Chemical Papers
https://doi.org/10.1007/s11696-021-01544-8

ORIGINAL PAPER

Evaluation and identification of antioxidative components of Radix Rhodomyrti by DPPH–UPLC–PDA coupled with UPLC–QTOF-MS/MS

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Received: 14 August 2020 / Accepted: 28 January 2021

Abstract

Radix Rhodomyrti is the dried root of *Rhodomyrtus tomentosa* (Aiton) Hassk., which is used as a herb in southeast Asian countries and regions. Its main active ingredients are phenols. To make efficient use of the active components in Radix Rhodomyrti, 2,2-diphenyl-1-picrylhydrazyl radical–ultra-high-performance liquid chromatography–photo diode array (DPPH–UPLC–PAD) analysis and UPLC–quadrupole time-of-flight tandem mass spectrometry (UPLC–QTOF-MS/MS) were applied to evaluate and identify the antioxidant phenolic components in Radix Rhodomyrti. Gallic acid and ellagic acid were identified as the major phenolic components in Radix Rhodomyrti. The levels of gallic acid and ellagic acid in Radix Rhodomyrti were 2.16 ± 0.27 mg/g and 0.17 ± 0.04 mg/g, respectively. Both compounds showed excellent free-radical scavenging activity in vitro. The known antioxidant capacity of ellagic acid is slightly higher than that of gallic acid, which is consistent with the results of the DPPH radical-scavenging assay. The results of this study indicate that the proposed method could be applied for rapid screening and identification of antioxidants from complex natural products.

Keywords Radix Rhodomyrti · Antioxidant · Gallic acid · Ellagic acid · DPPH–UPLC–PDA

Introduction

Rhodomyrtus tomentosa (Aiton) Hassk., known as Rose Myrtle and a member of the family Myrtaceae, is a wild resource plant that is widely distributed in southeast Asian countries and regions, especially in the southern parts of China and in Japan, Thailand, and Vietnam (Zhao et al. 2019; Hamid et al. 2017). This plant has been found to have therapeutic effects on inflammatory and infectious diseases, and has been used for the treatment of colic, diarrhea, heartburn, dysentery, abscesses, and hemorrhage (Mordmuang et al. 2019; Vo and Ngo 2019). The whole plant can be used as medicine, and different parts of *R. tomentosa* have been studied by many scholars. Leaf extract of *R. tomentosa* has been found to have therapeutic effects on streptococcosis in Nile tilapia and Staphylococcus infections in bovine udders (Na-Phatthalung et al. 2017). The fruit is rich in flavonoids, phenols, polysaccharides, and other active ingredients (Lai et al. 2015). Piceatannol, a promising health-promoting stilbene component with anti-leukemia activity, was found in the fruit (Lai et al. 2013). The fruit has also been used to prepare wine and beverages with good flavor. Radix Rhodomyrti is the dried root of *R. tomentosa*. In China, people use Radix Rhodomyrti to treat foot pain, and farmers use the root as a supplement in daily feed to improve the immunity of livestock and poultry. The roots have been found to have good antioxidant and anticancer activities (Hamid et al. 2016). However, there are few studies on the effective substances in Radix Rhodomyrti.

Phenolic compounds, widely found in plants, play important roles in antioxidation because of their redox properties and their potential as hydrogen donors, singlet oxygen quenching agents, reducing agents, and the ability to scavenge free radicals (Van Hung 2016). They can be divided into simple phenols, phenolic acids, hydroxycinnamic acid derivatives, and flavonoids (Lin et al. 2016). Some studies have shown that phenolic compounds such as gallic acid, ellagic acid, and resveratrol have antivirus, antitumor, and antioxidant effects (Zhang et al. 2018; Limmongkon et al. 2017).

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Published online: 25 February 2021

There are numerous studies on the antioxidant activity of phenolic compounds, but limited information about the analysis and screening of the specific antioxidants in the crude extracts of Radix Rhodomyrti has been performed. Further research is needed to analyze and evaluate the main antioxidant components in dried root of Rhodomyrtus tomentosa.

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity assay and the [2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] (ABTS) radical-scavenging assay are common methods used to determine the antioxidant activity of medicinal plants (Molyneux 2004; Re et al. 1999). However, these methods are usually applied to evaluate the total antioxidant capacity of extracts or certain components. To determine the antioxidant activity of a chemical component in medicinal plants, it is necessary to isolate and purify the relevant compound, sometimes requiring steps that may cause a decline in the antioxidant activity. Therefore, a significant need remains for a method that can provide rapid and accurate screening and evaluation of antioxidant components from complex mixtures in plant extracts.

DPPH–high-performance liquid chromatography (DPPH–HPLC) is a method for simultaneous analysis and evaluation of antioxidant activity of functional components in medicinal plants (Zhang et al. 2011). This method can quickly evaluate the antioxidant activity of a single component in complex samples without isolation and purification (Wang et al. 2016). In this study, ultra-high-performance liquid chromatography (UPLC) was applied to instead of HPLC to analyze the antioxidants. UPLC, a new-generation separation technology based on small particle packing, has been widely used in the analysis of natural plants. It has advantages over HPLC that include ultra-high speed, enhanced separation and sensitivity, and low consumption (Gumustas et al. 2013). This is the first report on rapid screening and evaluation of two antioxidants (Fig. 1) from Radix Rhodomyrti by DPPH–UPLC with photo diode array detection (DPPH–UPLC–PAD) coupled with UPLC–quadrupole time-of-flight tandem mass spectrometry (UPLC–QTOF-MS/MS). The scavenging rate of the antioxidant active component in the solution was calculated by comparing the peak area attenuation of the antioxidant active component before and after DPPH reaction to reflect the antioxidant capacity of the compound.

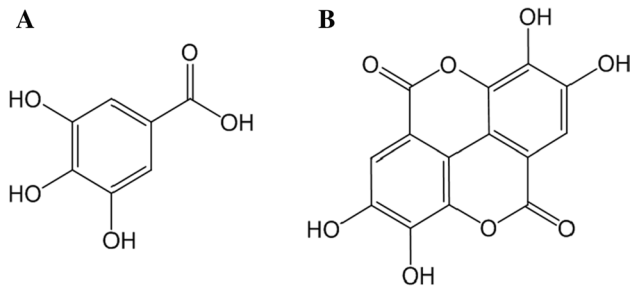

Fig. 1 Chemical structures of gallic acid **a** and ellagic acid **b** present in Radix Rhodomyrti

Materials and methods

Radix Rhodomyrti sample

Radix Rhodomyrti was collected from Rongxian, Guangxi Zhuang Autonomous Region, China, located in an area (22°51′32″N, 110°33′29″E) with abundant *R. tomentosa.* The species was identified by Dr. Fang Wen (Guangxi Institute of Botany, Chinese Academy of Sciences, Guilin, China). Radix Rhodomyrti was dried in natural wind and ground to powder with a multi-function grinder (LX-02, Lixiang, Shanghai, China) at 25,000 rpm to achieve particle sizes in the range of 250–300 μm. The ground samples were dried to constant dry weight and stored in a desiccator for further experimentation. The water content of Radix Rhodomyrti was 7.55%.

Chemicals and solvents

DPPH, ABTS, Folin–Ciocalteu reagent (FCR, 2 N), chlorogenic acid, gallic acid, and ellagic acid (Fig. 1) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, methanol, and formic acid were obtained from Fisher Scientific (Geel, Belgium). Unless otherwise stated, all chemicals were of analytical grade and all solvents were of HPLC grade. All reagents were obtained from commercial sources and used without further purification.

Phenolic component extraction

Dried and ground sample (0.3 g) was extracted with pure methanol (3 × 30 mL) under ultrasound for 30 min (350 W, 53 kHz, 45 °C). Fresh solvent was used for each extraction cycle. The collected supernatant solutions were combined, filtered, and dried by warming in a water bath (65 °C) (Putian, Changzhou, China). The residue was redissolved in pure methanol to a volume of 25 mL for further experimentation.

Standard solution preparation

Standard solutions (500 μg/mL) of gallic acid and ellagic acid were prepared in pure methanol. Standard solutions of gallic acid and ellagic acid were used to prepare diluted standard solutions (100 μg/mL). Chlorogenic acid solution (100 μg/mL) was prepared in pure methanol. All solutions were stored at 4 °C for further experimentation.

Total phenols analysis

Total phenols were detected in 96-well plates using the Folin–Ciocalteu method with a microplate reader. Gallic acid standard solution (0, 2, 4, 8, 10, 12, 14 µL of 100 µg/mL standard) was added to the well, followed by addition of 10 µL of 2 N Folin–Ciocalteu reagent. The 96-well microplate was swirled gently and allowed to stand for 5 min before 50 µL of 10% Na_2CO_3 solution was pipetted into each well and the volume topped up to 200 µL with water. A 5-µL aliquot of extracted sample was used to replace gallic acid standard solution as the sample group. The plate was covered with aluminum foil and allowed to stand for 30 min at room temperature in darkness before the absorbance was measured at 760 nm by a microplate reader (Infinite M200, Tecan, Männedorf, Switzerland). Total phenols were expressed in milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g sample).

Radical-scavenging capacity on microwell plate

DPPH radical-scavenging assay

Stable DPPH free radical was used to react with the crude extracted sample and the reference compounds, respectively, according to the DPPH radical-scavenging method reported previously (Parejo et al. 2000) with slight modifications. Different volume of sample was pipetted (in triplicate) into a 96-well enzyme plate, followed by 150 µL of 0.4 mg/mL DPPH solution. Each well was topped up with pure methanol to a final volume of 250 µL. The mixed reaction samples were allowed to stand for 30 min at room temperature in darkness, and the absorbance was monitored with a microplate reader at 515 nm. The DPPH solution was used as control. The DPPH radical-scavenging rate was calculated according to the following formula: $[1-(A_{\text{sample}}-A_{\text{blank}})/A_{\text{control}}]\times 100\%$, where A_{control} is the absorbance of the DPPH solution with methanol, A_{sample} is the absorbance of the mixed reaction sample group, and A_{blank} is the absorbance of the sample with methanol. The sample concentration providing 50% inhibition (IC_{50}) of DPPH radicals was calculated by plotting the inhibition percentage diagram. When the antioxidant compounds in the extract were confirmed, the antioxidant capacity of the corresponding reference substances was also determined by this method.

ABTS radical-scavenging assay

The ABTS radical-scavenging assay was used to evaluate the radical scavenging of Radix Rhodomyrti extract based on the procedure described by Re et al. (1999) with slight modifications. ABTS solution (7.40 mM) and potassium persulfate (2.60 mM) solution were mixed in a 1:1 ratio (v/v) and allowed to react in darkness at room temperature for 16 h to prepare the $ABTS^{\bullet+}$ solution. Different volume of sample was pipetted (in triplicate) into a 96-well plate, followed by 150 µL of diluted $ABTS^{\bullet+}$ solution. Each well was topped up with pure methanol to a final volume of 220 µL. The mixture was shaken gently and then allowed to stand for 5 min at room temperature in darkness. The absorbance was measured by microplate reader at 734 nm. The diluted $ABTS^{\bullet+}$ solution and pure methanol were used as control and blank, respectively. According to the recorded absorbance values, the $ABTS^{\bullet+}$ radical-scavenging rate was calculated by the following formula: $[1-(A_{\text{sample}}-A_{\text{blank}})/A_{\text{control}}]\times 100\%$, where A_{control} is the absorbance value of the diluted $ABTS^{\bullet+}$ solution, A_{sample} is the absorbance value of the mixed reaction sample group, and A_{blank} is the absorbance value of the sample with methanol. IC_{50} was calculated as described above.

DPPH–UPLC–PDA analysis for screening of main antioxidants in Radix Rhodomyrti extract

DPPH–UPLC–PDA was used to screen the main antioxidants in Radix Rhodomyrti. The extract of Radix Rhodomyrti sample was mixed with DPPH solution in a 1:1 ratio (v/v). Fresh DPPH solution of 1.0 mg/mL was prepared by dissolving 25.0 mg of DPPH in 25 mL of pure methanol every day and kept protected from light. The mixture was blended gently and allowed to react for 30 min at room temperature. For the control sample, pure methanol was added to the extract of Radix Rhodomyrti sample instead of DPPH solution. The reacted samples and control sample were filtered through a 0.2-µm filter paper for UPLC–PDA analysis. UPLC analysis used a Waters BEH C18 column (2.1 × 100 mm, 1.7 µm) and a mobile phase based on a combination of solvent A [0.1% (v/v) formic acid aqueous solution] and solvent B (acetonitrile). All flow rates were 0.25 mL/min. The injection volume was 3 µL, and the column temperature was set at 35 °C. PDA detection was performed at 260 nm after optimization by scanning the Radix Rhodomyrti sample from 200 to 400 nm (Fig. 2). The elution started with 6% solvent B for the first 2 min, increased to 16% solvent B from 2 to 3 min, increased to 20% solvent B from 3 to 8 min, and decreased to 6% solvent B from 8 to 10 min. Empower™3 chromatographic software was used for instrument control and data analysis. By comparing the chromatographic profiles of DPPH-reacted samples and control samples, the main antioxidants in Radix Rhodomyrti could be screened and collected.

Identification of main antioxidant components in Radix Rhodomyrti extract by UPLC–QTOF-MS/MS and UPLC–PDA

The main antioxidant components were identified by UPLC–QTOF-MS/MS. UPLC separation was achieved by

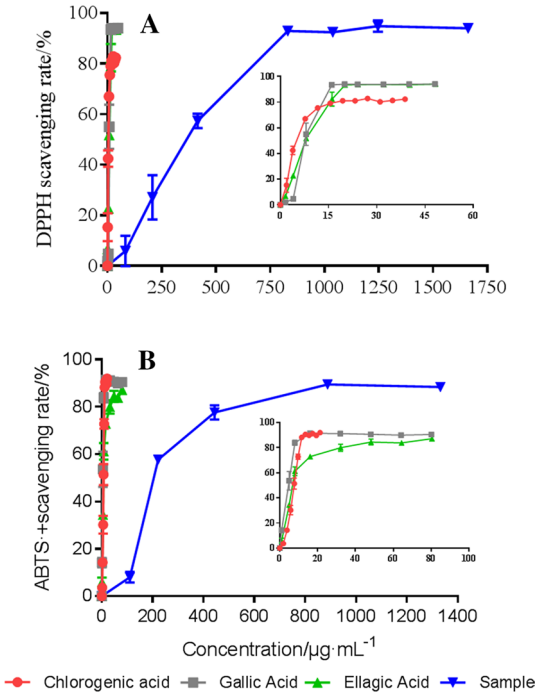

Fig. 2 Effect of concentration on DPPH radical-scavenging activity **a** and ABTS radical-scavenging activity **b**

using an Acquity UPLC HSS-T3 column (2.1 × 100 mm, 1.8 μm) with a flow rate of 0.5 mL/min. The mobile phase used a combination of solvent A [0.1% (v/v) formic acid aqueous solution] and solvent B (acetonitrile). The gradient applied was as follows: 0 min, 10% B; 1 min, 25% B; 3 min, 40% B; 7 min, 70% B; 10 min, 75% B; 12 min, 95% B; 15 min, 99% B; 15.1 min, 10% B; 30 min, 10% B. The injection volume was 0.5 μL, and the column temperature was set at 40 °C. Electrospray ionization was carried out in separate analyses under negative polarity by applying a capillary voltage of 2.80 kV. The source temperature was set at 100 °C, and the desolvation temperature was 400 °C. The sampling cone and source offset voltage were set at 40 V and 80 V, respectively. The desolvation gas flow was 700 L/h. 5-Leucine-enkephalin was used as the lockspray to ensure *m/z* accuracy. The mass spectrometry data were collected from 100 to 1500 Da in centroid mode for the MS^E mode.

Gallic acid and ellagic acid reference compounds having the same molecular weights as the screened antioxidants were employed for further identification of the main antioxidants in Radix Rhodomyrti extract by UPLC–PDA under the same conditions described above.

Quantitative analysis of main antioxidant components in Radix Rhodomyrti extract

Quantitative analysis of the main antioxidant components in Radix Rhodomyrti was monitored by UPLC–PDA. The UPLC–PDA chromatographic analysis conditions were consistent with the conditions for DPPH–UPLC–PDA analysis described above. The standard curves for the main phenolic components were drawn based on the linear relationship between the chromatographic peak area and the corresponding concentration. The contents in the extract of Radix Rhodomyrti were then calculated.

Antioxidant activity by DPPH–UPLC–PDA assay

The radical-scavenging capacity of Radix Rhodomyrti extract was evaluated by DPPH–UPLC–PDA. Different concentrations of DPPH solution were mixed with Radix Rhodomyrti extract at ratios of 1:1 (v/v), and the mixtures were allowed to react for 30 min at room temperature. The control sample was obtained by adding pure methanol to the extract of Radix Rhodomyrti sample instead of DPPH solution. All samples were filtered through a 0.2-μm filter paper for UPLC–PDA analysis under the same conditions described above. By comparing the chromatographic profiles and measuring the peak areas of the main compounds in the sample solution before and after reaction with DPPH, the clearance rate of each component involved in the reaction was calculated. The antioxidant activity was expressed as the percentage reduction of peak area by the following formula: $[(A_{blank} - A_{sample})/A_{blank}] \times 100\%$, where A_{blank} is the peak area of the blank DPPH solution and A_{sample} is the peak area of compound corresponding to the sample solution after reaction with DPPH.

Results and discussion

Total phenols and antioxidant activity

Phenolic compounds can be used as hydrogen donors, singlet oxygen quenchants, reducing agents, and free-radical scavengers, and play important roles as antioxidants. They can be divided into simple phenolic acids, hydroxycinnamic acid derivatives, and flavonoids. The main source of antioxidant components in the methanolic extract of Radix Rhodomyrti is phenols (Bazzaz et al. 2011). To investigate the antioxidant capacity of phenols extracted from Radix Rhodomyrti, the potential antioxidant components of total phenols were determined by the Folin–Ciocalteu method. The content of total phenols was 16.27 ± 1.33 μg/mL (Table 1), which is lower than that reported previously (Hamid et al. 2017). The difference could be caused by different growing conditions, different picking periods, genetic variations, extraction conditions, or other reasons.

Figure 3 shows the antioxidant capacity against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ in the extract of Radix Rhodomyrti with different concentration. With the increase in extract sample

Table 1 Total phenols, gallic acid, and ellagic acid in Radix Rhodomyrti sample

Total phenols (mg GAE/g)	Gallic acid (mg /g)	Ellagic acid (mg /g)
16.27 ± 1.33	2.16 ± 0.27	0.17 ± 0.04

Data expressed as mean ± SD ($n = 3$)

concentration, the clearance rate increased. Compared with the control substance chlorogenic acid (Naveed et al. 2018; Klaric et al. 2020), at lower concentrations, the free-radical scavenging abilities of extract sample against DPPH• (Fig. 3a) and ABTS•+ (Fig. 3b) were much lower than that of chlorogenic acid. At higher concentration, the free-radical scavenging ability of extract sample gradually approached that of chlorogenic acid against ABTS•+ and the maximum scavenging ability against DPPH• was higher than that of chlorogenic acid. The maximum free-radical scavenging abilities were 94.85% against DPPH• and 89.44% against ABTS•+, which were achieved at sample concentrations of 1245.6 μg/mL and 887.68 μg/mL, respectively. In this study, IC_{50} values were also calculated to measure the scavenging abilities. As shown in Table 2, the extract of Radix Rhodomyrti showed similar potency against DPPH• and ABTS•+ with respective IC_{50} values of 331.98 ± 29.50 μg/mL and 334.43 ± 23.10 μg/mL. The radical-scavenging activities of the extract against DPPH• and ABTS•+ were lower than those of chlorogenic acid ($IC_{50} = 4.55 \pm 0.40$ μg/ml against DPPH•, 7.91 ± 1.13 μg/ml against ABTS•+), which always showed excellent free-radical scavenging activity in vitro.

Table 2 DPPH and ABTS radical-scavenging activity

Compounds	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)
Chlorogenic acid	4.55 ± 0.40	7.91 ± 1.13
Gallic acid	8.09 ± 0.42	3.29 ± 0.21
Ellagic acid	7.16 ± 0.27	8.39 ± 1.59
Radix Rhodomyrti sample	331.98 ± 29.50	334.43 ± 23.10

Data expressed as mean ± SD ($n = 3$)

Separating potential antioxidants in methanolic extract of Radix Rhodomyrti by UPLC–PDA

The UPLC–PDA conditions for separating the main antioxidants in Radix Rhodomyrti were optimized in our previous research (Yin et al. 2015). In this study, two main peaks were detected and separated with retention times of 1.67 and 7.30 min (Fig. 4C). According to the chromatograms, the optimized UPLC conditions could be used to isolate potential antioxidants from Radix Rhodomyrti within 10 min. The compounds attributed to peaks 1 and 2 were considered as possible antioxidants in Radix Rhodomyrti.

DPPH–UPLC–PDA analysis for screening of main antioxidants in Radix Rhodomyrti

As a stable free radical, DPPH• can capture other free radicals. DPPH• appears dark purple in solution and becomes colorless or light yellow after being neutralized by antioxidants. In this study, DPPH–UPLC–PDA was employed to screen antioxidants in Radix Rhodomyrti extract. The peak

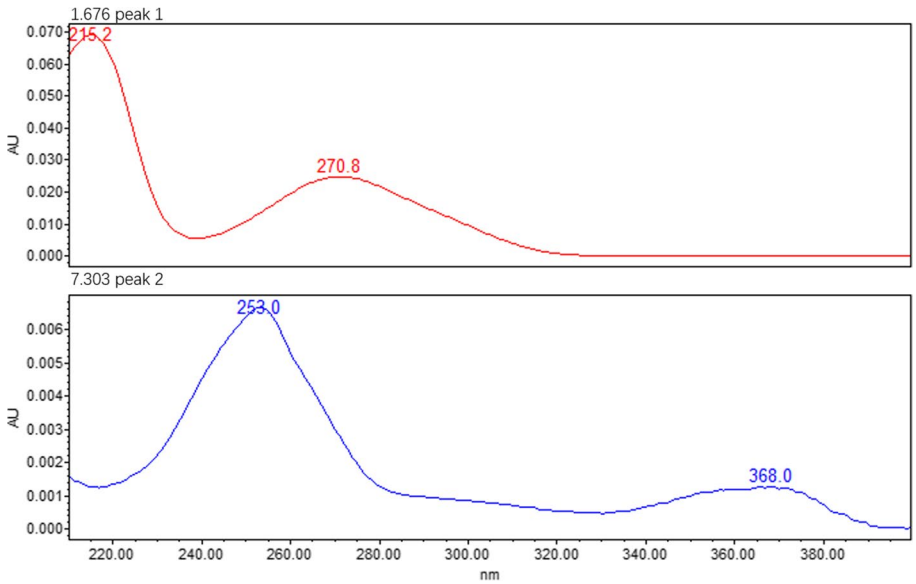

Fig. 3 Ultraviolet spectra of the chromatographically separated sample

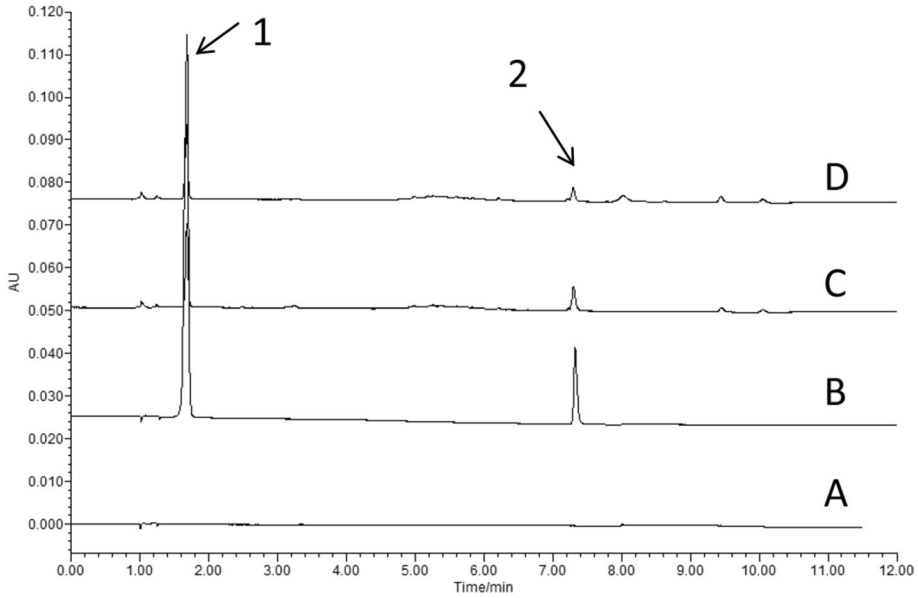

Fig. 4 UPLC chromatograms of pure methanol **a**, gallic acid and ellagic acid reference compounds **b**, methanolic extract of Radix Rhodomyrti before **c** and after **d** reaction with DPPH radicals. 1, gallic acid; 2, ellagic acid

areas of the antioxidant components decreased after reacting with DPPH solution and there was little change in the peak areas of compounds without antioxidant properties. The untreated and DPPH-treated extracts were analyzed by UPLC–PDA under the conditions described above. Comparing the UPLC chromatograms of untreated and DPPH-treated samples, peaks 1 and 2 were decreased after reaction with DPPH solutions (Fig. 4c, d), which confirmed that

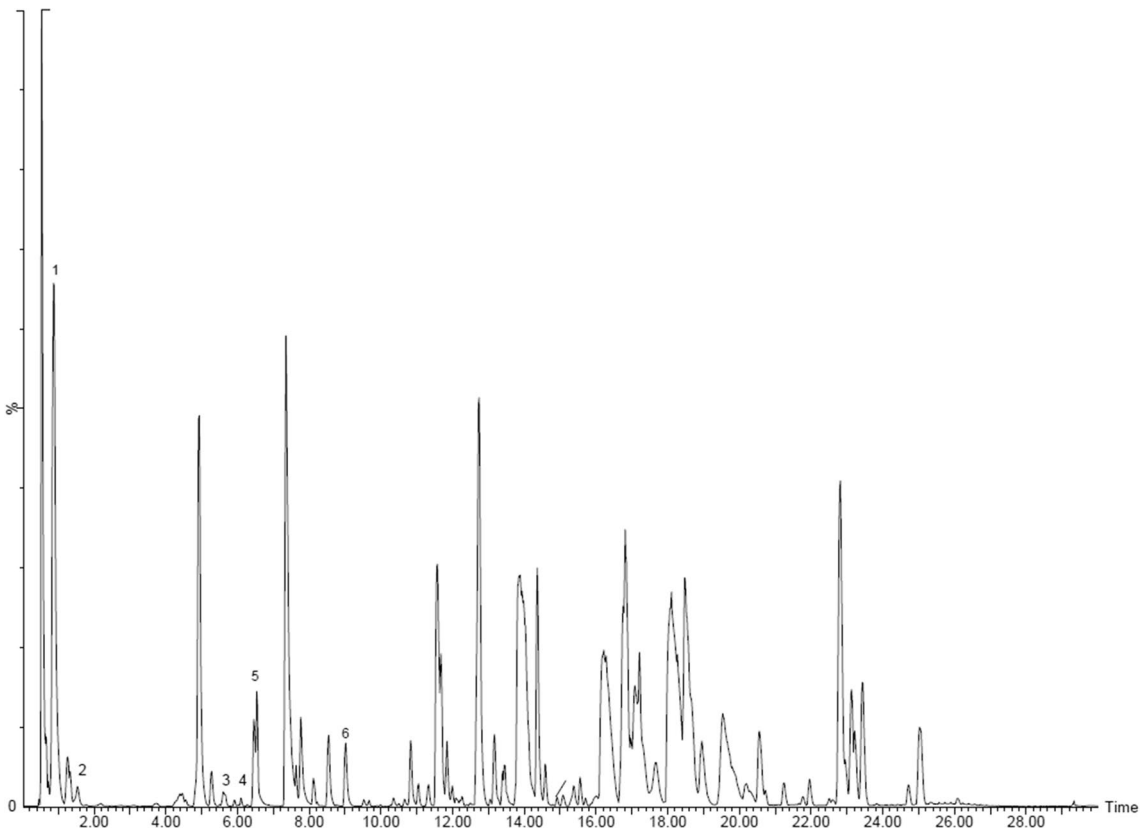

Fig. 5 Total ion chromatograms of Radix Rhodomyrti extract in negative mode by UPLC–QTOF–MS/MS

Table 3 Identification of phenolic compounds from Radix Rhodomyrti extract by UPLC–QTOF–MS/MS

No	Phenolic compounds	Retention time/min	Formula	Molecular ion $[M-H]^-$, *m/z*	MS/MS, *m/z*
1	Gallic acid	0.87	$C_7H_6O_5$	169.015	125.025
2	Protocatechuic acid	1.55	$C_7H_6O_4$	153.020	-
3	Kaempferol	5.63	$C_{15}H_{10}O_6$	285.041	151.003, 169.065
4	Isomyricitrin	6.11	$C_{21}H_{20}O_{13}$	479.090	316.028
5	Ellagic acid	6.49	$C_{14}H_6O_8$	300.999	283.997, 229.015
6	Quercetin	9.02	$C_{15}H_{10}O_7$	301.041	179.004, 151.009

the corresponding compounds were the antioxidants in the extract of Radix Rhodomyrti.

UPLC–QTOF-MS/MS identification

The structures of all phenolic compounds present in the root extract were identified by UPLC–QTOF-MS/MS in negative mode based on their retention time (t_R), and MS fragmentation patterns by comparison with standards or the published data (Zhao et al. 2019; Lai et al. 2015). Figure 5 shows UPLC–QTOF-MS/MS total ion chromatograms of Radix Rhodomyrti extract. In total, 6 phenolic compounds were successfully identified. Their mass information of $[M\text{-}H]^-$ ions, fragments, molecular formulas, and identification results are shown in Table 3. Peak 1, with $[M-H]^-$ ion at *m/z* 169.01, was tentatively assigned to gallic acid with a fragment ion at *m/z* 125.025 $[M-44-H]^-$ (Fig. 6a). Peak 2 yielded a molecular ion $[M-H]^-$ at *m/z* 300.99, and fragmentation resulted in the ions *m/z* 283.99 $[M-H_2O]^-$ and 229.01 $[M-HCO_2-CO]^-$ (Fig. 6b). The likely molecular formula was $C_{14}H_6O_8$. According to the Scifinder and Reaxys databases and references, compound 2 was assigned to ellagic acid (Fig. 1a). In comparing the UPLC chromatogram of Radix Rhodomyrti extract sample with those of gallic acid and ellagic acid reference compounds, peak 1 (see Fig. 4c) in the Radix Rhodomyrti extract matched gallic acid at 1.67 min, and peak 2 (see Fig. 4c) matched ellagic acid at 7.30 min. In detail, the structures of peaks 2 and 6 were identified as protocatechuic acid and quercetin, respectively, by fragment information analysis, and further confirmed by comparing with standard substances (see Supporting Information). Peaks 3 and 4 were identified as kaempferol and isomyricitrin, respectively, by comparing retention times and fragment information, which was consistent with the results of literature report (Wu et al. 2015; Hou et al. 1999; Zhou et al. 2016) and database information retrieval.

Quantitative analysis of main antioxidant components in Radix Rhodomyrti extract

Antioxidants in Radix Rhodomyrti extract were quantitatively analyzed by UPLC–PDA under the conditions described above. Different concentrations of gallic acid and ellagic acid mixed standard solution were precisely absorbed into the UPLC and analyzed to evaluate linearity. Gallic acid and ellagic acid reference compounds were eluted at 1.67 and 7.30 min, respectively, as seen in the chromatogram (Fig. 4b). In the Radix Rhodomyrti extract sample, these two compounds were eluted with matching retention times with good separation (Fig. 4c). The standard curves used the chromatographic peak area as the longitudinal coordinate (*y*) and the mass concentration as the transverse coordinate (*x*). After constructing the calibration curves, the correlation coefficient (*r*), linear range, and limits of quantitation (LOQ) and detection (LOD)

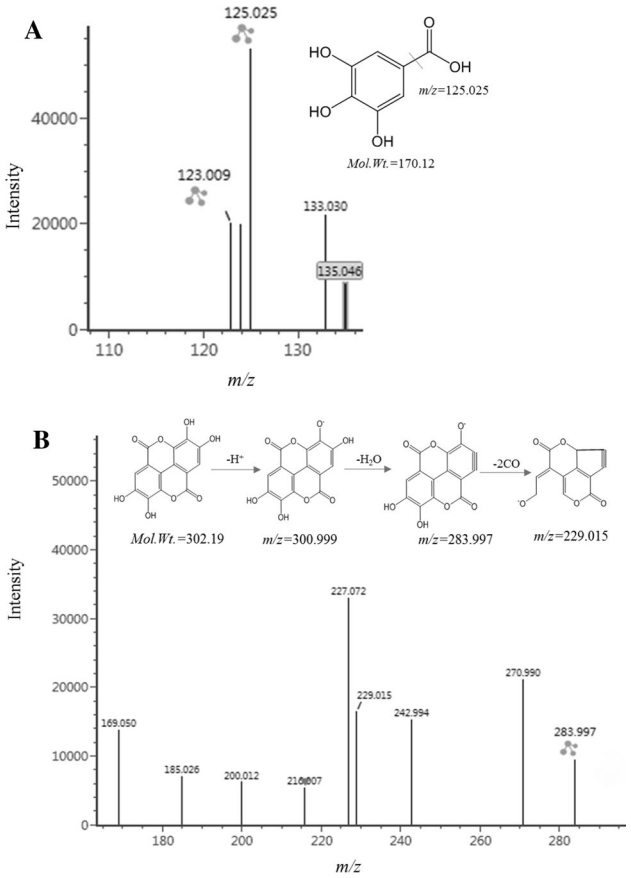

Fig. 6 MS/MS spectra of gallic acid **a**, ellagic acid **b**, and its fragmentation pathway

Table 4 Calibration curve, correlation coefficient (*r*), test range, limits of detection (LOD), and quantitation (LOQ) for main antioxidants determined by UPLC–PDA

Analyte	Calibration curve	r	Linear range (μg/mL)	LOD (ng/mL)	LOQ (ng/mL)
Gallic acid	$y = 4516.5x + 608.2$	0.9995	1.00 ~ 80.12	2.66	8.86
Ellagic acid	$y = 6032.9x + 369.99$	0.9994	1.06 ~ 80.44	1.74	5.80

of each compound were determined (Table 4). The LODs of gallic acid and ellagic acid (signal-to-noise ratio of 3) were 2.66 ng/mL and 1.74 ng/mL, respectively. The levels of gallic acid and ellagic acid in the Radix Rhodomyrti sample were 2.16 ± 0.27 mg/g and 0.17 ± 0.04 mg/g, respectively (Table 1). Gallic acid and ellagic acid have been demonstrated as important components of plant hydrolyzed tannins, with known biological activities including antivirus, antioxidant, and antitumor activities (Vu et al. 2018; Mehrzadi et al. 2019; de Moraes Alves et al. 2020). The content of gallic acid and ellagic acid can be used as an important index to evaluate the functional quality of Radix Rhodomyrti.

Evaluation of antioxidant capacity by DPPH–UPLC–PDA analysis

Radix Rhodomyrti extract possessed good radical-scavenging potency. Ellagic acid and gallic acid were confirmed as the main antioxidant compounds in the extract, and their antioxidant capacities in the extract were analyzed by DPPH–UPLC–PDA. The peak area of each compound in the untreated sample was set as 100%, and the relative peak areas of extract samples after reaction with different concentrations of DPPH solution were calculated (Fig. 7). With increase in DPPH concentration, the radical scavenging of the two compounds increased. The antioxidant capacities of phenols are related to their chemical structure, degree of hydroxylation, substituent type, conjugation, and degree of polymerization (Dai and Mumper 2010). From the trends observed for clearance rate, the two main antioxidants have good ability to scavenge DPPH free radicals. The antioxidant capacity of ellagic acid is slightly higher than that of gallic acid, which is consistent with the results of the DPPH radical-scavenging assay (Table 2). Ellagic acid is a dimer derivative of gallic acid. Ellagic acid contains four phenolic hydroxyl groups (see Fig. 1) and shows stronger proton donor ability than gallic acid, which has three phenolic hydroxyl groups.

Conclusions

In this study, a rapid and sensitive method using DPPH–UPLC–PDA coupled with UPLC–QTOF-MS/MS was applied to screen and identify the antioxidants from Radix Rhodomyrti for the first time. Ellagic acid and gallic acid were identified as the main antioxidant compounds in the extract of Radix Rhodomyrti and showed excellent free-radical scavenging activity in vitro. The DPPH–UPLC–PDA assay was developed into a high-throughput method for screening and quantitative analysis of the main antioxidants in *R. tomentosa* roots, which may help promote the development, utilization, and quality evaluation of *R. tomentosa* and other Chinese herbs of the same family.

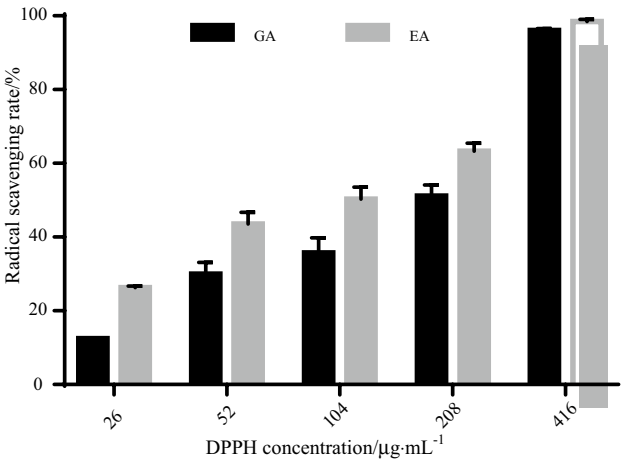

Fig. 7 Radical-scavenging rate after reaction with different concentrations of DPPH

Supplementary information The online version of this article (https://doi.org/10.1007/s11696-021-01544-8)

Acknowledgements This work was supported by the Guangxi aquatic animal husbandry and Veterinary Bureau Science and Technology Application Program (No. 201452044), the Guangxi Science and Technology Bureau Program (No. AA17204057), and the Guangxi Fundamental Research Funds (No. 20-5, 19-5).

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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