

Article

Rapid Discovery of Antioxidants in *Rosa rugosa* by UHPLC-PDA-Q-TOF/MS-TACD Integrated Technique

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Abstract

In this study, an integrated analytical method coupling ultra-high-performance liquid chromatography–photodiode array detection–quadrupole time-of-flight mass spectrometry with total antioxidant capacity determination (UHPLC-PDA-Q-TOF/MS-TACD) was developed for the rapid screening and identification of antioxidants in complex natural extracts. The system enables simultaneous chromatographic separation, mass spectrometric characterization, and on-line activity assessment by combining 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) and ferric ion reducing antioxidant power (FRAP) assays. When applied to *Rosa rugosa* samples from five different origins, the approach efficiently separated the extract and successfully localized antioxidants directly from the chromatographic profile. A total of 86 compounds were identified, including flavonoids, tannins, and phenolic acids. Among them, 60 exhibited free radical scavenging capacity and 59 showed reducing activity. Activity verification experiments showed that all seven compounds exhibited good antioxidant activity. The IC₅₀ values of gallic acid, ellagic acid, quercetin 3-*O*-rhamnoside, and rutin were 0.019, 0.025, 0.043, and 0.046 mM, respectively, which were significantly better than the positive control (vitamin C). This method provides methodological and technical support for the rapid discovery of antioxidant components in complex natural products.

Keywords: *Rosa rugosa* Thunb.; UHPLC-PDA-Q-TOF/MS-TACD; natural antioxidants; integrated active detection system



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1. Introduction

Current studies on the antioxidant activity of *Rosa rugosa* Thunb. (*R. rugosa*) largely remain at the level of total extracts, focusing primarily on the evaluation of total flavonoids and phenolic content. Research on the screening of specific antioxidant-active constituents in roses is relatively scarce, a limitation that is also commonly observed in antioxidant studies of other plant resources [1,2]. Furthermore, synthetic antioxidants such as propyl gallate have notable toxic side effects and are now restricted or prohibited [3,4]. Consequently, there is growing interest in the discovery of novel, highly effective, and low-toxicity antioxidants from natural plants, especially floral medicinal materials, to enhance human health and quality of life [5,6]. The conventional workflow for discovering natural bioactive components primarily involves separation, purification, and activity validation [7,8]. This

approach is constrained by limitations such as prolonged processing cycles, high energy consumption, and low efficiency. Furthermore, some bioactive components may be lost or degraded during the separation process. In recent years, researchers have developed various coupled chromatographic methods to assess the antioxidant capacity of complex plant matrices [9–11]. Among these, UHPLC coupled with antioxidant activity screening has gained widespread application in identifying bioactive compounds from natural products, owing to its high efficiency, speed, sensitivity, and low solvent consumption [12,13]. The HPLC-DPPH assay, in which HPLC separates antioxidant fractions and subsequently reacts with DPPH in a post-column reaction. The scavenging activity was quantified by monitoring the changes in the characteristic absorption peak at 517 nm. DPPH free radicals are neutralized, resulting in fading, and the degree of absorbance reduction is positively correlated with the antioxidant capacity. This method offers significant advantages for the analysis of antioxidant activity in complex matrices. However, it is important to note that the detection wavelength may interfere with the absorption of some antioxidant substances (carotenoids) in similar bands [14]. The HPLC-FRAP method is based on the ability of antioxidants to reduce Fe^{3+} -TPTZ to form a blue Fe^{2+} -TPTZ complex under acidic conditions, which produces a characteristic absorption peak at 510 nm. Using Fe^{2+} as a reference standard, the antioxidant capacity of the sample was quantitatively determined [15]. The HPLC-ABTS assay is based on the generation of a blue-green $\text{ABTS}^{\bullet+}$ radical by an oxidizing agent, which produces a characteristic absorption peak at 734 nm. In the presence of antioxidant compounds, the formation of $\text{ABTS}^{\bullet+}$ is inhibited, leading to a decrease in absorbance. Compared to the DPPH method, this approach is less susceptible to interference from the sample background absorption [16].

Under pathological conditions, reduced activity of antioxidant enzymes exacerbates oxidative stress injury. Reactive oxygen species, which participate in physiological regulation, can cause cellular damage when present in excess, contributing to chronic diseases such as cardiovascular and neurodegenerative disorders and accelerating organismal aging [17]. Traditional Chinese medicine represents a vital natural resource for the prevention of human diseases and holds potential as a pioneer in new drug development. *Rosa rugosa* Thunb., is a kind of traditional Chinese medicine with homology of medicine and food in China for a long history. The flowers of *R. rugosa* have long been used in traditional Chinese medicine to treat diarrhea, bleeding, and menstrual pain, as well as for their beautifying and skincare effects [18,19]. Modern scientific research has further revealed that the reason why *R. rugosa* has these traditional effects is closely related to its rich variety of bioactive components with antioxidant activity, such as polyphenols, flavonoids, polysaccharides, and vitamins [20,21]. These are associated with a wide range of health benefits, such as antioxidant, anti-inflammatory, antimicrobial, and antidiabetic activities, as well as protective effects on different tissues [22]. Importantly, these components can eliminate excessive free radicals in the body through multiple mechanisms, such as superoxide anion (O_2^-), hydroxyl radical ($\cdot\text{OH}$), and hydrogen peroxide (H_2O_2), thereby effectively inhibiting lipid peroxidation, preserving cell membrane integrity, and reducing oxidative damage to DNA, proteins, and lipids [23]. In vivo and in vitro studies have shown that *R. rugosa* extract demonstrates potent Total Antioxidant Capacity (TAC) and significantly enhances the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) [24]. Furthermore, its polyphenolic components can enhance the body's endogenous antioxidant defense system by activating the nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway and upregulating the expression of the antioxidant response element (ARE) [25]. Some studies suggest that long-term intake of *R. rugosa*-rich products may be associated with a reduced risk of chronic diseases, such as cardiovascular diseases, neurodegenerative

disorders, and certain cancers, whose pathogenesis is often closely linked to prolonged oxidative stress [26]. Although *R. rugosa* is widely cultivated and abundantly available in China, systematic studies on its antioxidant active constituents remain limited.

Building on previous research [10,11,27], this study established an integrated UHPLC-PDA-Q-TOF/MS-TACD system. This system combines two antioxidant assay methods, namely DPPH and FRAP, for the high-throughput screening of antioxidant components in complex *R. rugosa* samples. The 60% methanol extracts of *R. rugosa* samples from different regions were analyzed, enabling both compositional profiling and structural identification, as well as screening for antioxidant components, in a single run. Preliminary characterization identified 86 compounds, of which 59 exhibited FRAP binding activity and 60 exhibited DPPH radical scavenging activity. In the activity validation assay, gallic acid exhibited the lowest IC₅₀ value and the highest scavenging capacity against DPPH radicals. The rapid discovery of antioxidant components in *R. rugosa* is of great significance for the in-depth study of its pharmacological activities, further development, and utilization. It also provides technical support for the rapid analysis of antioxidant components in natural products with complex matrices.

2. Materials and Methods

2.1. Chemicals and Reagents

Ellagic acid, quercetin 3-O-sophoroside, guaijaverin, astragalin, rutin, quercetin 3-O-rhamnoside and kaempferol 3-O-arabinoside were purchased from Sichuan Weikeyi Biological Technology Co., Ltd. (Chengdu, China). Isoquercitrin and apigenin 7-O- β -D-glucoside were purchased from Chengdu Must Biological Technology Co., Ltd. (Chengdu, China). Hyperoside was purchased from Baoji Herbest Bio-Tech Co., Ltd. (Baoji, China). Quercetin, gallic acid and vitamin C were procured from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). The purities of each standard were determined to be $\geq 98\%$ by HPLC.

1,10-Phenanthroline was obtained from Beijing Solarbio Technology Co., Ltd. (Beijing, China) (Cat: P9950-5 g). Trolox was purchased from Beijing Xinxiyuan Biological Technology Co., Ltd. (Beijing, China) (Cat: B27170-20 mg). DPPH was purchased from Beijing Biotopped Technology Co., Ltd. (Beijing, China) (Cat: D4313G-250 mg). Ferric chloride hexahydrate was purchased from Beijing Ouhe Technology Co., Ltd. (Beijing, China) (BN: 20240409). Chromatographic grade methanol and acetonitrile were purchased from Merck Drugs & Biotechnology (Shanghai, China). Dimethyl sulfoxide was purchased from Tianjin Komeo Chemical Reagent Co., Ltd. (Tianjin, China). LC/MS grade formic acid (FA) was purchased from Fisher Scientific (Thermo Fisher Scientific, Shanghai, China). 0.1 M sodium acetate buffer (pH 4.75) was purchased from Shaanxi Zhonghui Hecai Biomedical Technology Co., Ltd. (Xi'an, China). Ultrapure Water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA). Electronic balance (ME204, Mettler-Toledo, Shanghai, China); High-speed refrigerated centrifuge (KH20R, Hunan Keda Scientific Instrument Co., Ltd., Changsha, China); Vortex mixer (VORTEX-5, Kylin-Bell Lab Instruments Co., Ltd., Nantong, China).

2.2. Sample Information

To ensure sample stability and prevent compound volatilization and degradation, all *R. rugosa* samples were stored sealed under light-protected conditions at 2–8 °C. The samples were sourced and numbered as follows: Shaanxi Rose (SR), Kushui Rose (KS), Pingyin Rose (PR), Phnom Penh Rose (PPR), and Hetian Rose (HR). To ensure sample consistency, each variety was sourced from the same batch, with the harvest time and related information detailed in Table S1. Figure S1 displays five *R. rugosa* samples from

different origins. These five samples were authenticated by Professor Chen Shizhong from Peking University and are stored in the sample repository of the Shaanxi Collaborative Innovation Center for Chinese Medicine Resources Industrialization.

2.3. Preparation of Solution

2.3.1. Sample Preparation

Accurately weigh 2.0 g of *R. rugosa* powder (passed through a 60-mesh sieve) into a stoppered conical flask. Add 25 mL of 60% methanol (*v/v*), record the total weight, and soak for 30 min. Perform ultrasonic extraction for 1 h (300 W, 40 kHz, in one cycle), then allow to cool. Replenish the lost weight with 60% methanol (*v/v*), shake well, and centrifuge for 10 min ($12000 \times g$, 27 °C). Finally, the supernatant was filtered and stored until use.

2.3.2. Preparation of DPPH and FRAP Solutions

Accurately weigh an appropriate amount of DPPH powder and dissolve it in 80% (*v/v*) aqueous acetonitrile to prepare a 1.0 mM stock solution. Dissolve $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in sodium acetate buffer (0.1 M, pH 4.75) to obtain a 1.0 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ stock solution. Prepare a 1.0 mM 1,10-phenanthroline stock solution by dissolving it in HPLC-grade methanol.

2.3.3. Preparation of Reference Standard Solutions

Accurately weigh Trolox powder and dissolve in methanol to prepare a 1.0 mM stock solution. Dissolve each reference substance in methanol to form a solution with a concentration of 1.0 mg/mL, which is to be diluted for use. Before use, filter all solutions through 0.22 μm organic membrane filters and inject 2 μL into the integrated system. All solutions need to be used immediately after preparation, stored in the dark at 4 °C, and diluted to the required concentration according to the needs of offline and coupled methods.

2.4. UHPLC-PDA-Q-TOF/MS-TACD Integrated System Device

The integrated analytical system comprises three modules. The UHPLC-PDA part consists of the Shimadzu analysis system (Kyoto, Japan) for chromatographic analysis, including: a DGU-20A_{5R} degassing unit, a SIL-20AC_{XR} autosampler, three LC-20AD_{XR} high-pressure pumps, an ACQUITY UPLC[®] BEH C18 column (2.1 $\times$ 100 mm, 1.7 μm , Waters, MA, USA) column, two CTO-20AC column ovens, an SPD-M20A diode array detector, a CBM-20A system controller, and an LC-Solution chromatography workstation. The mass spectrometry module incorporated an AB Sciex 5600+Triple-TOF system (Applied Biosystems, Waltham, MA, USA). Antioxidant activity was recorded using the DPPH and FRAP flow paths. This part was composed of two LC-20AD_{XR} pumps, two CTO-20AC column temperature chambers, two reaction coils, and two Shimadzu SPD-10AV_{vp} UV detectors (Shimadzu, Kyoto, Japan). Different parts of the instrument were connected by PEEK pipes of different lengths (0.25 mm i.d.), and each part of the instrument was accurately separated by threaded tees.

The integrated system flowchart is shown (Figure 1). After the sample solution was injected into the system through the autosampler, it passed through the column and PDA detector sequentially to obtain the ultra-high-performance liquid chromatography profile. Mobile phases (A and B) were delivered by infusion pump A at 0.3 mL/min and subsequently divided into three flow paths: the primary flow path (0.1 mL/min) was directed to the mass spectrometer (MS) for total ion current (TIC) chromatogram acquisition; the secondary flow path (0.1 mL/min) was mixed with DPPH solution and fully reacted through the reaction coil. The reaction mixture was detected using a UV-VIS detector to generate a DPPH activity map. After the active ingredients in *R. rugosa* reacted with DPPH, the characteristic absorption intensity of DPPH at 517 nm decreased, resulting

in an inverted peak in the spectrum. The third flow path reacted thoroughly with the FRAP solution in the reaction coil at a flow rate of 0.1 mL/min, yielding an FRAP activity chromatogram upon passing through the detector. The active constituents in *R. rugosa* reduce Fe^{3+} in the FRAP solution to Fe^{2+} , which then reacts with 1,10-phenanthroline to undergo a chromogenic reaction, which exhibits maximum absorption at 510 nm and a positive active spectrum.

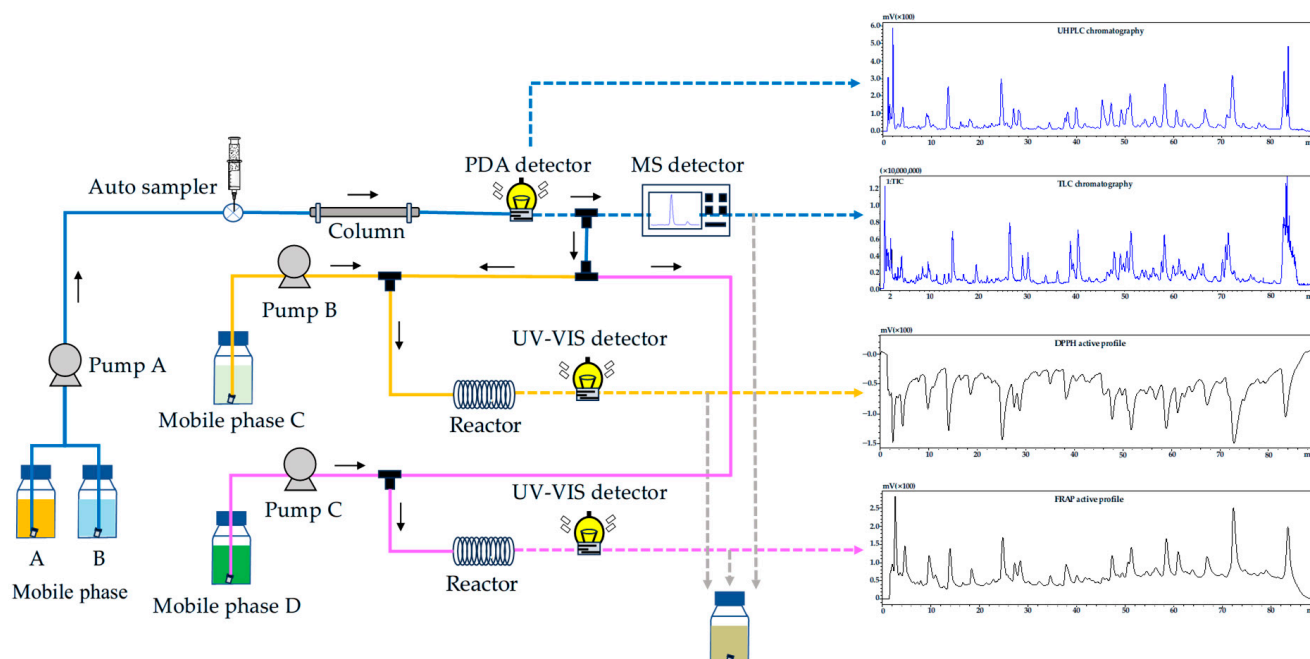


Figure 1. Schematic diagram of the integrated UHPLC-PDA-Q-TOF/MS-TACD system. (UHPLC: ultra-high performance liquid chromatography, PDA: photodiode array detection, MS: mass spectrometry, UV-VIS: ultraviolet-visible spectroscopy, DPPH: 1,1-Diphenyl-2-picrylhydrazyl, FRAP: ferric ion reducing antioxidant power).

2.4.1. UHPLC and Q-TOF/MSⁿ Analysis Conditions

The mobile phase gradient was systematically optimized, and the final chromatographic conditions were established as follows: separation was performed on an ACQUITY UPLC[®] BEH C18 column at a flow rate of 0.3 mL/min. The mobile phase consisted of water containing 0.05% formic acid (A) and acetonitrile (B), with the following gradient elution program: 0–80 min, 3–15% B; 80–84 min, 15–95% B; 84–87 min, 95–95% B; 87–88 min, 95–3% B; 88–90 min 3–3% B. The UV spectrum was scanned between 200 and 800 nm, and the PDA detector was set to monitor at 270 nm. The column temperature was maintained at 35 °C.

LC-MS analysis of *R. rugosa* was performed using an AB SCIEX 5600+ Q-TOF/MS detection system (SCIEX, Framingham, MA 01701, USA). After gradient optimization, data acquisition was performed in the negative ion mode, with a scanning range of m/z 100–2000 Da and a scanning time of 0.15 s. Information-dependent acquisition (IDA), dynamic background subtraction (DBS), and high-sensitivity modes were used for data collection. The conditions for the negative ion mode were as follows: collision energy (CE): −35 eV, −55 eV; collision energy dispersion (CES): 15 V; ion spray voltage floating (ISVF): −4500 V; declustering potential (DP): −80 V; collision gas: high-purity nitrogen (N_2); ion temperature (TEM): 500 °C. In addition, both the nebulizer gas pressure (GS1) and auxiliary gas pressure (GS2) were set to 50 psi; curtain gas (CUR) was set to 35 psi; ion release delay (IRD) and ion release width (IRW) were set to 67 ms and 25 ms, respectively. Finally, PeakView 1.2[®] software (Shanghai AB SCIEX Analytical Instrument Trading Co.,

Ltd., Shanghai, China) was used for data analysis and processing. The compounds were identified based on mass spectrometry data and literature references.

2.4.2. Conditions of Integrated TACD System

The solution entering the TACD system was evenly divided into two streams. The first stream was mixed with a 0.07 mM DPPH solution (flow rate: 0.25 mL/min) in a reaction coil (2 m length, 0.25 mm i.d.) maintained at 60 °C, generating DPPH radical scavenging activity profiles monitored at 517 nm. The second stream was mixed with a FRAP reagent solution (0.10 mM FeCl₃·6H₂O and 0.25 mM 1,10-phenanthroline, flow rate: 0.20 mL/min) delivered by pump C at 30 °C, and the FRAP activity peak spectrum was obtained at 510 nm.

2.5. Activity Validation

The DPPH radical is a stable organic free radical that exhibits a deep purple color in ethanol solution and shows strong absorption at a specific wavelength. When antioxidants are added, the absorption peak decreases, and the degree of decrease directly reflects the free radical scavenging ability of the sample [28]. Thus, the scavenging rate of a compound against DPPH radicals can serve as a key indicator for assessing its antioxidant activity, with a higher scavenging rate indicating greater antioxidant potency.

Each reference standard solution was diluted to seven different mass concentrations (0.50, 0.20, 0.10, 0.05, 0.025, 0.01, 0.005 mg/mL) and stored as test samples for subsequent analysis. For the sample group, 50 µL of the test solution and 100 µL of 0.10 mg/mL DPPH solution were added to a 96-well plate and mixed thoroughly. After incubation at room temperature in the dark for 30 min, the absorbance (A) was measured at 517 nm. The control group consisted of 50 µL of methanol-water (60% v/v) mixed with 100 µL of 0.10 mg/mL DPPH solution, and the blank group contained 150 µL of methanol alone. The DPPH free radical-scavenging rate was calculated using the following formula:

$$\text{Scavenging Rate (\%)} = (A_{\text{control}} - A_{\text{sample}}) / (A_{\text{control}} - A_{\text{blank}}) \times 100\%$$

A_{control} , A_{sample} and A_{blank} were the A values of the control group, the experimental group and the blank group, respectively.

A standard curve was drawn with the clearance rate as the ordinate (Y) and the mass concentration as the abscissa (X), and the IC₅₀ value of DPPH free radicals of each reference substance was calculated.

3. Results and Discussion

3.1. Optimization of Conditions for Integrated TACD Analysis

The types, contents, and activities of active ingredients vary significantly among different screening samples. The corresponding chromatographic separation solvent system and volumetric flow rate can greatly impact the sensitivity and baseline stability of the screening method. Therefore, systematic optimization of DPPH coupled screening conditions is necessary. In this study, the coil length, solvent type, concentration, reaction temperature, and volumetric flow rate were optimized.

3.1.1. Selection of Detection Wavelengths for DPPH and FRAP Activities

DPPH demonstrates a strong characteristic absorption peak at 517 nm. In contrast, the primary components of *R. rugosa*, including flavonoids, tannins, and phenolic acids, display their main ultraviolet absorption in the range of 190–400 nm, with almost no ultraviolet absorption above 500 nm, so it will not interfere with the detection results of the active peak. Finally, 517 nm was selected as the detection wavelength of DPPH activity [4].

The Fe^{2+} -phen complex generated in the FRAP assay exhibits a characteristic absorption peak at 510 nm. Compounds present in *R. rugosa* do not demonstrate absorption at this specific wavelength, thus ensuring no interference with the antioxidant activity detection in the FRAP method. The FRAP method involves the mixing of two solutions; therefore, solution stability was assessed. Specifically, a 0.05 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution (dissolved in 0.1 mM PH 4.75 NaAc buffer solution) and a 0.05 mM 1,10-phenanthroline methanol solution were mixed in equal volumes and stored in the dark at refrigerated conditions (2–8 °C) for subsequent use. Absorbance measurements were conducted hourly to monitor stability. The results demonstrated that under these conditions, the mixed solution of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 1,10-phenanthroline remained stable for 12 h, with no detectable absorbance at 510 nm.

3.1.2. Investigation of Reaction Coil and Solvent Type

The investigation of PEEK (0.25 mm i.d.) tubing with reaction coil lengths of 2, 5, and 10 m (Figure S2) revealed that longer coils resulted in higher active peak response values but also led to decreased chromatographic peak resolution, poor peak shape, and reduced sensitivity. Consequently, the 2 m PEEK tubing was selected as the optimal coil length. Additionally, three acetonitrile concentrations (50%, 80%, and 100%) were evaluated as DPPH solvents for their impact on the baseline (Figure S3). Ultimately, 80% acetonitrile-water was chosen as the solvent for DPPH because of its stable baseline and sharp peak shape.

3.1.3. Optimization of DPPH Conditions

To optimize the experimental conditions related to DPPH in the integrated system, this study investigated the effects of DPPH concentration (0.01, 0.03, 0.05, 0.07 and 0.09 mM), reaction temperature (30, 40, 50, 60 and 70 °C), and flow rate (0.1, 0.15, 0.20, 0.25 and 0.30 mL/min) (Figure 2). As shown in the figure, as the DPPH concentration increases, the inverted peak area shows an increasing trend. When the concentration reaches 0.07 mM, the increase in peak area slows down, so the optimal DPPH concentration is determined to be 0.07 mM. Regarding the reaction temperature, when the temperature of the reaction coil rises to 60 °C, the growth rate of the inverted peak area begins to slow down, so 60 °C is selected as the reaction temperature for the experiment. In the optimization of flow rate, when the flow rate is 0.25 mL/min, the inverted peak area gradually decreases, so 0.25 mL/min is chosen as the final flow rate for DPPH. The stability information of DPPH concentration, reaction temperature and flow rate was presented in Table S2.

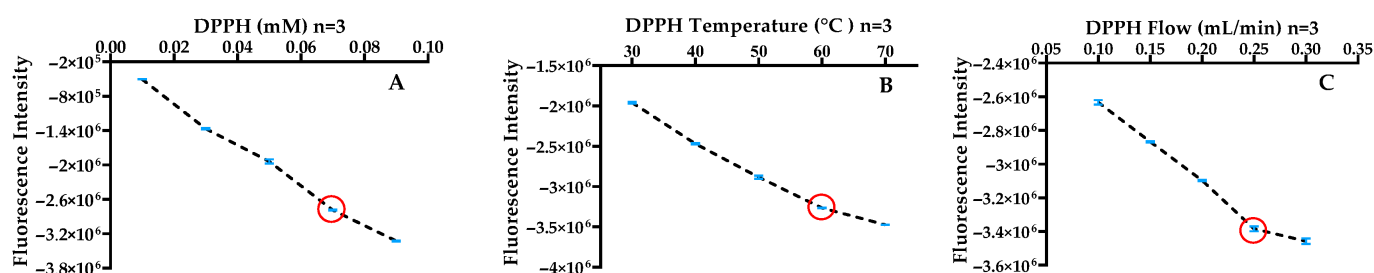


Figure 2. Optimization of DPPH concentration (A) and reaction temperature (B) as well as flow rate (C) in the integrated system. The red circles represent the final optimization results.

3.1.4. Optimization of FRAP Conditions

In addition to the concentrations of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.025, 0.05, 0.10, 0.15 and 0.20 mM) and 1,10-phenanthroline (0.05, 0.15, 0.25, 0.35, 0.45 mM), this study also investigated the reaction temperature (30, 35, 40, 45 and 50 °C) and flow rate (0.10, 0.15, 0.20, 0.25 and 0.30 mL/min) of the FRAP system. The relevant data are recorded in Figure 3 and Table S3. As shown in Figure 3, the peak area increases with the concentration of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. When the concentration reaches 0.10 mM, the increase in peak area levels off. When the concentration of 1,10-phenanthroline exceeds 0.25 mM, the growth rate of the positive peak area decreases significantly. Therefore, the final concentrations of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ and 1,10-phenanthroline are determined to be 0.10 mM and 0.25 mM, respectively. As the reaction temperature increases, the active peak area also increases. However, when the temperature exceeds 40 °C, the rate of increase in the peak area begins to plateau. The active peak area increases with the flow rate, but when the flow rate exceeds 0.20 mL/min, the growth rate of the peak area slows noticeably. Based on the above results, the optimal reaction temperature of the FRAP system was determined to be 40 °C, and the optimal flow rate was determined to be 0.2 mL/min.

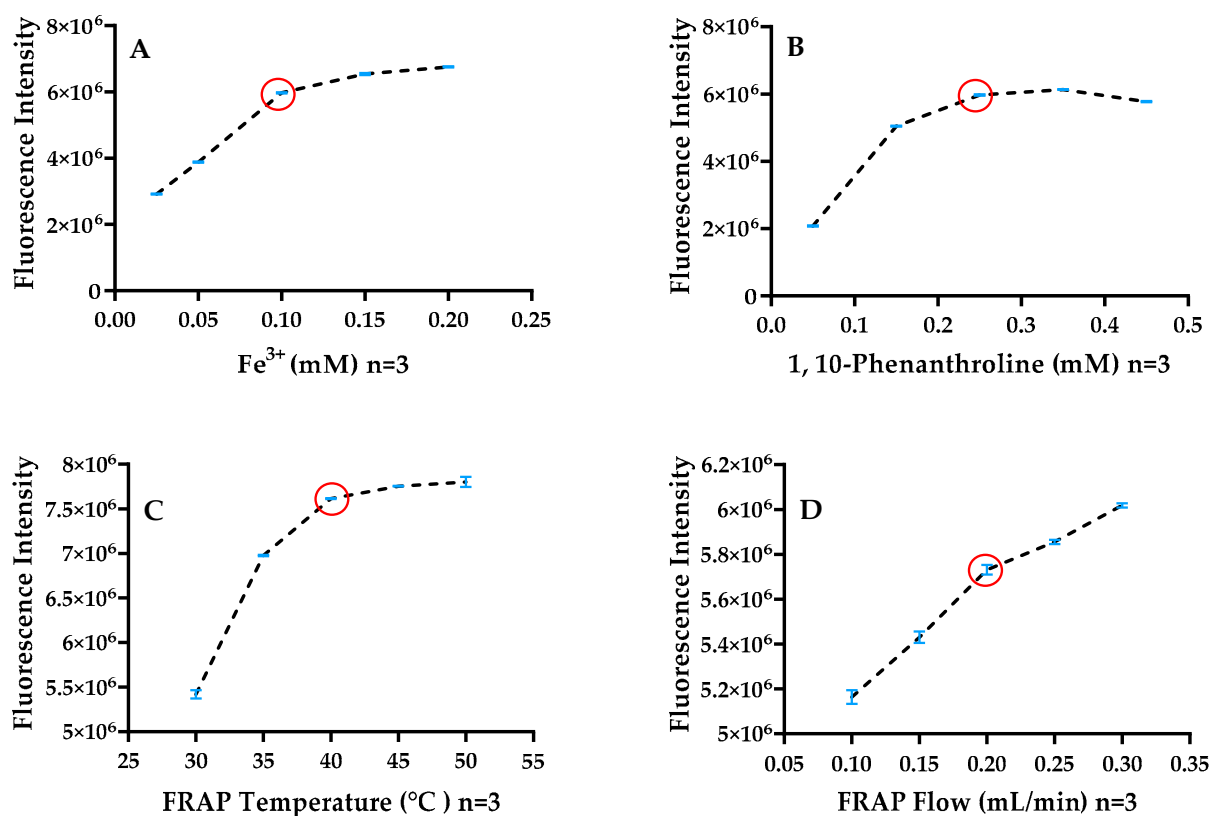


Figure 3. Optimization of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ concentration (A), 1,10-phenanthroline concentration (B), reaction temperature (C) and flow rate (D) of FRAP in the integrated system. The red circles represent the final optimization results.

3.2. Validation of the Integrated TACD Analysis Was Performed Using Positive Controls

The antioxidant capacity of Trolox was first reported in 1993 [29]. Trolox has been shown to inhibit oxidative stress-induced apoptosis, suppress peroxynitrite-mediated oxidative stress and apoptosis in rat thymocytes, and selectively enhance arsenic-induced oxidative stress and apoptosis in acute promyelocytic leukemia and other malignant cell lines. Additionally, it can reverse the motor defects and oxidative stress in the striatum of developing rats exposed to manganese [30–33], which fully shows that Trolox can inhibit oxidative stress. In this study, Trolox [4] was used as a positive control to validate the

established integrated antioxidant detection system (Figure S4). The results showed that Trolox exhibited good reactivity towards both DPPH and FRAP solutions, as evaluated in terms of linearity, precision, stability, and repeatability.

3.2.1. Investigation of Linearity

Linear regression analysis was performed using the amount of Trolox (x , mM) as the independent variable and the UHPLC peak area (y_1), DPPH active peak area (y_2), and FRAP active peak area (y_3) as dependent variables, respectively (Figure S5, Table S4). The results showed the following linear relationships: $y_1 = (363,551 \pm 1605) x$, $R^2 = 0.9998$, $y_2 = (-1,437,175 \pm 9565) x$, $R^2 = 0.9994$ and $y_3 = (1,387,203 \pm 7578) x$, $R^2 = 0.9996$.

3.2.2. Investigation of Precision, Repeatability and Stability

The precision of the integrated system was evaluated by injecting 2 μ L of Trolox solution (1.0 mM) six consecutive times, and the results are presented in Table S5. The RSD values for the UHPLC peak area, DPPH active peak area, and FRAP active peak area were 0.21%, 0.56%, and 0.35%, respectively. Six portions of the same reference substance were accurately weighed and injected in parallel three times to assess the repeatability of the integrated system (Table S5). The RSD values for the UHPLC peak area, DPPH active peak area, and FRAP active peak area were 0.29%, 0.24% and 0.14%, respectively. Meanwhile, the samples were injected at different time points (0 h, 2 h, 4 h, 8 h and 12 h) to investigate the stability of the Trolox solution (Table S5). The RSD values of the UHPLC peak area, DPPH active peak area, and FRAP active peak area within 12 h were calculated to be 0.14%, 0.35% and 0.37%, respectively. The results showed that the method had good precision, repeatability, and stability, and was suitable for analyzing compounds with DPPH and FRAP binding activity.

3.3. Identification of Major Compounds in *R. rugosa*

In this study, an integrated analytical system was employed to perform LC-MS analysis and antioxidant activity screening of *R. rugosa* samples. The UHPLC chromatogram, total ion current (TIC), DPPH radical scavenging activity profile, and FRAP ferric-reducing antioxidant power profile of *R. rugosa* (represented by the SR sample) were obtained and are shown in Figure 4. The detection results of samples from other regions are shown in Figure S6. A preliminary characterization of 86 compounds was achieved (Figure S7), with detailed fragment ion information provided in Table S6. These compounds included 26 flavonoids (flavones: 3; flavonols: 20; dihydroflavonols: 1; catechins: 2), 43 tannins (35 ellagitannins and 8 gallotannins), 9 phenolic acids, and 8 other types of compounds. The structural elucidation of the compounds was carried out as follows: based on the fragment ion information ($[M-H]^-$) and precise molecular masses provided by high-resolution mass spectrometry, the fragmentation patterns were clarified. The compound structures were ultimately identified by comparing the data with literature reports and reference standards. Based on mass spectrometric data and reference literature, the screened antioxidant active compounds were identified. Among them, 59 compounds exhibited FRAP binding activity, and 60 showed DPPH binding activity. Analysis of the characteristic profiles of various compound classes revealed that the antioxidant components identified in *R. rugosa* were predominantly flavonoids and tannins.

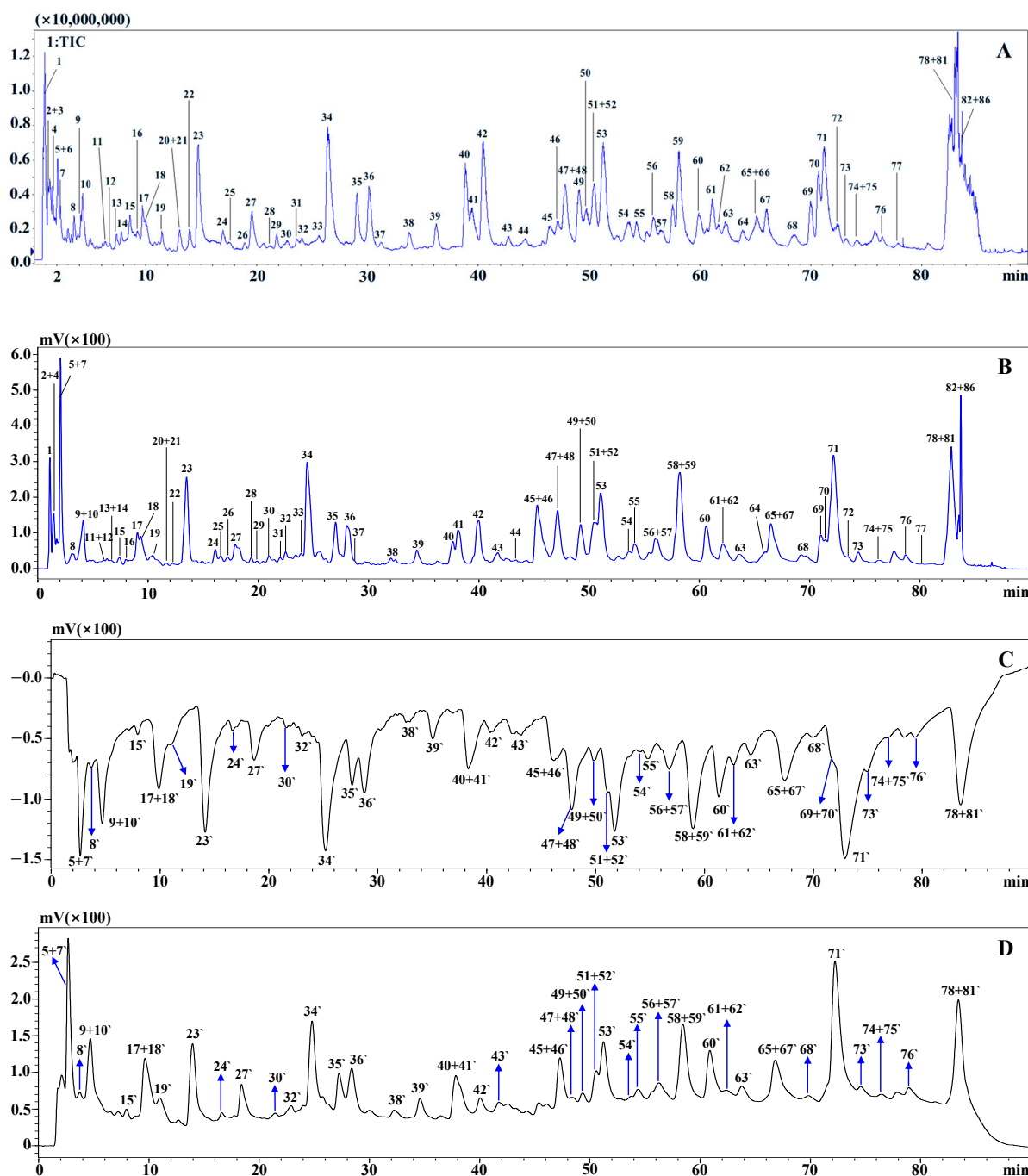


Figure 4. The total ion chromatogram (A), UHPLC chromatogram (B), DPPH active profile (C) and FRAP active profile (D) of *R. rugosa* in the negative ion mode.

3.3.1. Identification of Phenolic Acids

Phenolic acid compounds are prone to lose neutral molecules such as H_2O , $COOH$, and CO_2 during MS fragmentation. Nine phenolic acid compounds were identified in *R. rugosa*, as listed below: Compound 5 exhibited a $[M-H]^-$ ion at m/z 169.0145 ($C_7H_6O_5$). It lost a CO_2 molecule, generating a fragment ion at m/z 125.0245 $[M-H-H_2O-CO]^-$. Based on comparison with standards, compound 5 was tentatively identified as gallic acid [34]. Compound 6 was presented as $[M-H]^-$ at m/z 343.0667, corresponding to the molecular formula $C_{14}H_{13}O_{10}$. m/z 191.0523 $[M-H-galloyl]^-$ and m/z 173.0436 $[M-H-gallic\ acid]^-$ fragment ions were observed in MS^2 . Additionally, a characteristic ion of quinic acid at m/z 127.0365 $[M-H-galloyl-H_2O-CO_2-2H]^-$ was detected. These fragmentation patterns

suggest that the galloyl group is attached to a hydroxyl group of quinic acid. Therefore, compound **6** was tentatively identified as 4-O-galloylquinic acid [34]. Compound **45** showed $[M-H]^-$ at m/z 301.0037 ($C_{14}H_6O_8$), produced fragment ions m/z 285.0511 $[M-H-H_2O]^-$, m/z 257.0504 $[M-H-CO_2]^-$, m/z 229.0102 $[M-H-CO_2-CO]^-$ and m/z 185.0276 $[M-H-2CO_2-CO]^-$, which were consistent with the fragmentation pattern of ellagic acid [35]. Other compounds were shown in Table S6.

3.3.2. Identification of Flavonoids

Flavonoids are mainly composed of aglycones and glycosyl groups, and their general cleavage pattern involves first removing the glycosyl moiety to form the aglycone structure. The aglycone then undergoes further RDA cleavage, rearrangement cleavage, or decarbonylation, losing neutral fragment ions such as $-OCH_3$ and $-OH$. Characteristic fragment ions of quercetin and kaempferol with high relative abundance can be observed in the mass spectrum. A total of 26 flavonoid compounds were identified based on literature data, comparison with reference standards, and database analysis. These include dihydroflavonols (1), flavonols (21), flavonoid glycosides (2), and catechins (2), among which 10 compounds were confirmed using reference standards. The detailed identifications are as follows:

Compound **21** was identified as a dihydroflavonol-*O*-glycoside derivative, showing a $[M-H]^-$ ion at m/z 627.1578. The predicted molecular formula was $C_{27}H_{32}O_{17}$. In the MS^2 spectrum, compound **21** produced a fragment ion at m/z 465.1044 $[M-H-162]^-$ after losing a glucose residue. Subsequently, the loss of another glucose or galactose residue generated an ion peak at m/z 303.0522. Furthermore, fragment ions characteristic of taxifolin were detected at m/z 285, 241, 217, 199, and 175. After comparing the standards, we identified compound **21** as taxifolin 3-*O*-maltoside [36].

Compound **48** exhibits a $[M-H]^-$ ion at m/z 639.1525 ($C_{28}H_{32}O_{17}$). Binding $[M-H-2Glc-CH_3]^-$ m/z 299.9985, was inferred to be dactylin [34]. Compounds **50** and **63** were similarly cleaved by mass spectrometry and were probably isomer, showing $[M-H]^-$ at m/z 433.07 ($C_{20}H_{18}O_{11}$). The fragment ion at m/z 301.01 $[M-H-Xyl]^-$ was observed, indicating the loss of a pentose residue. Additionally, a deprotonated aglycone ion was detected at m/z 300.01. According to the literature [37], this type of radical aglycone ion is typically only observed in quercetin 3-*O*-glycosides. In reversed-phase chromatography, flavonoid-*O*-xylosides are eluted earlier. Thus, we speculated compound **50** was quercetin 3-*O*- β -D-xyloside. Compound **63** was further confirmed as guaijaverin by comparison with a reference standard.

Compound **60** was presented as $[M-H]^-$ at m/z 615.0989. And the molecular formula was calculated as $C_{28}H_{24}O_{16}$. Fragment ions were observed at m/z 463.1024 $[M-H-galloyl]^-$ and m/z 301.0431 $[M-H-galloyl-Glc]^-$, along with a characteristic fragment at m/z 445.0915 $[M-H-170]^-$. This indicates that the galloyl group is attached to the 2'-position of the glucose moiety [38,39]. Thus, compound **60** was speculated to be the quercetin 3-*O*-(2''-gallate)- β -D-glucoside. Compound **79** produced a $[M-H]^-$ ion at m/z 301.0352, corresponding to the molecular formula $C_{15}H_{10}O_7$. A fragment ion at m/z 271.0243 $[M-H-CH_2O]^-$ was observed. In combination with the characteristic fragment ions at m/z 151.0100, m/z 121.0310, and m/z 107.0144, compound **79** was identified as quercetin [40]. Other compounds were shown in Table S6.

Compound **61** gave a $[M-H]^-$ ion at m/z 579.1334 ($C_{26}H_{28}O_{15}$). Fragment ions at m/z 445.0610 $[M-H-Ara]^-$ and m/z 283.0486 $[M-H-Ara-Glu]^-$ were observed, followed by RDA cleavage, which generated a characteristic fragment ion at m/z 151. Thus compound **61** was preliminarily presumed to be carlinoside [34]. Compound **77** was presented as $[M-H]^-$ ion at m/z 431.0967 ($C_{21}H_{20}O_{10}$). The aglycone fragment $[M-H-162]^-$ at m/z 268.1192 was

formed by the loss of a glucose moiety. Thus we speculated compound **77** was apigenin 7-O- β -D-glucoside [41].

Compound **20** exhibited a $[M-H]^-$ ion at m/z 289.0718 ($C_{15}H_{14}O_6$). In the MS² spectrum, the generation of the fragment ion m/z 271.0593 is due to the loss of one H₂O molecule, followed by the loss of CO₂ to yield a fragment at m/z 245.0781. Additional fragment ions at m/z 205.0512, 179.0340, and 165.0202 were also observed, which are characteristic of epicatechin [34]. Compound **57** appeared a $[M-H]^-$ ion at m/z 421.1158 ($C_{20}H_{22}O_{10}$). During mass spectrometric fragmentation, it first lost a xylose moiety, generating a fragment ion at m/z 289.0712 $[M-H-C_5H_8O_4]^-$, followed by the loss of a H₂O molecule to produce a characteristic ion at m/z 271.0557 $[M-H-C_5H_8O_4-H_2O]^-$. compound **57** was finally presumed to be (+)-catechin-7-O- β -D-xyloside or its isomer [34].

3.3.3. Identification of Tannins

The tannins in *R. rugosa* are primarily categorized into gallotannins and ellagitannins. The latter often exist as doubly charged ions, and both classes tend to lose characteristic neutral moieties such as H₂O, CO₂, CO, and Glc [42]. The distinction between gallotannins and ellagitannins lies in the type of phenolic acids generated upon hydrolysis. Gallotannins primarily yield gallic acid upon hydrolysis, whereas ellagitannins produce ellagic acid, which arises from the presence of a hexahydroxydiphenoyl (HHDP) moiety in their hydrolysis products. In mass spectrometric analysis, ellagitannins typically exhibit an ion fragment at m/z 301 $[M-H]^-$, which is indicative of the presence of ellagic acid or the HHDP moiety. This characteristic ion fragment is absent in gallotannins. A total of 43 tannin compounds were identified in *R. rugosa*, including 8 gallotannins and 35 ellagitannins. The detailed identifications are as follows:

Compounds **7**, **9**, and **24** were identified as gallotannins, all showing a $[M-H]^-$ ion at m/z 483.07 ($C_{20}H_{20}O_{14}$). The presence of characteristic fragment ions at m/z 331.06 $[M-H-galloyl]^-$, m/z 169.01 $[M-H-galloyl-Glc]^-$, and m/z 125.02 $[M-H-galloyl-Glc-CO_2]^-$ indicates that all three compounds are di-O-galloylglucosides. Furthermore, compounds **7** and **9** displayed an additional fragment ion at m/z 313.05, corresponding to the loss of H₂O, suggesting the presence of a galloyl group at the 2'-position of the glucose moiety. In contrast, compound **24** lacked this m/z 313.05 fragment, indicating no galloyl substitution at the 2'-position. Therefore, compounds **7**, **9**, and **24** were judged to be di-O-galloylglucoside and its isomers [39,43]. Other compounds were shown in Table S6.

Compounds **10** and **17** exhibited $[M-H]^-$ ions at m/z 783.06 ($C_{34}H_{24}O_{22}$). Combined with the fragment ions at m/z 481.05 and 300.99, corresponding to the loss of an HHDP moiety and a glucose unit, respectively, compounds **10** and **17** were preliminarily identified as pedunculagin and casuariin [44], respectively. Compounds **18** and **27** both show $[M-H]^-$ ions at m/z 633.07 ($C_{27}H_{22}O_{18}$), and their MS² fragmentation patterns were similar, suggesting they are isomers. Key fragment ions were observed at m/z 463.05 $[M-H-galloyl-H_2O]^-$ and m/z 300.99 $[M-H-galloyl-Glc-H_2O]^-$, indicating the presence of a galloyl-HHDP-glucose structure. Thus, compounds **18** and **27** were presumed to be isostrictinin and corilagin [45], respectively.

The molecular ion peaks of compounds **23**, **32**, **34** and **39** exhibit two formation modes, $[M-H]^-$ at m/z 785.08 and $[M-2H]^{2-}$ at m/z 392.03, respectively, with strong signal peaks. These correspond to a doubly charged ion of a compound with a molecular weight of 786, and the calculated molecular formula is $C_{34}H_{26}O_{22}$. The fragment ions at m/z 633.07 and m/z 483.06 are formed by the loss of galloyl and HHDP groups, respectively. In addition, the characteristic fragments $[M-2H-galloyl]^{2-}$ at m/z 316.97, $[M-H-2galloyl-Glc-H_2O]^-$ at m/z 300.99 and $[M-H-galloyl-HHDP-Glc]^-$ at m/z 169.01 was observed. These match the

fragmentation pattern of di-O-galloyl-HHDP-glucose. Therefore, compounds **23**, **32**, **34** and **39** were tentatively speculated to be tellimagrandin I or its isomers [19,46].

Compound **35** and **36** produced the $[M-H]^-$ ions at m/z 953.09 ($C_{41}H_{30}O_{27}$). Characteristic fragment ions at m/z 454.05 $[M-2H-CO_2]^{2-}$ were observed in the MS^2 spectrum, along with diagnostic ions at m/z 392.04 $[M-2H-O-galloyl]^{2-}$, m/z 483.07 $[M-H-2galloyl-Glc]^-$, m/z 382.01 $[M-2H-galloyl-2H_2O]^{2-}$, m/z 300.99 $[M-H-3galloyl-H_2O-O-Glc]^-$, and m/z 169.01 $[M-H-2galloyl-HHDP-O-Glc]^-$ were detected in the MS^3 spectrum. These fragmentation patterns are consistent with those reported for rugosin B [19,46]. Compounds **35** and **36** was presumed to be rugosin B or its isomers.

Compound **41** gave a $[M-H]^-$ ions at m/z 935.0786 ($C_{41}H_{28}O_{26}$), and produced double charged ions $[M-2H]^{2-}$ at m/z 467.0447. The characteristic fragment ions m/z 391.0456, 301.0013 and 257.0170 in the MS^2 spectrum were consistent with casuarictin [47].

Compound **47** appeared $[M-H]^-/[M-2H]^{2-}$ ions at m/z 1103.1139/551.0527 ($C_{48}H_{32}O_{31}$). Fragment ions at m/z 467.0499, m/z 391.5427, and m/z 300.9967 were observed in the MS^2 spectrum. Furthermore, compared to casuarictin, compound **47** exhibited an additional neutral group of 168 Da and displayed a characteristic signal corresponding to the loss of CO_2 . Thus, compound **47** was tentatively identified as rugosin C [45].

Compound **53** produced $[M-H]^-/[M-2H]^{2-}$ at m/z 937.0945/468.0560 ($C_{41}H_{30}O_{26}$). Fragment ions at m/z 767.0854, m/z 615.0785, m/z 316.0287, m/z 300.9975, m/z 257.0126, m/z 229.0224, and m/z 201.0221 were found, respectively. Thus, compound **53** was preliminarily presumed to be tellimagrandin II or its isomer [19,48].

The $[M-H]^-/[M-2H]^{2-}$ of compound **55** and **58** both appeared at m/z 1721.1740/860.1090 ($C_{75}H_{54}O_{48}$). Fragment ions at m/z 937.1215 $[M-H-tellimagrandin I]^-$, m/z 785.0877 $[M-H-tellimagrandin I-galloyl]^-$, m/z 765.0594 $[M-H-tellimagrandin II-H_2O]^-$, m/z 483.0871 $[M-H-tellimagrandin II-HHDP]^-$, and m/z 301.0074 $[M-H-tellimagrandin I-3galloyl-Glc-H_2O]^-$ were observed in the MS^2 spectrum, which are characteristic fragment ions of rugosin E [49,50]. The $[M-H]^-/[M-2H]^{2-}$ of compound **59** was shown at m/z 1105.1039/552.0628 ($C_{48}H_{34}O_{31}$). Fragment ions at m/z 468.0544 $[M-2H-O-galloyl]^{2-}$, m/z 458.0317 $[M-2H-galloyl-2H_2O]^{2-}$, and m/z 300.9946 $[M-H-4galloyl-O-Glc-H_2O]^-$ were detected, respectively. In addition, m/z 530.0617 $[M-2H-CO_2]^-$ was the characteristic fragment ion of rugosin A. Therefore, compound **59** was identified as rugosin A [45,46]. Other compounds were shown in Table S6.

3.3.4. Other

The $[M-H]^-$ ion of compound **2** was shown at m/z 191.0190 ($C_6H_8O_7$). It generated fragment ions at m/z 173.0075 $[M-H-H_2O]^-$, m/z 154.9974 $[M-H-2H_2O]^-$, and m/z 111.0092 $[M-H-H_2O-H_2O-CO_2]^-$. Therefore, compound **2** was tentatively identified as citric acid [34]. The $[M-H]^-$ ion of compound **28** was observed at m/z 337.0929, and the molecular formula was calculated as $C_{16}H_{18}O_8$. Following the loss of the trans-p-hydroxycinnamoyl moiety, fragment ions at m/z 191.0508 $[M-H-Quinic acid-H]^-$, m/z 173.0356 $[M-H-Quinic acid-H_2O-H]^-$, and m/z 119.0514 $[M-H-C_8H_{11}O_7]^-$ were detected in MS^2 . Consequently, compound **28** was preliminarily identified as 3-O-coumaroylquinic acid [4]. The $[M-H]^-$ ion of compound **29** was produced at m/z 401.1476 ($C_{16}H_{18}O_8$). Loss of the arabinosyl moiety generates a fragment ion at m/z 269.1019 $[M-H-Ara]^-$. Additionally, a characteristic fragment ion at m/z 161.0497 $[M-H-Ara-pyrogallol]^-$ was detected, indicating the presence of a hexose residue. The ion at m/z 269.1019 is postulated to be a fragment of phenethyl glucoside. Based on previous studies [6,51], compound **29** was preliminarily presumed to be benzyl-6'-O- α -L-arabinofuranosyl- β -D-glucoside. The $[M-H]^-$ of compound **84** appeared at m/z 487.3409 ($C_{30}H_{48}O_5$). The fragment ions at m/z 469.3489 $[M-H-H_2O]^-$ and m/z 423.3410 $[M-H-H_2O-COOH]^-$ were generated, leading to its tentative identifi-

cation as asiatic acid [52]. Compounds **13**, **82**, **85**, and **86** were tentatively assigned as 3-(2-(β -D-glucopyranosyloxy)-3-hydroxyphenyl) propanoic acid, docosapentaenoic acid, 9-kode, and 3-O-coumaroylasiatic acid, respectively, based on their molecular formulas, fragment ion patterns, and supporting references [34,53,54].

3.4. Studies on Active Components in *R. rugosa* Samples

In the activity profile, the peak area of each peak represents the antioxidant capacity of the corresponding compound in scavenging DPPH radicals or reducing Fe^{3+} . The total area of all peaks reflects the overall antioxidant capacity of each sample. A total of 60 compounds with DPPH radical scavenging activity and 59 compounds with Fe^{3+} reducing capacity were screened from *R. rugosa*. The number of DPPH and FRAP active compounds across the five origins is shown in Table S7, with the highest number of active compounds observed in SR and KS. In total, 32 common active compounds were identified among the five *R. rugosa* origins. The identified active compounds were predominantly tannins and flavonoids. Among them, ellagitannins and gallotannins were the most potent contributors to antioxidant activity, accounting for more than 50% of the total antioxidant capacity of the samples (55.00% by DPPH assay and 52.54% by FRAP assay). Flavonoids were the second major contributors, representing 33.33% of the total antioxidant activity by the DPPH method and 35.59% by the FRAP method. Phenolic acids contributed 11.66% to the total antioxidant activity. The relative contributions of each active compound class are illustrated in Table S8. Interestingly, both the UHPLC chromatographic peaks and antioxidant activity peak areas of tellimagrandin I and its isomers were relatively large. In the SR samples, 58 and 56 compounds exhibited DPPH and FRAP activities, respectively. Among them, 56 active compounds were common to both assays, while 2 compounds displayed activity only in the DPPH assay and not in the FRAP assay. This indicates that the FRAP method is more selective, whereas the DPPH method has higher sensitivity. The mechanisms of antioxidant activity of different types of compounds may be different. In this study, two methods were used to screen the antioxidant active components in *R. rugosa* samples, and the antioxidant mechanism of active components was reflected from different angles, which could reflect the antioxidant capacity of active components more comprehensively.

3.5. Evaluation of Activity

The antioxidant activity of individual compounds was evaluated using an in vitro DPPH radical scavenging assay. Based on the cost and availability, we selected 7 commercially available compounds to determine the DPPH free radical scavenging activity and their IC_{50} values were calculated (Table 1). The results indicated that all seven compounds exhibited good antioxidant activity, showing a clear dose-dependent relationship within the tested concentration range. The IC_{50} value of gallic acid against DPPH free radicals was 0.019 mM, which was the smallest, indicating that its antioxidant activity was the strongest. Compared with the antioxidant activity of vitamin C, gallic acid, ellagic acid, quercetin 3-O-rhamnoside, and rutin exhibited better activity. The order of IC_{50} values (from smallest to largest) was as follows: gallic acid < ellagic acid < quercetin 3-O-rhamnoside < rutin < vitamin C < quercetin 3-O-sophoroside < quercetin < guaijaverin.

Table 1. Verification of antioxidant activity of compounds screened from *R. rugosa*.

	Compounds	DPPH IC ₅₀ (mM)	DPPH Clearance Rate/% ^a
5	Gallic acid	0.019 ± 0.0018	69 ± 0.41
40	Quercetin 3-O-sophoroside	0.050 ± 0.00035	22 ± 0.46
45	Ellagic acid	0.025 ± 0.0011	56 ± 0.85
49	Rutin	0.046 ± 0.0005	24 ± 0.51
63	Guaijaverin	0.057 ± 0.0037	29 ± 1.5
66	Quercetin 3-O-rhamnoside	0.043 ± 0.0041	34 ± 1.6
79	Quercetin	0.055 ± 0.0049	36 ± 2.9
Y	Vitamin C	0.046 ± 0.0011	55 ± 0.49

Note: ^a indicates DPPH clearance at a sample concentration of 10 mg·L⁻¹; Y denotes the positive control drug; Data are presented as mean ± SD.

4. Conclusions

In this study, an integrated antioxidant component screening method based on UHPLC-PDA-Q-TOF/MS-TACD technology was established and applied for the rapid identification of active antioxidant compounds in *R. rugosa* extracts. The established platform enables efficient separation at low flow rates, which not only reduces solvent consumption but also minimizes baseline noise typically associated with higher flow rates, thereby significantly improving both the resolution and sensitivity of the integrated screening process. Using this approach, a total of 86 compounds were characterized from *R. rugosa* extracts, including 26 flavonoids, 43 tannins, 9 phenolic acids, and 8 compounds from other structural classes. Using this platform, 60 and 59 compounds with DPPH and FRAP antioxidant activity, respectively, were screened from *R. rugosa* extracts sourced from five different origins. In vitro activity experiments of seven components were carried out. Gallic acid exhibited the lowest IC₅₀ value and the highest scavenging rate against DPPH radicals, indicating that it had the strongest antioxidant activity. Beyond its specific application to *R. rugosa*, this study establishes a robust and versatile methodological framework that can be extended to antioxidant screening in other complex natural matrices. The workflow demonstrated in this study combines high-resolution separation, component characterization, and activity detection, and has significant progress in speed, efficiency, and information richness compared to traditional bioactivity-guided methods. These findings not only underscore the value of *R. rugosa* as a source of bioactive compounds but also provide a reliable technical foundation for accelerating the discovery of natural antioxidants in plant extracts and other challenging samples.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/analytica6040045/s1>.

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References

1. Park, H.E.; Hyun, J.A.; Kang, E.B.; Kwon, H.J.; Beom, S.H.; Han, D.G.; An, B.J. Anti-inflammatory and antioxidant activities of *Rosa multiflora*. *Food Sci. Preserv.* **2022**, *29*, 327–338. [\[CrossRef\]](#)
2. Simatupang, N.T.; Limanan, D.; Yulianti, E.; Ferdinal, F. Methanolic Extracts of Rose Flowers (*Rosa chinensis* Jacq.): Phytochemical Evaluation and Total Antioxidant Capacity. In Proceedings of the 1st Tarumanagara International Conference on Medicine and Health (TICMIH 2021), Jakarta, Indonesia, 5–6 August 2021; Atlantis Press: Paris, France, 2021; pp. 29–32.
3. Niu, S.M.; Zhu, S.H.; Li, W.; Wang, D.; Wang, C.H.; Liu, F. Studies on antioxidant effects and mechanism of chinese herb *Rosa rugosa* Thunb. *Acta Sci. Nat. Univ. Nankaiensis* **2004**, *37*, 29–33.
4. Sun, M.M.; Lei, X.Y.; Lan, X.; Lin, Z.T.; Xu, H.B.; Chen, S.Z. Online identification of potential antioxidant components and evaluation of DNA oxidative damage protection ability in *Prunus persica* flowers. *Talanta* **2024**, *280*, 126702. [\[CrossRef\]](#)
5. Nong, S.S.; Gong, Z.L.; Zhou, J.H.; Li, R.; Huang, S.Y. Study on Tanshinone in Vitro Antioxidant Activity. *Chem. World* **2013**, *54*, 132–134. [\[CrossRef\]](#)
6. Luo, Y.K.; Wang, H.; Li, X.; He, T.; Wang, D.D.; Wang, W.W.; Jia, W.J.; Lin, Z.T.; Chen, S.Z. One injection to profile the chemical composition and dual-antioxidation activities of *Rosa chinensis* Jacq. *J. Chromatogr. A* **2020**, *1613*, 460663. [\[CrossRef\]](#)
7. Yang, H.D.; Tang, Z.S.; Xue, T.T.; Zhu, Y.Y.; Su, Z.H.; Xu, H.B. Acyl-quinic acids from the root bark of *Acanthopanax gracilistylus* and their inhibitory effects on neutrophil elastase and cyclooxygenase-2 in vitro. *Bioorganic Chem.* **2023**, *140*, 106798. [\[CrossRef\]](#)
8. Yang, H.D.; Tang, Z.S.; Xue, T.T.; Xu, H.L.; Hou, B.L.; Zhu, Y.Y.; Su, Z.H.; Xu, H.B. Monoterpenoids from the root bark of *Acanthopanax gracilistylus* and their inhibitory effects on neutrophil elastase, 5-lipoxygenase, and cyclooxygenase-2 in vitro. *Phytochemistry* **2023**, *215*, 113851. [\[CrossRef\]](#)
9. Zhu, Y.Y.; Yang, Y.G.; Tang, Z.S.; Xue, T.T.; Chen, S.Z.; Yang, H.D.; Su, Z.H.; Xu, H.B. UHPLC–PDA–Q-TOF–MS– α -amylase–FLD activity detection system and molecular docking. *Phytochem. Anal.* **2024**, *35*, 1457–1471. [\[CrossRef\]](#)
10. Su, Z.H.; Xu, H.B.; Chen, S.Z.; Li, S.M.; Weng, J.Y.; Yang, Y.G. Screening Inhibitors of α -Amylase in *Polygala Radix* Based on an Online Targeted Detection System and Molecular Docking. *Phytochem. Anal.* **2025**, *36*, 759–775. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Sun, M.M.; Yuan, M.M.; Wang, H.; Yin, R.X.; Yan, C.J.; Yin, M.H.; Su, Z.H.; Lei, X.Y.; Fu, X.Y.; Sun, S.F. Development of an online UPLC–PDA–ESI–Q-TOF–MS–LOX–FLD system for rapid screening of anti-inflammatory compounds in *Polygala tenuifolia* Willd. *J. Pharm. Biomed. Anal.* **2023**, *229*, 115353. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Xavier, J.d.A.; Valentim, I.B.; Camatari, F.O.; de Almeida, A.M.; Goulart, H.F.; Ferro, J.N.D.S.; Barreto, E.D.O.; Cavalcanti, B.C.; Bottoli, C.B.; Goulart, M.O.F. Polyphenol profile by UHPLC–MS/MS, anti-glycation, antioxidant and cytotoxic activities of several samples of propolis from the northeastern semi-arid region of Brazil. *Pharm. Biol.* **2017**, *55*, 1884–1893. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Vargas-Arana, G.; Merino-Zegarra, C.; Tang, M.; Pertino, M.W.; Simirgiotis, M. UHPLC–MS characterization, and antioxidant and nutritional analysis of cocoa waste flours from the Peruvian Amazon. *Antioxidants* **2022**, *11*, 595. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Geng, D.D.; Chi, X.F.; Dong, Q.; Hu, F.Z. Antioxidants screening in *Limonium aureum* by optimized on-line HPLC–DPPH assay. *Ind. Crops Prod.* **2015**, *67*, 492–497. [\[CrossRef\]](#)
15. Fan, W.F.; Zhou, J.Q.; Wu, Z.; Tan, G.Y.; Li, H.X.; Mei, Q.X.; Qian, Z.M. Analysis of antioxidants in *Chrysanthemum indic* flos by online gradient extraction and HPLC–FRAP. *Anal. Methods* **2021**, *13*, 2283–2289. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Le Grandois, J.; Guffond, D.; Hamon, E.; Marchioni, E.; Werner, D. Combined microplate-ABTS and HPLC-ABTS analysis of tomato and pepper extracts reveals synergetic and antagonist effects of their lipophilic antioxidative components. *Food Chem.* **2017**, *223*, 62–71. [\[CrossRef\]](#)
17. Zhang, M.M.; Cheng, S.P.; Zhao, Z.G.; Liu, Q.; Liu, W.; Wang, X.; Zhao, H.Q. Rapid discovery of antioxidants in *Salvia miltiorrhiza* by UPLC–DPPH–PDA–ESI–TOF/MS online technique. *Chin. Tradit. Herb. Drugs* **2020**, *51*, 2908–2913.
18. Kim, E.; Mok, H.K.; Hyun, T.K. Variations in the antioxidant, anticancer, and anti-inflammatory properties of different *Rosa rugosa* organ extracts. *Agronomy* **2022**, *12*, 238–246. [\[CrossRef\]](#)
19. Ayati, Z.; Amiri, M.S.; Ramezani, M.; Delshad, E.; Sahebkar, A.; Emami, S.A. Phytochemistry, traditional uses and pharmacological profile of rose hip: A review. *Curr. Pharm. Des.* **2018**, *24*, 4101–4124. [\[CrossRef\]](#)
20. Um, M.; Han, T.H.; Lee, J.W. Ultrasound-assisted extraction and antioxidant activity of phenolic and flavonoid compounds and ascorbic acid from rugosa rose (*Rosa rugosa* Thunb.) fruit. *Food Sci. Biotechnol.* **2018**, *27*, 375–382. [\[CrossRef\]](#)
21. Czyzowska, A.; Klewicka, E.; Pogorzelski, E.; Nowak, A. Polyphenols, vitamin C and antioxidant activity in wines from *Rosa canina* L. and *Rosa rugosa* Thunb. *J. Food Compos. Anal.* **2015**, *39*, 62–68. [\[CrossRef\]](#)
22. Li, C.; Luo, Y.; Zhang, W.; Cai, Q.; Wu, X.; Tan, Z.; Chen, R.; Chen, Z.; Wang, S.; Zhang, L. A comparative study on chemical compositions and biological activities of four essential oils: *Cymbopogon citratus* (DC.) Stapf, *Cinnamomum cassia* (L.) Presl, *Salvia japonica* Thunb. and *Rosa rugosa* Thunb. *J. Ethnopharmacol.* **2021**, *280*, 114472–114481. [\[CrossRef\]](#)
23. Schieber, M.; Chandel, N.S. ROS function in redox signaling and oxidative stress. *Curr. Biol.* **2014**, *24*, R453–R462. [\[CrossRef\]](#)
24. Zhang, J.; Yu, X.; Guan, Y.; Rui, X.; Zhang, Y.; Dong, M.; Ma, W. An aqueous polyphenol extract from *Rosa rugosa* tea has antiaging effects on *Caenorhabditis elegans*. *J. Food Biochem.* **2019**, *43*, e12796. [\[CrossRef\]](#)

25. Li, H.; Xie, Z.; Zhang, Y.; Liu, Y.; Niu, A.; Liu, Y.; Zhang, L.; Guan, L. *Rosa rugosa* polysaccharide attenuates alcoholic liver disease in mice through the gut-liver axis. *Food Biosci.* **2021**, *44*, 101385. [\[CrossRef\]](#)
26. Zhao, X.; Jiang, Y.; Qiao, M.; Lin, F.; Miao, B. Recent Advances in Bioactive Compounds, Health Functions, and Utilization of Rose (*Rosa* spp.). *Molecules* **2025**, *30*, 3869. [\[CrossRef\]](#)
27. Zhang, X.X.; Lin, Z.T.; Fang, J.G.; Liu, M.X.; Niu, Y.Y.; Chen, S.Z.; Wang, H. An on-line high-performance liquid chromatography–diode-array detector–electrospray ionization–ion-trap–time-of-flight–mass spectrometry–total antioxidant capacity detection system applying two antioxidant methods for activity evaluation of the edible flowers from *Prunus mume*. *J. Chromatogr. A* **2015**, *1414*, 88–102. [\[CrossRef\]](#)
28. Chen, Y.; Wang, M.F.; Rosen, R.T.; Ho, C.T. 2,2-Diphenyl-1-picrylhydrazyl radical-scavenging active components from *Polygonum multiflorum* thunb. *J. Agric. Food Chem.* **1999**, *47*, 2226–2228. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Huang, D.J.; Ou, B.X.; Prior, R.L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Forrest, V.J.; Kang, Y.H.; McClain, D.E.; Robinson, D.H.; Ramakrishnan, N. Oxidative stress-induced apoptosis prevented by Trolox. *Free Radic. Biol. Med.* **1994**, *16*, 675–684. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Salgo, M.G.; Pryor, W.A. Trolox inhibits peroxynitrite-mediated oxidative stress and apoptosis in rat thymocytes. *Arch. Biochem. Biophys.* **1996**, *333*, 482–488. [\[CrossRef\]](#)
32. Diaz, Z.; Colombo, M.; Mann, K.K.; Su, H.X.; Smith, K.N.; Bohle, D.S.; Schipper, H.M.; Miller, W.H., Jr. Trolox selectively enhances arsenic-mediated oxidative stress and apoptosis in APL and other malignant cell lines. *Blood* **2005**, *105*, 1237–1245. [\[CrossRef\]](#)
33. Cordova, F.M.; Aguiar, A.S.; Peres, T.V.; Lopes, M.W.; Gonçalves, F.M.; Pedro, D.Z.; Lopes, S.C.; Pilati, C.; Prediger, R.D.; Farina, M. Manganese-exposed developing rats display motor deficits and striatal oxidative stress that are reversed by Trolox. *Arch. Toxicol.* **2013**, *87*, 1231–1244. [\[CrossRef\]](#)
34. Zhao, L.L.; Sun, J.L.; Zhao, S.Y.; Zhao, P.; Liu, J.T.; Liu, H.Y. Qualitative and Quantitative Analysis of Chemical Constituents in *Rosa rugosa* by UPLC-Q-Exactive Orbitrap-MS and UPLC-QQQ-MS Methods. *Mod. Food Sci. Technol.* **2024**, *40*, 379–397. [\[CrossRef\]](#)
35. Yisimayili, Z.; Abdulla, R.; Tian, Q.; Wang, Y.Y.; Chen, M.C.; Sun, Z.L.; Li, Z.X.; Liu, F.; Aisa, H.A.; Huang, C.G. A comprehensive study of pomegranate flowers polyphenols and metabolites in rat biological samples by high-performance liquid chromatography quadrupole time-of-flight mass spectrometry. *J. Chromatogr. A* **2019**, *1604*, 460472. [\[CrossRef\]](#)
36. Desta, K.T.; Kim, G.S.; Abd El-Aty, A.; Raha, S.; Kim, M.B.; Jeong, J.H.; Warda, M.; Hacımüftüoğlu, A.; Shin, H.C.; Shim, J.H. Flavone polyphenols dominate in *Thymus schimperii* Ronniger: LC–ESI–MS/MS characterization and study of anti-proliferative effects of plant extract on AGS and HepG2 cancer cells. *J. Chromatogr. B* **2017**, *1053*, 1–8. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Yang, W.Z.; Qiao, X.; Bo, T.; Wang, Q.; Guo, D.; Ye, M. Low energy induced homolytic fragmentation of flavonol 3-O-glycosides by negative electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **2014**, *28*, 385–395. [\[CrossRef\]](#)
38. Qing, L.S.; Xue, Y.; Zhang, J.G.; Zhang, Z.F.; Liang, J.; Jiang, Y.; Liu, Y.M.; Liao, X. Identification of flavonoid glycosides in *Rosa chinensis* flowers by liquid chromatography–tandem mass spectrometry in combination with ¹³C nuclear magnetic resonance. *J. Chromatogr. A* **2012**, *1249*, 130–137. [\[CrossRef\]](#)
39. Ferreres, F.; Llorach, R.; Gil-Izquierdo, A. Characterization of the interglycosidic linkage in di-, tri-, tetra- and pentaglycosylated flavonoids and differentiation of positional isomers by liquid chromatography/electrospray ionization tandem mass spectrometry. *J. Mass Spectrom.* **2004**, *39*, 312–321. [\[CrossRef\]](#) [\[PubMed\]](#)
40. Guan, Q.H.; Tang, L.H.; Xu, M.; Zhang, L.L.; Huang, L.X.; Khan, M.S. Comparison of binding sites and affinity of flavonol–Cu (II) complexes with the same parent nucleus: Synthesis, DFT prediction, and coordination pattern. *Food Chem.* **2024**, *442*, 138453. [\[CrossRef\]](#)
41. Karaźniewicz-Łada, M.; Wójtowski, J.A.; Głowska, F.; Danków, R.; Pikul, J.; Gryszczyńska, A.; Foksowicz-Flaczyk, J.; Mikołajczak, P.Ł. Application of UPLC-MS/MS method for analysis of apigenin, apigenin 7-glucoside and chlorogenic acid in goat serum. *Chromatographia* **2023**, *86*, 401–411. [\[CrossRef\]](#)
42. Cao, D.; Zhao, C.J.; Wang, H.Y.; Wei, B.W.; Jiang, Y.Y.; Lin, R.C.; Li, J. Chemical constituents in She medicine *Melastoma dodecandrum* Lour. by UPLC-Q-TOF-MS. *Cent. South Pharm.* **2021**, *19*, 1806–1810.
43. Zan, L.F.; Yang, X.Y.; Guo, H.Y.; Wang, Y.D.; Ye, J. Characterization of Chemical Constituents from Fruits of *Rosa xanthina* by UPLC-Q-TOF-MS. *Sci. Technol. Food Ind.* **2021**, *42*, 251–258. [\[CrossRef\]](#)
44. Yoshida, T.; Tanaka, K.; Chen, X.M.; Okuda, T. Tannins of Rosaceus Medicinal Plants. V.: Hydrolyzable Tannins with Dehydrodi-galloyl Group from *Rosa laevigata* MICHX. *Chem. Pharm. Bull.* **1989**, *37*, 920–924. [\[CrossRef\]](#)
45. Okuda, T.; Hatano, T.; Yazaki, K.; Ogawa, N. Rugosin A, B, C and praecoxin A, tannins having a valoneoyl group. *Chem. Pharm. Bull.* **1982**, *30*, 4230–4233. [\[CrossRef\]](#)
46. Kato, E.; Uenishi, Y.; Inagaki, Y.; Kurokawa, M.; Kawabata, J. Isolation of rugosin A, B and related compounds as dipeptidyl peptidase-IV inhibitors from rose bud extract powder. *Biosci. Biotechnol. Biochem.* **2016**, *80*, 2087–2092. [\[CrossRef\]](#)

47. Yoshida, T.; Feng, W.S.; Okuda, T. Two polyphenol glycosides and tannins from *Rosa cymosa*. *Phytochemistry* **1993**, *32*, 1033–1036. [\[CrossRef\]](#)
48. D'Urso, G.; Sarais, G.; Lai, C.; Pizza, C.; Montoro, P. LC-MS based metabolomics study of different parts of myrtle berry from Sardinia (Italy). *J. Berry Res.* **2017**, *7*, 217–229. [\[CrossRef\]](#)
49. Cendrowski, A.; Ścibisz, I.; Mitek, M.; Kieliszek, M.; Kolniak-Ostek, J. Profile of the phenolic compounds of *Rosa rugosa* petals. *J. Food Qual.* **2017**, *2017*, 7941347. [\[CrossRef\]](#)
50. Oracz, J.; Żyżelewicz, D.; Pacholczyk Sienicka, B. UHPLC-DAD-ESI-HRMS/MS profile of phenolic compounds in northern red oak (*Quercus rubra* L., syn. *Q. borealis* F. Michx) seeds and its transformation during thermal processing. *Ind. Crops Prod.* **2022**, *189*, 115860. [\[CrossRef\]](#)
51. Liu, M.Z.; Song, X.; Jiang, Y.Y.; Cao, Y.B.; Yan, T.H. Research progress on chemical constituents and pharmacological activities of *Rosa chinensis*. *J. Pharm. Pract.* **2015**, *33*, 198–200. [\[CrossRef\]](#)
52. Liang, G.P.; Wang, W.; Wu, J.L.; Liu, J.; Yang, J. Rapid identification on chemical compounds in the extracts from viburnum foetidum fruits with anti-tumor activities based on UPLC-ESI-HRMSⁿ. *J. Shaanxi Univ. Science Technol.* **2022**, *40*, 70–77. [\[CrossRef\]](#)
53. Faqueti, L.G.; Sandjo, L.P.; Biavatti, M.W. Simultaneous identification and quantification of polymethoxyflavones, coumarin and phenolic acids in *Ageratum conyzoides* by UPLC-ESI-QToF-MS and UPLC-PDA. *J. Pharm. Biomed. Anal.* **2017**, *145*, 621–628. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Du, S.B.; Chen, X.W.; Dong, Z.Q.; Xie, H.C. Analysis of chemical components in alcohol extract of *Rosa sertata* × *Rosa rugosa* based on UPLC-Q-TOF-MS. *Nat. Prod. Res. Dev.* **2022**, *34*, 1129–1142. [\[CrossRef\]](#)

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