Original Article

Composition analysis and antioxidant activities of the Rhus typhina L. stem

Ting Liu a, b, Zhaoqin Li a, Ruiyun Li a, Yue Cui a, Yunli Zhao a,*, Zhiguo Yu a,*,

a School of Pharmacy, Shenyang Pharmaceutical University, 103 Wenhu Road, Shenhe District, Shenyang 110016, China
b The Precise Medicine Center, Key Laboratory of Environmental Pollution and Microecology, Liaoning Province; College of Basic Medical Sciences, Shenyang Medical College, No. 146, North Huanghe Street, Huanggu District, Shenyang 110034, China

A R T I C L E  I N F O

Article history:
Received 26 July 2018
Received in revised form 1 January 2019
Accepted 2 January 2019
Available online 2 January 2019

Keywords:
Rhus typhina L. stem
Antioxidant activity
Chemical composition
UPLC-ESI-MS/MS

A B S T R A C T

The present investigation reports the chemical composition of the Rhus typhina L. stem identified via mass spectrometry and NMR as gallic acid, 1-O-galloyl-β-D-glucose, tryptophan, scopolin, methyl gallate, fustin, quercetin, rutin, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose. The antioxidant properties and the chemical composition contents of the R. typhina L. stem grown in different regions in China were determined. To determine the antioxidant activity, a total phenolic content analysis, 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity assay, ferric reducing antioxidant power assay, and β-carotene linoleic acid model system were conducted. The results showed that the Rhus typhina L. stem possessed high antioxidant capacities due to its high phenolic content. The contents of the nine isolated compounds were determined by UPLC-ESI-MS/MS. The calibration curves of the nine isolated compounds were linear within the concentration range and the average recoveries were high. The result showed that 1-O-galloyl-β-D-glucose, gallic acid, methyl gallate, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose could be the compounds mainly responsible for the antioxidant capacity of the R. typhina L. stem. This reveals that the R. typhina L. stem is a good source of antioxidants.

© 2019 Xi’an Jiaotong University. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

1. Introduction

Rhus typhina L., originated from North America, is a deciduous shrub to a small tree. It is distributed primarily throughout midwestern and eastern North America, and now it is widely grown in northwest China and other regions [1]. The fruits of this plant are used for the preparation of beverages like lemonade and it has been reported to display antimicrobial and antioxidant activities [2]. However, other parts of the plant, with a variety of medicinal properties, are used for treating asthma, bacterial diseases, inflammation and bacterial infections [3]. Polyphenols from the bark of another Rhus species (Rhus verniciflua) show antitumor and anti-inflammatory activities [4], its bark extract and active flavonoids have potent neuroprotective and anti-inflammatory effects and may be good therapeutic candidates as cognitive-enhancers [5]. R. typhina L. shows that it is a valuable source with various kinds of biological activity and has a potential for use in pharmaceutical products. Thus, it is important to explore the chemical composition and biological activity of the Rhus typhina L. stem.

In recent years, there has been an increasing interest in natural antioxidant compounds offered by the plant. The objective of this study was to purify and characterize the chemical composition, investigate the antioxidant capacity of the extracts and the chemical composition, and determine the contents of the nine compounds of the R. typhina L. stem in China.

2. Materials and methods

2.1. Chemicals and reagents

Ferric chloride (FeCl3) and sodium carbonate were purchased from Tianjin Chemical Factory (Tianjin, China). 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from TCI Development Company Limited (Shanghai, China). 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ) was obtained from Shanghai Aladdin Reagent Company Limited (Shanghai, China). Folin-Ciocalteu’s phenol reagent was obtained from ShangHai LabAide Biotechnology Company Limited (Shanghai, China). Gallic acid was purchased from Shanghai Yueye Biotechnology Company (Shanghai, China). 2,6-di-tet-butyl-4-methylphenol (BHT), ascorbic acid, β-carotene, linoleic acid, Tween 40, and acetonitrile of HPLC grade were obtained from Sigma (St. Louis, MO, USA). Formic acid of HPLC grade was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).
purchased from Concord Technology (Tianjin, China). Water was purified by redistillation and passed through a 0.22 μm membrane filter before use. All other chemical reagents were of analytical grade.

2.2. Plant materials

Stems of R. typhina L. were collected from ten different locations in August 2014: Shenyang city (Liaoning province, China)-L1; Dalian city (Liaoning province, China)-L2; Anshan city (Liaoning province, China)-L3; Jinhzhou city (Liaoning province, China)-L4; Huiluado city (Liaoning province, China)-L5; Yingkou city (Liaoning province, China)-L6; Suqian city (Jiangsu province, China)-L7; Baoding city (Hebei province, China)-L8; Xuchang (Henan province, China)-L9; and Zaozhuang city (Shandong province, China)-L10. The samples were authenticated by Professor Jingming Jia (School of Traditional Chinese Medicine, Shenyang Pharmaceutical University, China). The stems were cleaned thoroughly to remove foreign matters such as dust and leaf. The dried stems were ground into a fine homogeneous powder using a commercial blender, passed through a 0.45 mm sieve, and stored in the shade until further use.

2.3. Extraction of plant material

The dried and powdered stems (100 g) of R. typhina L. were extracted three times in succession with MeOH and the combined extract was evaporated to dryness by rotary evaporation (Shanghai Yarong Biochemistry Instrument Factory, Shanghai, China). The methanol extract (10 g) was subjected to ODS eluted successively with MeOH–H2O in a stepwise manner (5:100, 10:100, 20:100, 30:100, 40:100, 50:100, v/v, each 900 mL) to obtain 549 fractions. Fr.3–10 was further separated by C18 semi-preparative HPLC using a solvent system of MeOH-0.05% phosphoric acid H2O (1:10) at a flow rate of 1.5 mL/min (CAPCELL PAK C18 column; 250 mm × 10 mm; 5 μm particle size) to yield compound 1 (gallic acid). Similarly, Fr.40–50 was further separated using a solvent system of MeOH-H2O (1:9) to yield compound 2 (1-O-galloyl-β-D-glucose). Fr.135–141 was further separated using a solvent system of MeOH-H2O (7:3) to yield compound 3 (tryptophan). Fr.178–195 was further separated using a solvent system of MeOH-H2O (7:3) to yield compound 4 (scopolin). Fr.239–262 was further separated using a solvent system of MeOH-H2O (7:3) to yield compound 5 (methyl gallate). Fr.319–328 was further separated using a solvent system of MeOH-H2O (4:6) to yield compound 6 (fustin). Fr.362–372 was further separated using a solvent system of MeOH-H2O (5:5) to yield compound 7 (1,2,3,4,6-penta-O-galloyl-β-D-glucose). Fr.410–421 was further separated using a solvent system of MeOH-H2O (50:50) to yield compound 8 (rutin). Fr.449–457 was further separated using a solvent system of MeOH-H2O (50:50) to yield compound 9 (quercetin).

2.4. Determination of total phenolic content by Folin Ciocalteau assay

The determination of the total phenolic content was carried out according to the method by Wong et al.[6]. 25 μL of gallic acid standard solutions (5, 10, 20, 30, 40, and 50 μg/mL) or R. typhina L. extracts were mixed with 40 μL of Folin-Ciocalteau reagent. After 5 min, 200 μL of sodium carbonate solution (7.5%, w/v) was added and allowed to stand at 20 °C in a water bath for 90 min. The reaction mixture was measured at 765 nm on a Varioskan Flash microplate reader (Thermo Scientific, USA). The results were expressed as mg gallic acid equivalent/g dry weight (mg GAE/g DW) based on the calibration curve.

2.5. Antioxidant capacity determination

2.5.1. DPPH assay

The DPPH radicals scavenging activity assay was measured according to Kossah et al. [2]. 120 μL of various concentrations of R. typhina L. extracts were mixed with 120 μL of DPPH ethanol solution. After mixing vigorously, the mixture was allowed to stand in the dark at room temperature for 30 min. The reaction mixture was measured at 525 nm on a Varioskan Flash microplate reader (Thermo Scientific, USA). The antioxidant capacity was represented as the percent of radical scavenging capacity and calculated based on the percentage of scavenged DPPH using the equation: scavenging activity (%) = \( \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \), where \( A_{control} \) is the absorbance of the solution when the extract sample has been added at a certain level, and \( A_{sample} \) is the absorbance of the solution containing the same volume of ethanol instead of DPPH solution. \( \frac{A_{control} - A_{sample}}{A_{control}} \times 100 \) was defined as the concentration of compound required to scavenge 50% of the DPPH radicals. Ascobic acid was used as a control.

2.5.2. Ferric reducing antioxidant power assay

The ferric reducing antioxidant power (FRAP) was determined according to the method described by Benzie and Strain [7], with some modifications. Briefly, the FRAP reagent was prepared from 20 mmol/L iron (III) chloride solution, 10 mmol/L TPTZ solution in 40 mmol/L HCl and 300 mmol/L sodium acetate buffer (pH 3.6) in a volume ratio of 1:1:10, respectively. FRAP reagent should be prepared fresh daily and incubated in a water bath at 37 °C before use. 30 μL of various concentrations of R. typhina L. extracts and 225 μL of FRAP reagent were put into each well. The absorbance was recorded at 593 nm after 30 min on a Varioskan Flash microplate reader (Thermo Scientific, USA). The antioxidant activity was measured based on a calibration curve plotted using FeSO4·7H2O at a concentration ranging between 50 and 2000 mM, and the results were expressed as mmol Fe (II)/g dry weight of plant material.

2.5.3. β-Carotene linoleic acid model system

The β-carotene linoleic acid model system was performed as previously described by Wu et al. [11], with some modifications. 2 mg of β-carotene was mixed with 20 mg of linoleic acid and 200 mg of Tween 40 in 1 mL of chloroform. After mixing vigorously, chloroform was completely evaporated under vacuum, and 50 mL of oxygenated distilled water was added. Gently shaking the mixture, a clear yellowish emulsion was formed and freshly prepared before each experiment. 30 μL of R. typhina L. extract was mixed with 250 μL of the β-carotene and linoleic acid emulsion. After sufficient and rapid mixing, the absorbance values of the reaction mixtures were measured at 470 nm immediately and then measured after 120 min at 50 °C on a Varioskan Flash microplate reader (Thermo Scientific, USA). The negative control was methanol while butylated hydroxytoluene (BHT) was included as a positive control. The antioxidant activity (AA) of the samples was calculated by using the following equation: AA (%) = \( \left( 1 \right) - \left( \frac{A_{sample} - A_{blank}}{A_{control} - A_{blank}} \right) \times 100 \). Extract concentration providing IC50 value was defined by plotting the inhibition percentage versus the extract concentrations.

2.6. UPLC-ESI-MS/MS analysis of phenolic acids

2.6.1. Ultra-performance liquid chromatography

UPLC analysis was performed using an ACQUITY UPLC I-Class system (Waters Corp., Milford, MA, USA) with conditioned autosampler at 10 °C. Chromatographic separation was achieved on a
Thermo Hypersil GOLD C18 column (2.1 mm × 50 mm, 1.9 μm). The column temperature was maintained at 30 °C and the injection volume was 1 μL. A mobile system with 0.2% formic acid aqueous solution (phase A) and acetonitrile (phase B) was applied for the separation. With a flow rate of 0.20 mL/min, the gradient condition of the mobile phase was as follows: an isocratic elution of 10% B, 0–18 min; 10%–40% B, 18–30 min; an isocratic elution of 20% B, 30–4.0 min; 20%–60% B, 4.0–6.0 min; 60–90% B, 6.0–7.0 min; then quickly returned to the initial 10% B and maintained until 9.0 min for column balance.

2.6.2. Mass spectrometry
Mass spectrometric detection was carried out using a Waters Xevo TQ-S triple quadrupole tandem mass spectrometer (Waters Corp., Milford, MA, USA) with an ESI interface. The ESI source was operated in negative-ionization mode. The optimal ESI source parameters for the analytes were as follows: capillary voltage 3.0 kV, source temperature 150 °C, desolvation temperature 350 °C, and desolvation and cone gas at a flow rate of 700 and 150 L/h, respectively. For the collision induced dissociation (CID), argon was used as the collision gas at a flow rate of 0.13 mL/min. Quantitation was carried out in the selected ion recording mode and multiple reaction monitoring (MRM) mode. Transition reactions and other parameters of the analytes are given in Table 1.

2.7. Statistical analysis
Pearson’s correlation coefficient and Student’s t-test were calculated through SPSS17.0 program (IBM, New York, USA).

3. Results and discussion
3.1. Identification of the isolated compounds
The structures of the nine compounds (Fig. 1), gallic acid, 1-O-galloyl-β-D-glucose, tryptophan, scopolin, methyl gallate, fustin, rutin, quercetin, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose, were identified by spectroscopic means with comparison to literature data [8–20]. All these nine compounds were isolated and reported for the first time from the stems of this plant. NMR data are shown below.

| Compounds     | monitoring mode | Retention time (min) | m/z of precursor ion (Da) | m/z of product ion (Da) | Cone voltage (eV) | Collision voltage (eV) |
|---------------|-----------------|----------------------|--------------------------|------------------------|------------------|------------------------|
| Compound 1    | MRM             | 0.91                 | 168.92                   | 125.08                 | 58               | 14                     |
| Compound 2    | MRM             | 0.70                 | 331.07                   | 169.04                 | 29               | 18                     |
| Compound 3    | MRM             | 1.64                 | 203.05                   | 116.28                 | 46               | 18                     |
| Compound 4    | MRM             | 1.90                 | 399.05                   | 191.00                 | 60               | 25                     |
| Compound 5    | MRM             | 2.10                 | 183.00                   | 124.03                 | 5                | 20                     |
| Compound 6    | MRM             | 3.48                 | 287.05                   | 268.79                 | 5                | 12                     |
| Compound 7    | SRM             | 4.13                 | 939.10                   |                       |                  |                        |
| Compound 8    | MRM             | 3.73                 | 609.17                   | 300.15                 | 16               | 40                     |
| Compound 9    | MRM             | 5.82                 | 301.05                   | 151.05                 | 18               |                        |

Table 1 Parameters of SRM mode used for determination.
Table 2
Antioxidant activity of three different antioxidant capacity methods.

| Sample source                      | DPPH radical (μg/mL) | FRAP value (μmol Fe(II)/g) | Antioxidant capacity (μg/mL) |
|------------------------------------|----------------------|-----------------------------|-----------------------------|
| L1                                 | 18.58                | 1.05                        | 228.11                      |
| L2                                 | 17.69                | 1.13                        | 181.26                      |
| L3                                 | 28.15                | 0.79                        | 256.19                      |
| L4                                 | 29.26                | 0.81                        | 298.10                      |
| L5                                 | 26.19                | 0.92                        | 326.33                      |
| L6                                 | 32.12                | 0.59                        | 319.95                      |
| L7                                 | 22.25                | 0.98                        | 245.65                      |
| L8                                 | 20.89                | 0.95                        | 209.86                      |
| L9                                 | 28.25                | 0.55                        | 322.55                      |
| L10                                | 25.19                | 0.88                        | 238.79                      |
| Gallic acid                        | 0.92                 | 19.48                       | na                          |
| 1-O-galloyl-β-D-glucose            | 2.58                 | 11.64                       | 108.81                      |
| Methyl gallate                     | 1.23                 | 20.21                       | 119.19                      |
| Tryptophan                         | na                   | na                          | na                          |
| Scopolin                           | na                   | na                          | na                          |
| Fustin                             | 2.85                 | 11.02                       | 420.41                      |
| Rutin                              | 3.29                 | 9.31                        | na                          |
| Quercetin                          | 1.86                 | 18.16                       | 165.78                      |
| 1,2,3,4,6-penta-O-galloyl-β-D-glucose| 1.64                | 12.64                       | 37.18                       |
| Vc                                 | 2.91                 | 10.77                       | na                          |
| BHT                                | na                   | na                          | 18.49                       |

Table 3
Relative antioxidant capacity of nine compounds.

| Compound                      | DPPH radical (μg/mL) | FRAP value (μmol Fe(II)/g) | β-carotene linoleic acid model system |
|-------------------------------|----------------------|-----------------------------|-------------------------------------|
| Gallic acid                   | 1.78                 | 1.54                        | 0.00                                |
| 1-O-galloyl-β-D-glucose       | 0.64                 | 0.92                        | 0.34                                |
| Tryptophan                    | 0.00                 | 0.00                        | 0.00                                |
| Scopolin                      | 0.00                 | 0.00                        | 0.00                                |
| Methyl gallate                | 1.33                 | 1.60                        | 0.31                                |
| Fustin                        | 0.58                 | 0.87                        | 0.09                                |
| Rutin                         | 0.50                 | 0.74                        | 0.00                                |
| Quercetin                     | 0.88                 | 1.44                        | 0.22                                |
| 1,2,3,4,6-penta-O-galloyl-β-D-glucose | 1.00                | 1.00                        | 1.00                                |

3.2. Total phenolic contents (TPC)

The phenolic compounds contain a hydroxyl group on an aromatic ring and interrupt chain oxidation reactions by donating a hydrogen atom or chelating metals. In this way, they act as antioxidants. The TPC of methanol aqueous solutions with different methanol concentrations (i.e., 0, 30%, 50%, 70%, 90% and 100%, v/v) were tested. By comparison, the method using 70% methanol as an extraction solvent was more efficient than other methanol concentrations in the extraction of phenolic of R. typhina L. stems. The TPC of 62 fruits varied from 11.88 ± 0.11 to 585.52 ± 18.59 mg GAE/100 g [21], which are lower than those of extracts of R. typhina L. stem, which varied from 2596 to 5226 mg GAE/100 g.

3.3. Antioxidant activities

Similar studies on 1-O-galloyl-β-D-glucose, gallic acid [22], methyl gallate [23], quercetin [24, 25], and rutin [26] showed that they have antioxidant properties. In the study, the antioxidant capacity of the extracts and the nine isolated compounds was evaluated using three commonly used colorimetric methods, namely, DPPH, FRAP assay and β-carotene linoleic acid model system (Table 2). DPPH is a compound consisting of a nitrogen free radical which is purple and disappears by abstracting a hydrogen atom from the antioxidant. The IC50 value for the extracts varied from 17.69 to 32.12 μg/mL and the IC50 value for ascorbic acid was 2.91 μg/mL. The IC50 values of 7 compounds and Vc ranged from 0.92 to 3.29 μg/mL, and the antioxidant activities of them were in the order of gallic acid > methyl gallate > 1,2,3,4,6-penta-O-galloyl-β-D-glucose > quercetin > 1-O-galloyl-β-D-glucose > fustin > Vc > rutin.

The reducing power had a good relationship with its antioxidant, and the antioxidants had the ability of scavenging free radicals due to their hydrogen donation. Thus, the antioxidant activity was evaluated by measuring the absorbance of the reaction mixture, and the higher absorbance indicated a high reducing power of the sample. The mean FRAP values of the studied samples showed L2 > L1 > L7 > L8 > L5 > L4 > L3 > L6 > L9. The antioxidant activities of the studied compounds were in the order of methyl gallate > gallic acid > quercetin > 1,2,3,4,6-penta-O-galloyl-β-D-glucose > 1-O-galloyl-β-D-glucose > fustin > Vc > rutin.

In the β-carotene linoleic acid model system, the ability to inhibit the oxidation of linoleic acid of the R. typhina L. stem was tested. The R. typhina L. stem extracts could inhibit the oxidation of linoleic acid with an IC50 value between 181.26 and 326.33 μg/mL, and the lower AA value reflected better protective action. Moreover, R. typhina L. has the potential to be a good antioxidant for high-fat foods. The antioxidant activities of the studied

Fig. 1. Chemical structures of (A) gallic acid, (B) 1-O-galloyl-β-D-glucose, (C) tryptophan, (D) scopolin, (E) methyl gallate, (F) fustin, (G) 1,2,3,4,6-penta-O-galloyl-β-D-glucose, (H) rutin, and (I) quercetin.
compounds were in the order of BHT > 1,2,3,4,6-penta-O-galloyl-β-D-glucose > 1-O-galloyl-β-D-glucose > methyl gallate > quercetin > fustin, and gallic acid and rutin were not found to inhibit the oxidation of linoleic acid.

There are many factors, including agronomic and environmental, contributing to the antioxidant components and capacity. For this reason, the antioxidant properties of the extracts of the R. typhina L. stems grown in different regions in China were compared. The antioxidant activity and the TPC in the extracts showed positive linear relationships. After analyzing, we found that DPPH values correlated well with the TPC with the correlation coefficient value of 0.920 for the extracts. Similarly, the reducing power showed a highly positive correlation with the TPC with the correlation coefficient value of 0.915 for the extracts. In the β-carotene linoleic acid model system, AA values correlated well with the TPC, with the correlation coefficient value of 0.880 for the extracts. Although there are many components that may contribute to antioxidant activity, the strong correlation between the antioxidant activities and the phenolic content indicates that polyphenol compounds largely contributed to the antioxidant capacities of the R. typhina L. stem extracts.

In the study, we found that methyl gallate, 1-O-galloyl-β-D-glucose and 1,2,3,4,6-penta-O-galloyl-β-D-glucose possessed high antioxidant activity, too. Simultaneously, gallic acid was proved to have high antioxidant activity. This finding is consistent with those reported by other authors [22,27]. In a different anti-oxidation system, tryptophan and scopolin showed no antioxidant activity, while gallic acid, 1-O-galloyl-β-D-glucose, methyl gallate, fustin, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, rutin, and quercetin showed different levels of antioxidant activities. In the DPPH assay,
the antioxidant activity of gallic acid was higher than those of methyl gallate, 1-O-galloyl-β-D-glucose and 1,2,3,4,6-penta-O-galloyl-β-D-glucose. In the FRAP assay, the antioxidant activity of gallic acid was lower than that of methyl gallate, but higher than those of 1-O-galloyl-β-D-glucose and 1,2,3,4,6-penta-O-galloyl-β-D-glucose. In the β-carotene linoleic acid model system, methyl gallate, 1-O-galloyl-β-D-glucose, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose showed antioxidant activity, but gallic acid was not found to inhibit the oxidation of linoleic acid. Therefore, it can be concluded that after the carboxyl groups of gallic acid are esterified, including methyl esterification and glycosylation, the esterified product still has high antioxidant activity or even higher antioxidant activity. The relative antioxidant capacity of the nine compounds is shown in Table 3.

3.4. UPLC-ESI-MS/MS analysis of phenolic acids

The UPLC-ESI-MS/MS chromatogram (Fig. 2) shows that the nine isolated constituents of the R. typhina L. stem extracts included gallic acid, 1-O-galloyl-β-D-glucose, tryptophan, scopolin, methyl gallate, fustin, quercetin, rutin, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose. A calibration curve for gallic acid, 1-O-galloyl-β-D-glucose, tryptophan, scopolin, methyl gallate, fustin, 1,2,3,4,6-penta-O-galloyl-β-D-glucose, rutin and quercetin ranged from 0.078 to 2.50, 0.135 to 4.32, 0.013 to 0.42, 0.053 to 1.70, 0.126 to 4.03, 0.013 to 0.42, 0.995 to 31.84, 0.013 to 0.42 and 0.003 to 0.10 mg/L, respectively. All nine constituents average recoveries varied from 98.6% to 102.6% with the relative standard deviation being < 4%.

Table 4 shows the content of the nine isolated constituents in the stem of the R. typhina L. stem extracts obtained by UPLC-ESI-MS/MS. The antioxidant activity of an extract cannot be evaluated solely on its phenolic content, the content and antioxidant capacity of individual phenolic compounds also need to be considered. In addition, the contents of gallic acid, 1-O-galloyl-β-D-glucose, methyl gallate, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose were high. In order to determine the contribution of the seven isolated compounds to the antioxidant capacity, the correlation between the antioxidant capacity estimated by the three methods and the seven isolated constituents is shown in Table 5. A positive correlation was found between antioxidant capacity and the compounds including gallic acid, 1-O-galloyl-β-D-glucose, methyl gallate, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose. The major reasons for the positive correlation were the high contents and high antioxidant activities of the compounds. Negative or low correlation was found between the antioxidant capacity and the compounds including fustin, quercetin and rutin. The major reasons for the low contents and low antioxidant activities of these compounds were the low contents and low antioxidant activities of these. This shows that gallic acid, 1-O-galloyl-β-D-glucose, methyl gallate, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose seem to make a major contribution to the antioxidant activity of the R. typhina L. stem. Hence, gallic acid, 1-O-galloyl-β-D-glucose, methyl gallate, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose are the compounds mainly responsible for the antioxidant capacity of the R. typhina L. stem. After analysis, Table 6 shows that the correlation between the total content of the nine compounds and the weighted polyphenol contents and the different antioxidant capacity methods is high, but the correlation coefficient between antioxidant activity and the weighted polyphenol contents is greater than the correlation coefficient between antioxidant activity and the total content of galloyl-D compounds is shown in Table 3.

Table 4
The content of the nine constituents in the stem of the R. typhina L. by UPLC-ESI-MS/MS.

| Sample source | X (mg/g) | 1-O-galloyl-β-D-glucose | Tryptophan | Scopolin | Methyl gallate | Fustin | Rutin | Quercetin | 1,2,3,4,6-penta-O-galloyl-β-D-glucose | X_\text{corr} (mg/g) |
|---------------|---------|------------------------|----------|---------|---------------|-------|-------|----------|-------------------------------------|------------------|
| L1            | 1.31    | 3.28                   | 0.32     | 0.28    | 4.07          | 0.134 | 0.144 | 0.0074  | 8.53                                | 18.08            |
| L2            | 1.57    | 3.30                   | 0.27     | 0.43    | 3.00          | 0.142 | 0.092 | 0.0110  | 10.13                               | 18.95            |
| L3            | 1.11    | 2.32                   | 0.07     | 1.20    | 1.50          | 0.182 | 0.073 | 0.0198  | 5.81                                | 10.29            |
| L4            | 1.18    | 3.19                   | 0.09     | 1.38    | 2.06          | 0.100 | 0.067 | 0.0276  | 5.08                                | 13.17            |
| L5            | 1.35    | 2.19                   | 0.08     | 1.31    | 1.47          | 0.130 | 0.114 | 0.0258  | 3.37                                | 10.04            |
| L6            | 0.71    | 2.57                   | 0.08     | 0.94    | 1.23          | 0.196 | 0.136 | 0.0433  | 3.34                                | 9.25             |
| L7            | 0.59    | 1.44                   | 0.13     | 0.79    | 2.44          | 0.191 | 0.131 | 0.0123  | 5.32                                | 10.05            |
| L8            | 1.44    | 2.85                   | 0.09     | 1.25    | 1.93          | 0.072 | 0.070 | 0.0277  | 5.93                                | 13.66            |
| L9            | 0.68    | 1.75                   | 0.08     | 0.55    | 0.59          | 0.459 | 0.094 | 0.0652  | 2.66                                | 6.93             |
| L10           | 1.01    | 2.79                   | 0.16     | 1.08    | 1.84          | 0.168 | 0.179 | 0.0173  | 4.58                                | 11.82            |
| Mean          | 1.57    | 3.30                   | 0.27     | 0.43    | 3.00          | 0.142 | 0.092 | 0.0110  | 10.13                               | 18.95            |
| SD            | 0.34    | 0.64                   | 0.09     | 0.39    | 0.98          | 0.107 | 0.037 | 0.0173  | 2.39                                | –                |
| RSD (%)       | 31.1    | 25.0                   | 64.6     | 42.5    | 48.6          | 60.0  | 33.8  | 67.5    | 45.3                                | –                |

X_\text{corr}—Add the contents of nine compounds together; X_\text{corr}—Add the contents of nine compounds \times weight coefficient determined by DPPH assay together; X_\text{corr}—Add the contents of nine compounds \times weight coefficient determined by FRAP assay together; X_\text{corr}—Add the contents of nine compounds \times weight coefficient determined by β-carotene linoleic acid model system together.

Table 5
Correlation between seven compounds and the different antioxidant capacity methods.

| Compound                          | DPPH radical | FRAP value | β-carotene linoleic acid model system |
|-----------------------------------|--------------|------------|-------------------------------------|
| Gallic acid                       | 0.559        | 0.666      | 0.537                               |
| 1-O-galloyl-β-D-glucose           | 0.329        | 0.413      | 0.454                               |
| Methyl gallate                    | 0.789        | 0.824      | 0.686                               |
| Fustin                            | 0.350        | 0.659      | 0.447                               |
| Rutin                             | 0.080        | 0.077      | 0.006                               |
| Quercetin                         | 0.637        | 0.884      | 0.703                               |
| 1,2,3,4,6-penta-O-galloyl-β-D-glucose | 0.860  | 0.827      | 0.827                               |

* P < 0.05,
** P < 0.01.

Table 6
Correlation between the total content of nine compounds and weighten nine compounds contents and the different antioxidant capacity methods.

| X     | DPPH radical | FRAP value | β-carotene linoleic acid model system |
|-------|--------------|------------|-------------------------------------|
| X_\text{corr} | 0.778**     | 0.807**    | 0.767**                             |
| X_\text{corr} | 0.835**     | –          | –                                   |
| X_\text{corr} | 0.836**     | –          | –                                   |
| X_\text{corr} | 0.813**     | –          | –                                   |

* P < 0.01.
the nine compounds, suggesting the seven phenolic constituents may be the main basis of the antioxidant activity of the *R. typhina* L. stem. In addition, the strong correlation between the antioxidant activity and content indicated that gallic acid, 1-O-galloyl-β-D-glucose, methyl gallate, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose were major contributors to the antioxidant activity of the *R. typhina* L. stem.

4. Conclusions

The nine compounds isolated from the *R. typhina* L. stem including gallic acid, 1-O-galloyl-β-D-glucose, tryptophan, scopolin, methyl gallate, fustin, rutin, quercetin, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose were identified; their antioxidant properties and contents were investigated as well. Results of this study showed that the *R. typhina* L. stem contains a considerable amount of phenolic compounds with significant antioxidant activity, which might be developed as natural antioxidants to replace the synthetic preservatives. Results also revealed that the antioxidant capacity correlated well with the total phenolic content of the stem extracts. In addition, the strong correlation between the antioxidant activity and content indicated that 1-O-galloyl-β-D-glucose, gallic acid, methyl gallate, and 1,2,3,4,6-penta-O-galloyl-β-D-glucose were major contributors to the antioxidant activity of the *R. typhina* L. stem. In conclusion, this is the research that investigated the antioxidant capacity and composition of the *R. typhina* L. stem extracts. Their strong antioxidant activities and wide distributions make them promising sources of natural antioxidants and other bioactive compounds in food and other allied industries. The results of this study will contribute to the potential application of *R. typhina* L. as an economic natural antioxidant in China.

Acknowledgments

The authors are grateful to Dr. Jingming Jia for the assistance provided in authenticating the stem of *R. typhina* L.

Conflicts of interest

The authors declare that there are no conflicts of interest.

References

[1] T. Wu, J.L. Mccallum, S. Wang, et al., Evaluation of antioxidant activities and chemical characterisation of staghorn sumac fruit (*Rhus typhina* L.), Food Chem. 138 (2013) 1333–1340.

[2] R. Kossah, H. Zhang, W. Chen, Antimicrobial and antioxidant activities of Chinese sumac (*Rhus typhina*) fruit extract, Food Control 22 (2011) 128–132.

[3] E. Olchowik, A. Sciepuk, S. Mavlyanov, et al., Antioxidant capacities of polyphenols from sumac (*Rhus typhina*) leaves in protection of erythrocytes against oxidative damage, Biomed. Prev. Nutr. 2 (2012) 99–105.

[4] K.H. Kim, E. Moon, S.J. Choi, et al., Polyphenols from the bark of *Rhus verniciflua* and their biological evaluation on antitumor and anti-inflammatory activities, Phytochemistry 92 (2013) 113–121.

[5] N. Cho, K.Y. Lee, J. Huh, et al., Cognitive-enhancing effects of *Rhus verniciflua* bark extract and its active flavonoids with neuroprotective and anti-inflammatory activities, Food Chem. Toxicol. 58 (2013) 355–361.

[6] C.C. Wong, H.B. Li, K.W. Cheng, et al., A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay, Food Chem. 97 (2006) 705–711.

[7] J.F. Benzie, J.J. Strain, The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, Anal. Biochem. 239 (1996) 70–76.

[8] T.H. Lee, J.L. Chiou, C.K. Lee, et al., Separation and determination of chemical constituents in the roots of *Rhus javanica* L. var. roburghiana, J. Chin. Chem. Soc-Taip. 52 (2005) 833–841.

[9] Y. Kashiwada, G. Nonaka, I. Nishiohka, Tannins and related compounds, XXIII. rhubarb (4): isolation and structures of new classes of gallotannins, Chem. Pharm. Bull. 32 (1984) 3461–3470.

[10] Y.J. Zhang, D.L. Dewitt, S. Murugesan, et al., Novel lipid-peroxidation and cy-clooxygenase-inhibitory tannins from Pirocrhiza kurroa seeds, Chem. Biodivers. 1 (2004) 426–441.

[11] H.L. Ge, J.G. Dai, Chemical constituents of endophytic fungus from *Annona muricata*, China J. Chin. Mater. Med. 35 (2010) 3151–3155.

[12] Y.K. Wang, W.J. He, W.B. Zhou, et al., Two new phenylpropanoids from *Micromelium integerrimum* original research article, Chin. J. Nat. Med. 12 (2014) 619–622.

[13] A. Kumaran, R.J. Karunakaran, Nitric oxide radical scavenging active compo-nents from *Phyllanthus emblica* L Plant Food Hum. Nutr. 61 (2006) 1–5.

[14] J.J. Tan, Q.C. Zhao, L. Yang, et al., Chemical constituents in roots of *Porana lactifloran*, Chin. Tradit. Herb. Drugs 41 (2010) 1245–1247.

[15] S. Shrestha, D.Y. Lee, J.H. Park, et al., Flavonoids from the fruits of Nepalese Sumac (*Rhus parviflora*) attenuate glutamate-induced neurotoxicity in HT22 cells, Food Sci. Biotechnol. 22 (2013) 895–902.

[16] S. Yang, S. Park, D. Ahn, et al., DPPH free radical scavenging effect of the aerial parts of desmodium oldhami, Korean J. Pharmacogn. 41 (2010) 180–184.

[17] Z.W. Wang, X.H. Xu, X.T. Chen, et al., Chemical constituents from the Aeria Part of Sibiraea angustata, J. Chin. Med. Mater. 37 (2014) 60–63.

[18] M. Jehle, J. Bano, E.P. Ellmerer, Natural products from Scorzonera aristata (Asteraceae), Nat. Prod. Commun. 5 (2010) 725–727.

[19] Y.J. Zeng, S.H. Li, X.J. Wu, et al., Flavonoid compounds of whole plant of Crop-talania sessilifora L, Chin. Pharm. J. 49 (2014) 1190–1193.

[20] J.Y. Cho, M.J. Sohn, J. Lee, et al., Isolation and identification of penta-galloylgucose with broad-spectrum antibacterial activity from *Rhus tricho-carpa* Miquel, Food Chem. 123 (2010) 501–506.

[21] L. Fu, B.T. Xu, X.R. Xu, et al., Antioxidant capacities and total phenolic contents of 62 fruits, Food Chem 129 (2011) 345–350.

[22] G.M. Zheng, L.X. Xu, P.W. Wu, et al., Polyphenols from longan seeds and their radical-scavenging activity, Food Chem. 116 (2009) 433–436.

[23] M. Ainsahari, R. Farhoosh, A. Sharif, Antioxidant activity of gallic acid and methyl gallate in triacylglycerols of Kilka oil and its oil-in-water emulsion, Food Chem. 159 (2014) 439–444.

[24] J.M. Davis, E.A. Murphy, M.D. Carmichael, et al., Quercetin increases brain and muscle mitochondrial biogenesis and exercise tolerance, Am. J. Physiol. Regul. Integr. Comp. Physiol. 296 (2009) 1071–1077.

[25] V. Sakanoi, K. Oyama, H. Matsu, et al., Possible use of quercetin, an anti-oxidant, for protection of cells suffering from overload of intracellular Ca2+?: a model experiment, Life Sci. 83 (2008) 164–169.

[26] J.X. Yang, J. Guo, J.F. Yuan, In vitro antioxidant properties of rutin, LWT - Food Sci. Technol. 41 (2008) 1060–1066.

[27] S. Canas, V. Casanova, A.P. Belchior, Antioxidant activity and phenolic content of Portuguese wine aged brandsies, J. Food Compos. Anal. 21 (2008) 626–633.