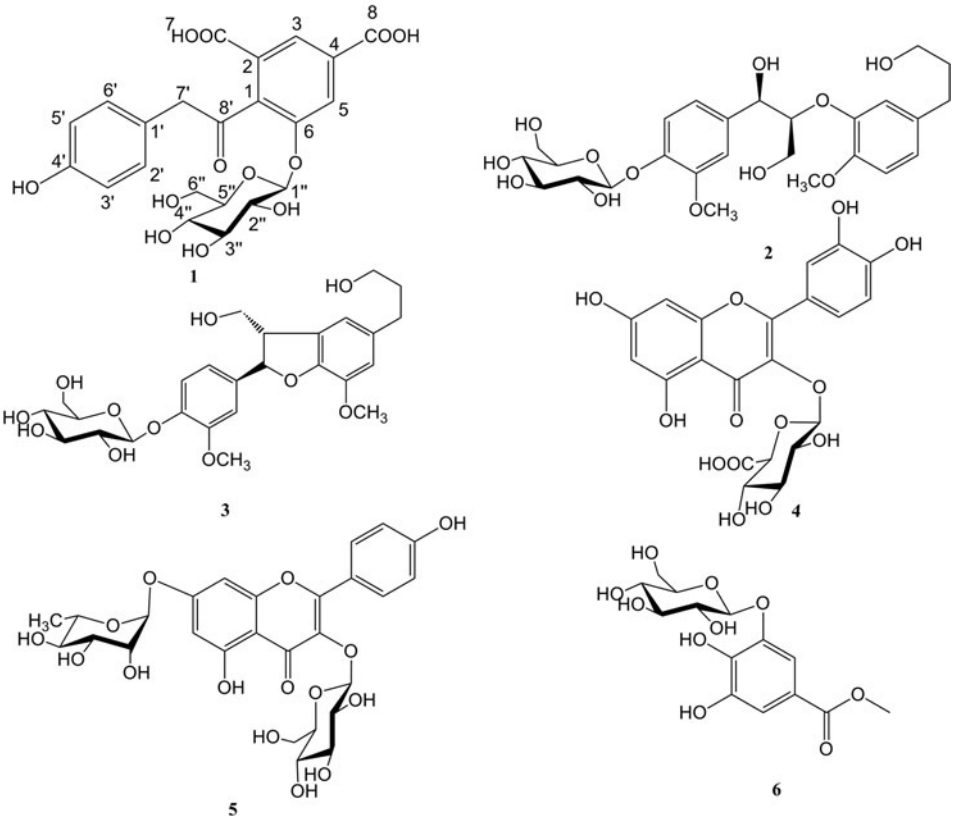

Figure 1. Chemical structures of compounds **1**–**6**.

Compound **6** displayed the most excellent antioxidant activity due to the presence of a catechol group and its low molecular weight. It was reported that small molecules had better access to the radical site, leading to high antioxidant activity (Huang et al. 2005). The carboxyl

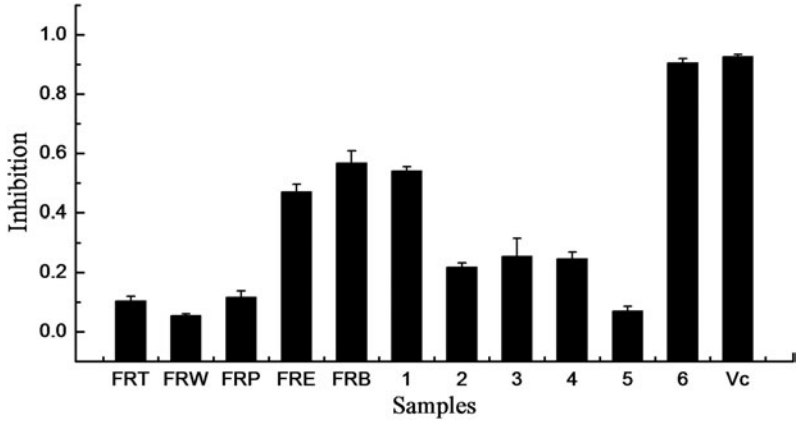

Figure 2. ABTS radical scavenging activities of the crude samples and the isolated compounds from the fruits of *R. soulieana*. The concentration of the tested samples was 50–μg/mL. FRT, the methanol extract; FRW, the aqueous extract; FRP; the petroleum ether soluble extract; FRE; the ethyl acetate soluble extract; FRB, the *n*-butanol-soluble extract.

Table 1. ABTS radical-scavenging activity of the isolated compounds from the fruits of *R. soulieana*.

Compounds	Molecular weight	SC_{50} values (μM)
1	478	102.10
2	540	274.10
3	522	193.85
4	478	262.32
5	592	>500.00
6	346	65.38
Vc	176	117.16

groups on the aromatic ring in compound **1** may be responsible for its strong activity. Compound **4** exhibited better antioxidant activity than compound **5** due to the presence of the catechol group at C-3′ and C-4′. Moreover, the glycosylation at C-7 OH in compound **5** also reduced its activity. As a result, compound **5** exhibited very poor antioxidant activity. Compounds **2** and **3** possessed similar antioxidant activity because of their similar chemical structures.

3. Experimental

3.1. *General experimental procedures*

The solvents, which were used for extraction and isolation, were of analytical grade (Chengdu Kenong Chemical Reagent Factory, Chengdu, China). Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), silica gel 60 (40–63 μm, Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia, Uppsala, Sweden) were used for open CC. TLC was performed on pre-coated silica gel plates (GF254, 0.25 mm, Kang-Bi-Nuo Silysia Chemical Ltd., Yantai, China). IR spectrum was acquired with a Perkin-Elmer 1725X-FT spectrometer (PerkinElmer Instruments LLC, Granville, Hamilton City Metro, DE, USA) with a KBr disk. NMR spectra were recorded on a Bruker Avance-600 spectrometer (Bruker Corporation, Stockholm, Sweden). The chemical shift (δ) values are given in ppm with TMS as internal standard, and coupling constants (J) are in Hz. ESI-MS and HR-ESI-MS were measured on Finnigan LCQ^{DECA} (San Jose, CA, USA) and Bruker Apex-III mass spectrometer (Bruker Corporation, Stockholm, Sweden), respectively. 2,2′-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), potassium persulphate ($K_2S_2O_8$) and all the chromatographic solvents (methanol and acetonitrile) for analytical HPLC were purchased from Sigma (St Louis, MO, USA).

3.2. *Plant material*

The fruits of *R. soulieana* were sampled from the dry valley in Sichuan province, China, in June 2011 and identified by Professor Xinfen Gao, Chengdu Institute of Biology, Chinese Academy of Sciences. A voucher specimen (20110622) has been deposited at the Natural Product Research Center, Chengdu Institute of Biology, Chinese Academy of Sciences.

3.3. *Extraction and isolation*

The dried and powdered fruits (4.5 kg) of *R. soulieana* were extracted with methanol at room temperature for three times in an ultrasonic bath. The extracted solution was concentrated under reduced pressure to yield a dark brown residue (252.6 g), which was suspended in water and extracted with petroleum ether, ethyl acetate and *n*-butanol, successively. The *n*-BuOH fraction (62.1 g) was subjected to a silica gel (200–300 mesh) column, eluting with increasing polarity of $CHCl_3$–MeOH (100:0 → 0:100) to obtain six fractions (Fr. 1–Fr. 6) combined by TLC. Fr. 2 was subjected to a Sephadex LH-20 column with a step gradient of CH_3OH–H_2O (1:50, 1:40,

1:30, 1:20, 1:10), followed by preparative RP-HPLC to yield compounds **2** (40 mg) and **3** (22 mg). Fr. 3 was chromatographed over a C_{18} column using mixtures of CH_3OH-H_2O as eluent to yield compound **1** (15 mg). Fr. 4 was purified by RP-C18 column to yield three subfractions (Fr. 4-1, Fr. 4-2 and Fr. 4-3). Fr. 4-2 was further separated by preparative RP-HPLC to yield compounds **4** (36 mg), **5** (20 mg) and **6** (25 mg).

3.4. *Acid hydrolysis of compound 1 and measurement of optical rotation of the solution*

Compound **1** (5 mg) was hydrolysed in HCl (0.5 mL)/H_2O (1.5 mL)/dioxane (3 mL) under reflux for 2 h. After diluting with H_2O, the hydrolysate mixture was extracted with EtOAc. The H_2O layer was neutralised with $NaHCO_3$ and then concentrated to dryness under reduced pressure. The residue was re-dissolved with H_2O (2 mL) for optical rotation measurement and TLC analysis. The sugar from the water layer was identified as glucose by TLC analysis. The optical rotation of the water solution was determined to be $[\alpha]_D^{20} + 48.9°$ ($c = 0.09$ H_2O), indicating that the absolute configuration of the glucose was D.

3.5. *Spectroscopic data*

Compound **1**: $C_{22}H_{22}O_{12}$, yellow powder (MeOH); $[\alpha]_D^{20}$ -0.137° ($c = 1.00$, MeOH); UV λ_{max} (MeOH) nm(ϵ): 200 (13,734), 290 nm (7950); IR (KBr) ν_{max} 3389, 2922, 1688, 1616, 1515, 1099, 1075 cm^{-1}; ESI-MS *m/z* 501 $[M + Na]^+$; HR-ESI-MS *m/z* 501.1007 (calcd for $[M + Na]^+$, 501.1003). ^1H NMR (600 MHz, CD_3OD-d_4) δ (ppm): 5.83 (1H, s, H-3), 5.94 (1H, s, H-5), 6.89 (1H, d, $J = 7.2$ Hz, H-2′), 6.46 (1H, d, $J = 7.2$ Hz, H-3′), 6.46 (1H, d, $J = 7.2$ Hz, H-5′), 6.98 (1H, d, $J = 7.2$ Hz, H-6′), 4.76 (1H, d, $J = 7.8$ Hz, H-1″), 3.41 (1H, m, H-2″), 3.31(1H, m, 3″), 3.30 (1H, m, H-4″), 3.34 (1H, t, $J = 9.0$ Hz, H-5″), 3.37 (1H, brs, $J = 12.0$ Hz, H-6″-a), 3.57 (1H, m, H-6″-b). ^{13}C NMR (150 MHz, CD_3OD-d_4) δ (ppm): 106.2 (C-1), 101.8 (C-2), 91.9 (C-3), 102.1 (C-4), 96.1 (C-5), 156.9 (C-6), 173.2 (C-7), 170.3 (C-8), 124.3 (C-1′), 131.2 (C-2′, 6′), 114.4 (C-3′, 5′), 155.9 (C-4′), 40.1 (C-7′), 195.5 (C-8′), 100.3 (C-1″), 72.6 (C-2″), 76.9 (C-3″), 69.8 (C-4″), 75.9 (C-5″), 61.0 (C-6″).

Compound **2**: $C_{26}H_{36}O_{12}$, white amorphous powder, ESI-MS *m/z* 563 $[M + Na]^+$. ^1H NMR (600 MHz, CD_3OD-d_4) δ (ppm): 7.12 (1H, brs, H-2), 6.99 (1H, d, $J = 8.2$ Hz, H-5), 6.97 (1H, d, $J = 8.2$ Hz, H-6), 4.87 (1H, d, $J = 5.2$ Hz, H-7), 4.24 (1H, m, H-8), 3.47 (1H, d, $J = 4.9$ Hz, H-9a), 3.83 (1H, dd, $J = 12.0$, 4.2 Hz, H-9b), 6.83 (1H, brs, H-2′), 6.94 (1H, d, $J = 8.4$ Hz, H-5′), 6.68 (1H, d, $J = 8.4$ Hz, H-6′), 2.70 (2H, t, $J = 8.2$ Hz, H-7′), 1.88 (2H, m, H-8′), 3.63 (2H, t, $J = 6.5$ Hz, H-9′), 3.92 (3H, s, H-3-OCH_3), 3.91 (3H, s, H-3′-OCH_3), 4.92 (1H, d, $J = 5.4$ Hz, H-1″), 3.49 (1H, m, H-2″), 3.54 (1H, m, H-3″), 3.39 (1H, m, H-4″), 3.56 (1H, m, H-5″), 3.57 (1H, m, H-6″a), 3.76 (1H, dd, $J = 10.6$, 2.0 Hz, H-6″b).

Compound **3**: $C_{26}H_{34}O_{11}$, white amorphous powder, ESI-MS *m/z* 545 $[M + Na]^+$. ^1H NMR (600 MHz, CD_3OD-d_4) δ (ppm): 7.02 (1H, brs, H-2), 7.14 (1H, d, $J = 8.3$ Hz, H-5), 6.93 (1H, dd, $J = 8.3$, 1.8 Hz, H-6), 5.54 (1H, d, $J = 5.9$ Hz, H-7), 3.38 (1H, m, H-8), 3.83 (1H, m, H-9a), 3.66 (1H, m, H-9b), 6.72 (1H, brs, H-2′), 6.70 (1H, brs, H-6′), 2.61 (1H, t, $J = 7.7$ Hz, H-7′), 1.81 (1H, m, H-8′), 3.56 (1H, t, $J = 6.3$ Hz, H-9′), 4.89 (1H, d, $J = 7.3$ Hz, H-1″), 3.49 (1H, m, H-2″), 3.48 (1H, m, H-3″), 3.45 (1H, m, H-4″), 3.75 (1H, m, H-5″), 3.38 (2H, d, $J = 4.8$ Hz, H-6″), 3.85, 3.81 (each 3H, s, -OMe).

Compound **4**: $C_{21}H_{18}O_{13}$, yellow amorphous powder, ESI-MS *m/z* 501 $[M + Na]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 6.22 (1H, s, H-6), 6.42 (1H, s, H-8), 7.56 (1H, s, H-2′), 6.85 (1H, d, $J = 8.4$ Hz, H-5′), 7.61 (1H, d, $J = 8.4$ Hz, H-6′), 5.50 (1H, d, $J = 7.2$ Hz, H-1″), 3.50 (1H, m, H-2″), 3.43 (1H, m, H-3″), 3.57 (1H, m, H-4″), 3.58 (1H, d, $J = 9.6$ Hz, H-5″).

Compounds **5**: $C_{27}H_{30}O_{15}$, yellow amorphous powder, ESI-MS *m/z* 615 $[M + Na]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 12.59 (1H, s, 5-OH), 10.20 (1H, s, 4′-OH), 8.05 (2H, d, $J = 8.9$ Hz, H-2′, 6′), 6.90 (2H, d, $J = 8.9$ Hz, H-3′, 5′), 6.80 (1H, d, $J = 2.0$ Hz, H-8), 6.43 (1H, d, $J = 2.0$ Hz, H-6), 5.55 (1H, d, $J = 1.2$ Hz, H-Rha-1‴), 1.22 (3H, d, $J = 8.2$ Hz, H-Rha-6‴), 5.43 (1H, d, $J = 6.0$ Hz, H-Glc-1″).

Compounds **6**: $C_{14}H_{18}O_{10}$, pale yellow powder, ESI-MS *m/z* 369 $[M + Na]^+$. ^1H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.45, 7.25 (each 1H, d, $J = 1.7$ Hz, aromatic H), 4.85 (1H, d, $J = 7.2$ Hz, anomeric H), 3.85 (3H, s, -OMe).

3.6. *ABTS free radical-scavenging activities*

The scavenging activity of the tested samples on $ABTS^{\cdot+}$ radical was measured according to the method as previously described (Chung et al. 2011). L-Ascorbic acid was used as the positive control. Briefly, $ABTS^{\cdot+}$ radical cation was prepared by mixing 10 mL of 2 mM $ABTS^{\cdot+}$ water solution with 100 μL of 70 mM potassium persulphate, and the mixture was allowed to stand in the dark at room temperature for 12 h before use. Prior to the assay, the solution was diluted with ethanol to give an absorbance at 734 nm of 0.70 ± 0.02 in a 96-well flat-bottom microtitre plates and was equilibrated at 25°C. The $ABTS^{\cdot+}$ solution (110 μL) was added to the sample solution (total volume: 200 μL) and incubated at room temperature for 10 min, and the absorbance of *AA* fractions (A_i) were read at 734 nm. Measurements were performed at least in triplicate. The blank absorbance (A_0) was measured using ethanol. The $ABTS^{\cdot+}$ radical cation solution was prepared daily. The scavenging activity was expressed as percentage inhibition of the $ABTS^{\cdot+}$ radical cation and was determined by the following equation:

$$AA(\%) = [1 - (A_i/A_0)] \times 100\%.$$

The SC_{50} values (the concentration of sample required to scavenge 50% of $ABTS^{\cdot+}$ radicals) were obtained through extrapolation from linear regression analysis.

Supplementary material

Supplementary material relating to this article is available online, alongside Figures S1–S3.

Acknowledgements

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