Determination of the volatile and polyphenol constituents and the antimicrobial, antioxidant, and tyrosinase inhibitory activities of the bioactive compounds from the by-product of *Rosa rugosa* Thunb. var. *plena* Regal tea

Guixing Ren1,4*, Peng Xue2,4†, Xiaoyan Sun4 and Gang Zhao3*

**Abstract**

**Background:** The phytochemical constituents and biological activities of *Rosa rugosa* Thunb. var. *plena* Regal flower cell sap (RFCS) were investigated.

**Methods:** Volatile constituent, such as linalool, phenylethyl alcohol, citronellol, α-bisabolol, were identified by GC-MS. The contents of hyperoside, kaempferol-3-O-rutinosid, rutin, and luteolin as well as the total flavonoid content in RFCS were determined by HPLC and HPLC-MS. The total polyphenol content was evaluated by the Folin-Ciocalteu colorimetric method. The antioxidant activities of RFCS and the standards were evaluated by DPPH and ABTS radical scavenging assays. The tyrosinase inhibitory activities of the rose samples and standard substances were determined by a spectrophotometric method. The antimicrobial effects of RFCS were evaluated in terms of minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) or minimum Fungicidal concentrations (MFCs).

**Results:** The rose fraction exhibited a high content of biologically active ingredients. The total content of volatile compounds in RFCS was approximately 48.21 ± 2.76 ng/mL. The total phenolic acid content and total flavonoid content were 0.31 ± 0.01 mg/mL and 0.43 ± 0.01 mg/mL, respectively. Its IC50 value in the DPPH assay was 1120 ± 42 μg/mL, and its IC50 value for ABTS radical scavenging activity was 1430 ± 42 μg/mL. RFCS strongly inhibited L-tyrosine oxidation with an IC50 value of 570 ± 21 μg/mL. Every compound identified in RFCS exhibited broad-spectrum antimicrobial activity. *F. nucleatum* was most susceptible to RFCS with an MIC of 64 μg/mL and MBC of 250 μg/mL.

**Conclusions:** Due to its rose-like aroma, phenylethyl alcohol may be combined with linalool for use as a natural skin-whitening agent and skin care additive in the and pharmaceutical industries.

**Keywords:** RFCS, Phytochemical constituents, Antioxidant, Antimicrobial, Tyrosinase inhibitory activities

* Correspondence: renguixing@cdue.edu.cn; zhaogang@cdue.edu.cn
† Guixing Ren and Peng Xue contributed equally to this work.
1 College of Pharmacy and Biological Engineering, Chengdu University, Chengdu 610000, People’s Republic of China
2 Key Laboratory of Coarse Cereal Processing, Ministry of Agriculture, No.2025 Chengluo Road, Longquanly District, Chengdu 610106, People’s Republic of China
3 Full list of author information is available at the end of the article

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Background
The flower of *Rosa rugosa* Thunb. var. plena Regal is not only used in perfume production but has also been used as a health food and medicine in Asian countries for thousands of years. Additionally, roses contain active materials, such as essential oils, polyphenols, flavonoids and anthocyanin, which are known for their antimicrobial, anti-inflammatory, hypoglycemic, and antioxidant activities [1–4]. Roses can be consumed in many forms, such as rose teas, rose cookies, and rose oils. The production of rose tea or dried flower petals via low-temperature drying of rose flowers (*Rosa rugosa* cv. *Plena*) yields a condensate called “rose flower cell sap” (RFCS). The disposal of RFCS represents a great waste of resources due to its high content of polyphenols and rose essential oil, which has very high biological activity. In addition, environmental pollution may be caused by the improper disposal of RFCS because it is difficult to decompose. In addition, essential oils and polyphenols are active ingredients in the pharmaceutical, cosmetic, and food industries. The drying of 1 kg of raw rose petals or flower bud material can produce approximately 0.2 L of condensate. Approximately 40,000 kg of rose flower buds and 20,000 kg of petals are used per cycle of industrial microwave-drying in Pingyin alone. To date, no studies have reported a suitable method for the disposal of RFCS and the bioactive compounds contained therein.

Rose oil distillation wastewater (RODW) is another by-product of the steam distillation of dried rose flowers to product rose oil. In previous studies, RODW has been concentrated to generate a polyphenol-enriched residue containing non-volatile phenolic compounds [5]. Moreover, the polyphenol fraction of RODW can strongly inhibit mushroom tyrosinase (IC$_{50}$ value of 0.41 μg/mL) [6]. Thus, the polyphenols in RODW may be used as a bioactive substance to relieve hyperpigmentation.

Food-related pathogenic bacteria cause foodborne illnesses in millions of people and even hundreds of deaths every year in the USA alone, and the associated cost total approximately $2.4 billion [7]. Thus, the increasing demand for healthy, non-toxic, and effective antimicrobial agents has inspired research on multifunctional, naturally produced food additives. Although rose oil primarily contains essential oils known for their antimicrobial activities [8], the antimicrobial effects of RFCS have not been investigated.

The phenolic compounds and volatile substances in flowers have strong biological activities such as antioxidant and tyrosinase inhibitory effects [9]. The development of additional methods for inhibiting tyrosinase activity is an active area of research in the functional cosmetics and food industries due to tyrosinase’s whitening effect and ability to control browning [10, 11]. Antioxidants may lower the risks of health concerns such as cancer, aging, and atherosclerosis by reducing the level of reactive oxygen species (ROS) [12]. Some antioxidants, such as ascorbic acid, also have been reported to have whitening effects [11].

In our preliminary test, antimicrobial, antioxidant, and tyrosinase inhibitory activities of RODW from *Rosa rugosa* Thunb. var. *plena* Regal were evaluated [13]. However, there are no reports in the literature investigating the phytochemical composition and biological activities of RFCS from *Rosa rugosa* cv. *Plena*. In this study, (1) contents of the total phenolics, flavonoids, total solid and volatile contents were investigated; (2) the antibacterial (six strains) and anti fungal (one strain) activity, antioxidant, and tyrosinase inhibitory activities of each active compound and RFCS were examined. Our results will help to improve the value of roses in the fields of medicinal and cosmetic products [13–16].

Methods

Chemicals
Phenylethyl alcohol, α-bisabolol, α-terpineol, citronellol, miconazole nitrate, hydrochloride tetracycline, menthol and camphor were purchased from J&K Scientific Ltd. (Beijing). Kojic acid, hyperoside, quercetin, gallic acid, kaempferol-3-O-acetylglucosylrhamnoside and kaempferol-3-O-glucoside were purchased from Sigma (Shanghai, China), Anaerobic blood agar base medium (CDC), actinomycete broth medium (GAM broth), brain heart infusion (BHI) broth, and nutrient agar were obtained from Suolaibao Biotech Co., Ltd. (Beijing, China). The remaining chemicals were analytical or chromatographic grade.

Sample preparation
RFCS of *Rosa rugosa* Thunb. var. *plena* Regal was obtained from Fragrant Rose Biological Technology Co., LTD in Pingyin. The samples were filtered through a 0.42 μm microfiltration membrane prior to analyses. The total solid content of RFCS was evaluated by freeze-drying. The identification of *Rosa rugosa* Thunb. var. *plena* Regal was identified by senior agronomist Guo and confirmed in voucher sample (Ser. No. 0712) deposited at Herbarium, Pingyin Institute of Rose Sciences.

HPLC analyses
The concentration of polyphenol constituents in the extract was determined by HPLC and UV analyses. The HPLC apparatus was an LC-20A HPLC system (Shimadzu Corporation, Kyoto, Japan), and it was equipped with an Ultrasphere 5 C$_{18}$ column (4.6 mm × 250 mm, Ultrasphere Co., Ltd., Berkshire, UK). The mobile phase was a gradient elution of water (A) and acetonitrile (B) and was programmed as follows: starting with 10% B for 10 min, 10–25% B between 15 and 20 min, 25–30% B between
were identified based on their mass spectral data and retention time.

**HPLC-ESI-MS conditions**

The electrospray ionization (ESI) mass spectrometry (MS) data were recorded on an Agilent-LC-1100 instrument (Agilent, USA). The HPLC conditions for the HPLC-ESI-MS analysis were as described above. The ESI parameters were as follows: the collision gas (N₂) flow rate was maintained at 10 mL/min, the column oven was set at 25 °C, and the sample injection volume was 10 μL.

**GC/MS analysis**

The volatile constituents in RFCS were determined by a Shimadzu GC/MS model QP2010 Ultra system equipped with an Rtx-5MS (30 m × 0.25 mm, film thickness 0.25 μm) capillary column. The oven program was as follows: starting at 60 °C, heating to 120 °C at a rate of 1.7 °C/min, heating to 200 °C at 2.5 °C/min, heating to 260 °C at a rate of 8 °C/min, and finally holding at 260 °C for 2 min. Helium was used as the carrier gas, and the flow rate was 1.0 mL/min. The injector and detector temperatures were held at 250 °C and 280 °C, respectively. A split injection was conducted in splitless mode. The ion source temperature was 250 °C and its ionization energy was 70 eV. The mass range was 35–500 Da. The components in the sample were identified based on their mass spectral data and retention time.

**Preparation of standard curves**

Solutions of phenylethyl alcohol (2.23 mg), α-bisabolol (2.1 mg), α-terpineol (5.23 mg), citronellol (1.52 mg), menthol (1.32 mg), and camphor were separately prepared in 1 mL of acetonitrile. Next, the stock solutions were diluted by factors of ten thousand to one billion with ethyl acetate, and 1 μL of each solution was analyzed by HPLC. Solutions of kojic acid (1.12 mg), hyperoside (1.07 mg), quercetin (1.07 mg), gallic acid (1.29 mg), and kaempferol-3-O-acetylglucosylrhamnoside (1.15 mg) were separately prepared in 1 mL of methyl alcohol. The stock solutions were diluted by factors of 2 with methyl alcohol, and 10 μL of each solution was analyzed by HPLC. Each concentration of working solution was analyzed in three times. The calibration curves were plotted as the peak areas against the concentration of each standard. The content of the reference substance in each sample was calculated using the calibration curves.

**Determination of total phenolics, flavonoids and total solid content**

The total phenolic contents of the RFCS were evaluated by the Folin-Ciocalteu colorimetric method [17]. The total content of phenolic substance was determined by comparison to a standard curve of gallic acid. The total flavonoid contents of the RFCS samples were evaluated by HPLC, which provided the total amount of all tested flavonoid compounds. A 10-mL sample of RFCS was freeze-dried to determine the total solid content. Every determination was carried out in triplicate.

**Antioxidant properties**

**DPPH radical scavenging activity**

The antioxidant activity of RFCS and the standards were evaluated by DPPH radical scavenging activity using a slightly modified version of a previously reported method [18]. Briefly, 10 μL aliquots of the rose samples (1000 μg/mL to 62.5 μg/mL) were mixed with 190 μL of 50% ethanol containing 0.4 mM DPPH and incubated in the dark for 30 min. Aliquots (100 μL) of the supernatants were transferred into a 96-well microplate, and the absorbance of each was recorded at 517 nm using a Spectramax Plus384 UV-Vis spectrophotometer (Molecular Devices, Sunnyvale, California, USA). Ascorbic acid (1000 μg/mL to 0.05 μg/mL) was used as a positive control, and DPPH solution without sample was used as the negative control. The IC₅₀ values, which represent the concentrations of rose samples and standard substance at which 50% of the DPPH radical was inhibited, were determined. The tests were performed in triplicate, and the percentage of DPPH scavenging was calculated using the following equation.

\[
\text{Inhibition} \% = \left\{ \frac{(H_0 - H)}{H_0} \right\} \times 100
\]

\( H \): Absorbance of RFCS and the standards; \\
\( H_0 \): Absorbance of the blank

**Determination of ABTS radical scavenging**

The ABTS assay of RFCS was performed according to a modified version of a previously reported method [19]. Briefly, the stock solutions were generated by mixing equal quantities of 7.4 mM ABTS⁺ solution and 2.6 mM potassium persulfate solution, and the mixture was incubated at room temperature for 12 h in the dark. Then, the solution was equilibrated with 1 mL of ABTS** solution with 50% ethanol serving as a positive control. The absorbance of the solution at 734 nm was 1.17 ± 0.02 units. Aliquots (10 μL) of the rose samples
(1000 μg/mL to 62.5 μg/mL) were mixed with 1.0 mL of the diluted ABTS⁺⁺ solution. The mixture was mixed vigorously and incubated at 30 °C for 30 min. The absorbance was then measured at 520 nm with an excitation wavelength of 734 nm using the spectrophotometer. The positive standard was Trolox (2000 μg/mL to 0.05 μg/mL).

\[
\text{Inhibition} \% = \left(\frac{\text{Absorbance of blank} - \text{Absorbance of sample}}{\text{Absorbance of blank}}\right) \times 100
\]

### Determination of the Tyrosinase inhibitory activity

The tyrosinase inhibitory activities of the rose samples and standard substance were determined by a spectrophotometric method [17]. First, 300 μL aliquots of different concentrations (1000 μg/mL to 62.5 μg/mL) of each sample were diluted with 700 μL of 0.175 M sodium phosphate buffer (pH 6.8), then 1.0 mL of 10 mM DOPA solution and 1.0 mL of mushroom tyrosinase (220 units/mL) were added. Ethanol (300 μL, 50%) and kojic acid (2000 μg/mL to 0.1 μg/mL) were used as the blank reference and positive standard, respectively. The reaction mixture was vortexed and maintained at 37 °C for 15 min, and then the absorption maximum of dopachrome (set at 479 nm) was measured using a microplate reader (Molecular Devices, Sunnyvale, California, USA). The tests were performed in triplicate, and the value of tyrosinase inhibition activity was calculated as described above.

### Antimicrobial properties

#### Antibacterial and antifungal assays

The antimicrobial activity was measured by the method described by Xue [20]. All standard strains were obtained from the Guangdong Microbiology Culture Center (Guangzhou, China). _Listeria ivanovii_ (ATCC 19119) was cultured in BHI, _Salmonella enteritidis enteritidis_ (ATCC 14028) _Staphylococcus aureus_ (ATCC 25923) and _Escherichia coli_ (ATCC 25922) were cultured in nutrient agar (NA) for 24 h and at 37 °C. _candida albicans_ (ATCC 10231) was cultured in PHB at 37 °C for 24 h. _Propionibacterium acnes_ (ATCC 6919) and _Fusobacterium nucleatum_ (ATCC 10953) were cultured in CDC broth at 37 °C for 24 h in a YQX-II anaerobic incubator (Shanghai, China). The final cell counts in 1 mL of broth were approximately 10⁶ colony-forming units (CFU/mL). A 10 mg/mL solution of miconazole nitrate and hydrochloride tetracycline in water was used as a positive control against fungi and bacteria, respectively.

### Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) or minimum fungicidal concentration (MFC)

The MIC and MBC or MFC values were determined as described previously by Xue. Briefly, 100 μL dilutions (approximately 100,000 CFU/mL) of _Staphylococcus aureus_, _Escherichia coli_, _Salmonella enteritidis enteritidis_, _Fusobacterium nucleatum_, and _Candida albicans_ in nutrient broth and _Listeria ivanovii_ and _Propionibacterium acnes_ in GMA broth were inoculated into microtiter plates. Then, 100 μL aliquots of the solutions of the test compound were added after a two-fold serial dilution with nutrient broth (from 2 mg/mL to 3 μg/mL). Broths with 5% (v/v) DMSO were used as controls. The petri dishes were incubated at 37 °C for 24 h with the exception of _Propionibacterium acnes_ and _Fusobacterium nucleatum_, which were incubated at 37 °C for 48 h. The MIC was recorded as the lowest concentration of sample showing no detectable growth. To determine the MBC or MFC values for no bacterial or fungus growth, 10 μL of sub-inhibitory concentrations of the test compounds were incubated on CDC or GMA agar plates for 24 or 48 h. Every determination was carried out in triplicate.

### Date analysis

Data are presented as the mean of three replicates ± standard deviation. One-way ANOVA with Duncan's multiple range test was used to analyze the results with SPSS 13.0 and Sigma Plot 10.0, respectively, using a computer (Lenovo, Yangtian B 41) equipped with the Win 7 operating system. A p value of <0.05 was determined to be statistically significant.

### Results and discussion

#### The contents of volatile substance

Since the RFCS samples had a specific rose fragrance, we analyzed and compared the volatile components of its ethyl acetate extract. The contents of the volatile components of the ethyl acetate extract of RFCS were determined by GC/MS and analyzed by comparison to four standard curves, and the results are expressed as ng/mL.

Six principal components were simultaneously identified according to their standard retention times and MS ion fragments. The GC chromatograms of the reference substance in RFCS are shown in Fig. 1. The content of each element in every sample is presented in Table 1. As shown in Fig. 1, six compounds were successfully separated under the gradient temperature program. The total content of volatile compounds in RFCS was approximately 48.21 ± 2.76 ng/mL, and six major kinds of volatile compounds, including phenylethyl alcohol (40.48 ± 2.24 ng/mL), citronellol (7.83 ± 0.77 ng/mL), α-bisabolol (0.08 ± 0.01 ng/mL) and phenylethyl acetate (11.20 ± 0.89 ng/mL) were identified (two peaks have not been identified and the content of linalool is rare). In previous studies on the volatile compounds in RODW, GC-MS, more specifically HS-SPME/GC/MS, techniques have been widely utilized [13, 21–24].
contrast to these previous studies, our study reports
the absolute contents of the components. Although
there was a broad range of volatile compounds, there
were no differences in the dominant components. The
major volatile compounds in RFCS were monoterpen
e alcohols (citronellol, linalool, and phenylethyl alcohol,
which is specific to small roses). The types of domi-
nant components in RFCS are similar to those of
RODW, but there is a significant difference in the con-
tents of the components [13]. One possible reason for
these differences is that most of the volatile compo-
nents were lost in the rose tea drying process.

**Total phenolic, flavonoid and solid contents**
The flavonoids, their retention times, and the calibration
curves of standard compounds in RFCS, as determined
using HPLC, are shown in Fig. 2. Four compounds were
successfully separated under the gradient temperature
program, as shown in Fig. 2. The linearity of calibration
curves and regression coefficients of flavonoids were dem-
onstrated in Table 2. It was found that the reference com-
ounds showed good linearity ($R^2 \geq 0.997$). RFCS was
found to contain three main components, namely,
hyperoside (0.18 ± 0.01 mg/mL), kaempferol-3-O-ruta-
nosid (0.12 ± 0.01 mg/mL), and rutin (0.23 ± 0.01 mg/mL).
The total phenolic content and total flavonoid content were
0.31 ± 0.01 mg/mL and 0.43 ± 0.01 mg/mL, respectively.
The total solid content in RFCS was 1.45 ± 0.04 mg/mL.

Previous studies have reported that the dominant
phenolic and flavonoid compounds in rose rugosa tea
were gallic acid, catechin, epicatechin, and quercetin and
the total polyphenol content and flavonoid content in
the *Rosa rugosa* tea polyphenol extract were 875.2 mg/g
and 610.3 mg/g, respectively [1]. In addition, rutin, mul-
tiflorin B, hyperoside, kaempferol, and ellagic acid were
also found in the resin fractions of RODW [6]. Further-
more, unlike previous studies, although our study used
HPLC-MS to determine the phenol and flavonoid com-
ponents in RODW, only one of the dominant compo-
nents, kaempferol-3-O-rutinosid, was found in this
study and in previous studies [6, 13]. Neither of the phe-
nol compounds was detected in RFCS by HPLC primar-
ily because the concentrations of phenols and flavonoids
in RFCS are very low, and thus they are undetectable by
HPLC. Those solids in RFCS were mixture of small
molecules.

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**Table 1** Analytical characteristics of volatile substances in rose
waste. (ng/mL)

| Compound              | RT     | Formula structure | Weight  | RFCS       |
|-----------------------|--------|-------------------|---------|------------|
| Linalool              | 12.163 | C_{10}H_{18}O     | 154.25  | n.d        |
| Phenylethyl alcohol   | 12.921 | C_{8}H_{10}O      | 122.16  | 40.48 ± 2.24 |
| Citronellol           | 20.393 | C_{10}H_{20}O     | 156.27  | 7.83 ± 0.77 |
| Phenylethyl acetate   | 21.923 | C_{10}H_{12}O_{2} | 164.2   | 11.20 ± 0.89 |
| Citronellol acetate   | 29.029 | C_{12}H_{22}O_{2} | 198.3   | n.d        |
| α-bisabolol           | 49.909 | C_{15}H_{26}O     | 222.36  | 0.08 ± 0.01 |
| Total content         |        |                   | 48.21 ± 2.76 |

*nd* not detected; Data are expressed as mean ± standard deviation of triplicate samples; RFCS rose flower cell sap

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**Fig. 1** a Total ion chromatogram of volatile compounds in ethyl acetate from standard substance (a), and rose flowers cell sap (b). RFCS: rose flower cell sap. Identification of peaks. 1, linalool; 2, phenylethyl alcohol; 3, citronellol; 4, ester phenylethyl acetate; 5, citronellol acetate; 6, α-bisabolol
Antioxidant capacity

Table 3 presents the DPPH IC_{50} values of RFCS and the standard compounds. The flavonoids with IC_{50} values < 1 μg/mL, including hyperoside (IC_{50} value of 0.695 ± 0.021 μg/mL), kaempferol-3-O-rutinoside (IC_{50} value of 0.808 ± 0.024 μg/mL), rutin (IC_{50} value of 0.715 ± 0.017 μg/mL), and luteolin (IC_{50} value of 0.507 ± 0.015 μg/mL), showed stronger DPPH radical scavenging activities than RFCS (IC_{50} value of 1120 ± 42 μg/mL). Single volatile compounds, such as linalool, phenylethyl alcohol, citronellol, and α-bisabolol, showed weak radical scavenging activity with IC_{50} values of > 10,000 μg/mL. In previous reports, the antioxidant activities of various natural products, including those from rose, have been attributed to the contents of phenolic compounds [25, 26]. The ABTS radical assay is also used to evaluate the radical scavenging activity of hydrogen-donating and chain-breaking antioxidants in

| Table 2 | Analytical characteristics of compounds in rose waste. (mg/mL) |
|---------|---------------------------------------------------------------|
| Peak    | Ginsenoside | Retention time | Calibration curve | R^2  | RFCS            |
| 1       | hyperoside   | 16.981         | y = 14,955x - 5214 | 0.9989 | 0.18 ± 0.01     |
| 2       | kaempferol-3-O-rutinoside | 17.737      | y = 7102x + 3256  | 0.9984 | 0.12 ± 0.01     |
| 3       | rutin        | 19.01          | y = 41,285x - 43,792 | 0.9979 | 0.23 ± 0.01     |
| 4       | luteolin     | 22.01          | y = 31,527x - 5241 | 0.9991 | n.d             |
|         | total phenolic content |         |                  |      | 0.31 ± 0.01    |
|         | total flavonoid content |         |                  |      | 0.43 ± 0.01    |
|         | total solid content |         |                  |      | 1.45 ± 0.04    |

n.d not detected; Data are expressed as mean ± standard deviation of triplicate samples; RFCS rose flower cell sap.
many natural products [27, 28]. As shown in Table 3, the ABTS radical scavenging activities of single compounds and RFCS are expressed as μg/mL. Consistent with previous works, flavonoids exhibited significantly higher antioxidative activities and antioxidant capacities than volatile compounds [8, 13]. In the present study, the results of ABTS scavenging were similar to those of DPPH; flavonoids, hyperoside, kaempferol-3-O-rutinoside, luteolin (IC50 value of 0.436 ± 0.026 μg/mL), and RFCS are expressed as mean ± standard deviation of triplicate samples; RFCS rose flower cell sap values in each column followed by different letters are significantly different (P < 0.01).

**Table 3** Total solid content and IC50 values of single compounds in rose products. (μg/mL)

| Compound               | DPPH radical scavenging activity | Tyrosinase inhibition | ABTS radical scavenging activity |
|------------------------|---------------------------------|-----------------------|----------------------------------|
| RFCS                   | 1120 ± 42 b                     | 570 ± 21 ab           | 1430 ± 49 b                      |
| Linalool               | > 10,000 a                      | 730 ± 44 a            | > 10,000 a                       |
| Phenylethyl alcohol    | > 10,000 a                      | 315 ± 13 b            | > 10,000 a                       |
| Citronellol            | > 10000a                        | 825 ± 31 a            | > 10,000 a                       |
| α-bisabolol            | > 10,000 a                      | 635 ± 22 a            | > 10,000 a                       |
| Hyperoside             | 0.695 ± 0.021 c                 | 0.762 ± 0.018 d       | 0.526 ± 0.014 c                  |
| Kaempferol-3-O-Rutinoside | 0.808 ± 0.024 c               | 0.908 ± 0.021 d       | 0.719 ± 0.016 c                  |
| Rutin                  | 0.715 ± 0.017 c                 | 0.856 ± 0.014 d       | 0.621 ± 0.024 c                  |
| Luteolin               | 0.507 ± 0.015 c                 | 0.613 ± 0.016 d       | 0.436 ± 0.026 c                  |
| Positive control       | 0.449 ± 0.013 c                 | 80 ± 17 c             | 0.324 ± 0.019 c                  |

Data are expressed as mean ± standard deviation of triplicate samples; RFCS rose flower cell sap.

**Tyrosinase inhibitory activities**

Tyrosinase is a multifunctional copper-containing enzyme found in fungi, mammals, and plants [29]. Tyrosinase has two distinct enzyme activities, namely, monophenolase activity and diphenolase activity [30]. We conducted an initial study of the tyrosinase inhibitory activities of RFCS. As per this assay, RFCS showed strongly tyrosinase inhibitory activities with an IC50 value of 0.41 ± 0.01 μg/mL [6]. Meanwhile, the tyrosinase inhibitory effects of RFCS is stronger than RODW from Pingyin [13]. The flavonoid content contributes to the overall tyrosinase inhibitory effect of RODW.

**Antimicrobial activities**

The results of the antimicrobial activity studies of the different rose fractions, RFCS, and the standard antibiotics (tetracycline and hydrochloride) are presented in Table 4. *F. nucleatum* was most susceptible to RFCS and showed an MIC of 64 μg/mL and MBC of 250 μg/mL. The MIC values for RFCS against both *P. acnes* and *S. aureus* were 125 μg/mL. The MIC values against other bacteria were 250 μg/mL. The MIC and MBC or MFC values of the nine components of RFCS were determined to identify the constituents responsible for the antimicrobial effects of RFCS. *L. ivanovii* and *F. nucleatum* were found to be the most susceptible to α-bisabolol, and it showed MIC values of 8 μg/mL and MBC values of 32 μg/mL against these species (Table 4). After α-bisabolol, phenylethyl alcohol showed the lowest MIC and MBC or MFC values among all the constituents of RFCS. Overall, the volatile constituents played a more important role than the flavonoid compounds in the antimicrobial activity of RFCS.

Previous investigations of the antimicrobial effects of the various fractions of rose have reported similar results [8, 28, 31]. The essential oil and various extracts of rose, including the aqueous extract, ethanol extract, chloroform extract, ethyl acetate fraction, and butanol fraction, exhibit broad-spectrum antimicrobial activities. With the exception of the ethyl acetate fraction, rose essential oil is comparatively more active against the tested bacteria [28]. The absolute and essential oils of rose contain high levels of polyphenols and phenylethyl alcohol, which result in outstanding antimicrobial properties [8]. Because...
the content of volatile oil in RODW is higher than RFCS, the antimicrobial effect of RODW better than RFCS [13]. The polyphenolic-enriched fraction from rugosa tea could inhibit Escherichia coli and Pseudomonas aeruginosa quorum sensing and biofilm formationsignificance [1]. The antimicrobial effects of some of the active ingredients of rose oil such as linalool, citronellol, and geraniol have been confirmed [32, 33]. To date, the antimicrobial activity of RFCS of R. fenghua has not been evaluated. This result explicitly supports the fact that high contents of phenylethyl alcohol and other volatile components contribute to the antimicrobial activities of RFCS [34].

## Conclusions

Our study demonstrated the strong antioxidant, anti-microbial, and tyrosinase inhibitory activities of RFCS. Due to the rose-like aroma of phenylethyl alcohol in combination with the tyrosinase inhibitory activities and antimicrobial effect against S. enteritidis subspecies enteritidis, C. albicans, and P. acnes, RFCS may be used as a natural skin-whitening and skin care additive in the cosmetics industry. Additionally, due to its antioxidant activities and antimicrobial effects against L. ivanovii, S. subspecies, E. coli, and S. aureus, RFCS can be used as a natural preservative and antimicrobial agent in the food and pharmaceutical industries.

## Abbreviations

BHI: Brain heart infusion; CDC: Anaerobic blood agar base medium; ESI: Electrospray ionization; GAM broth: Actinomycote broth medium; MBC: Minimum bactericidal concentration; MIC: Minimum inhibitory concentration; MS: Mass spectrometer; RFCS: Rosa rugosa Thunb. var. plena Regal flower cell sap; RODW: Rose oil distillation wastewater; ROS: Reactive oxygen species

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## Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Authors’ contributions

GR, GZ and PX conceived the study. GR and GZ were responsible for data collection and data entry. PX and XS analyzed data and wrote the manuscript. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

This chapter does not contain any studies with human participants or animals performed by any of the authors and there is no informed consent involved.

## Consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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## Author details

1College of Pharmacy and Biological Engineering, Chengdu University, Chengdu 610000, People’s Republic of China. 2Social Risk Prediction and Management, School of Public Health and Management, Weifang Medical University, No.7166 Baotong West Street Weicheng District, Weifang 261053, People’s Republic of China. 3Key Laboratory of Coarse Cereal Processing, Ministry of Agriculture, No.2025 Chenglu Road, Longquanyi District, Chengdu 610106, People’s Republic of China. 4Institute of Crop Sciences, Chinese Academy of Agricultural Sciences, No.80 XUEYUAN South Road, Handian District, Beijing 100081, People’s Republic of China.

## Table 4 MIC and MBC or MFC of RFCS and different monomers against pathogenic bacteria (μg/mL)

| Compound               | L. ivanovii MIC | L. ivanovii MBC | S. enteritidis enteritidis MIC | S. enteritidis enteritidis MBC | S. aureus MIC | S. aureus MBC | E. coli MIC | E. coli MBC | C. albicans MIC | C. albicans MBC | P. acnes MIC | P. acnes MBC | F. nucleatum MIC | F. nucleatum MBC |
|------------------------|----------------|----------------|-------------------------------|-------------------------------|--------------|--------------|-------------|-------------|----------------|----------------|--------------|--------------|----------------|----------------|
| RFCS                   | 500 > 1000     | 500 > 1000     | 125 1000                      | 500 1000                      | 1000 > 1000  | 125 500      | 64 250      | 125 500     | 250 1000       | 250 1000       | 64 250       | 125 500      | 64 250        | 125 500       |
| Linalool               | 500 > 1000     | 500 > 1000     | 250 1000                      | 250 1000                      | 1000 > 1000  | 125 500      | 250 1000    | 250 1000    | 250 1000       | 250 1000       | 125 500      | 8 32         | 125 500       | 125 500       |
| Phenylethyl alcohol    | 250 500        | 125 500        | 125 250                       | 250 1000                      | 250 1000     | 125 500      | 8 32        | 8 32        | 125 500        | 125 500        | 125 500      | 8 32         | 125 500       | 125 500       |
| Citronellol            | 250 500        | 250 1000       | 125 500                       | 250 1000                      | 250 1000     | 125 500      | 125 500     | 125 500     | 250 1000       | 250 1000       | 125 500      | 8 32         | 125 500       | 125 500       |
| α-bisabolol            | 8 32           | 500 > 1000     | 250 1000                      | 250 1000                      | 1000 > 1000  | 125 500      | 8 32        | 8 32        | 125 500        | 125 500        | 125 500      | 8 32         | 125 500       | 125 500       |
| Hyperoside             | 250 500        | 250 500        | 250 1000                      | 250 1000                      | 1000 > 1000  | 125 500      | 8 32        | 8 32        | 125 500        | 125 500        | 125 500      | 8 32         | 125 500       | 125 500       |
| Kaempferol-3-O-rutinosid | 500 1000      | 500 > 1000     | 250 1000                      | 250 1000                      | 1000 > 1000  | 125 500      | 8 32        | 8 32        | 125 500        | 125 500        | 125 500      | 8 32         | 125 500       | 125 500       |
| Rutin                  | 500 1000       | 125 500        | 250 500                       | 250 1000                      | 500 1000     | 125 250      | 62 500      | 125 500     | 250 1000       | 250 1000       | 125 500      | 125 500      | 125 500       | 125 500       |
| Luteolin               | 500 1000       | 250 1000       | 500 > 1000                    | 250 1000                      | 1000 > 1000  | 125 500      | 125 500     | 125 500     | 250 1000       | 250 1000       | 125 500      | 125 500      | 125 500       | 125 500       |
| Miconazole Nitrate     | – – – – – – – – | – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – | – – – – – – – – – – – – – – – – |
| Hydrochloride tetracycline | < 0.1 < 0.1 | 8 16          | 16 16                        | 16 16                        | 16 –        | – – –        | 16 8        | 8 – – – – – – | 16 8 – – – – – – | 16 8 – – – – – – | 16 8 – – – – – – | 16 8 – – – – – – | 16 8 – – – – – – | 16 8 – – – – – – |

Data are expressed as mean ± standard deviation of triplicate samples

MIC: Minimum inhibitory concentration; MBC: Minimum bactericidal concentration; MFC: Minimum Fungicidal concentration; RFCS: Rose oil distillation wastewater; ROS: Reactive oxygen species spectrometer; RFCS: Rosa rugosa Thunb. var. plena Regal flower cell sap; RODW: Rose oil distillation wastewater; ROS: Reactive oxygen species.
