JPP Journal of Pharmacy and Pharmacology

JPP 2004, 56: 537–545

Received July 01, 2003
Accepted December 11, 2003
DOI 10.1211/0022357022944
ISSN 0022-3573

# A gallic acid derivative and polysaccharides with antioxidative activity from rose (*Rosa rugosa*) flowers

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## Abstract

In this study, the major antioxidant components of rose flower were identified. An aqueous extract of rose flowers was chromatographed on CM-cellulose in ammonium acetate buffer (10 mM, pH 4.5) to yield three un-adsorbed peaks F1, F2 and F3. Each of these peaks was subjected to gel filtration on Sephadex G75. F1 yielded two peaks, whereas both F2 and F3 gave rise to only a single peak. Spectroscopic studies using NMR and FTIR revealed that F3 is a gallic acid derivative. It exhibited the highest antioxidative potency. F1-a derived from F1 by gel filtration is mainly a polysaccharide–peptide complex with less potent antioxidative activity. F2 is a polysaccharide also with reduced antioxidant activity. This study demonstrates, for the first time, the presence of both gallic acid derivatives and polysaccharides as major antioxidant principles of the aqueous extract of rose flowers.

## Introduction

Reactive oxygen species derived from molecular oxygen are highly reactive metabolites. These species can be generated by cellular or acellular mechanisms. They react with all biological molecules such as proteins, lipids and carbohydrates (Santanam et al 1998). Studies have shown that oxidative stress can mediate a wide variety of degenerative processes and diseases, such as aging, age-related neurodegenerative diseases, cardiovascular disease, carcinogenesis and diabetes (Dhalla et al 2000; Pimental et al 2000; Kovacic & Jacintho 2001; Rosen et al 2001; Sayre et al 2001). Therefore, research on antioxidants, especially the exploration of potent natural antioxidants with low cytotoxicity from plants, has become a new branch of biomedicine (Vinson et al 2002).

The rose plant is a source of phytochemicals, some of which have interesting biological activity. Sesquiterpenes are found in rose leaves (Hashidoho et al 2001). Rose flowers contain essential oils (Zhou et al 2002). The following compounds are found in the oils: phenylethyl alcohol, citronellol, geraniol, linalool, benzyl alcohol, nerol, nonyl alcohol, haptyl alcohol, phenylethyl acetate, benzyl formate, nonyl aldehyde, benzaldehyde, nonalactone, eugenol, phenyl acetic acid and benzoic acid. The traditional use of rose flowers is for controlling pain and diarrhoea, and for treating neurotic gastritis, chronic gastritis, hepatitis, acute mastitis, trauma and blood disorders (Hsu et al 1986). Rose root extracts reduced hepatic triglyceride content but not serum triglyceride level (Lee et al 1991). Small fruit juice from *Rosa rugosa* exerted an anti-proliferative activity toward cancer cell lines (Yoshizawa et al 2000a, b). Rugosa E, an ellagitannin from rose, induced platelet aggregation (Teng et al 1997). Cho et al (2003) demonstrated that the rose extract scavenged the 1,1-diphenyl-2-picryllhydrazyl radical. Flavonoids extracted from rose expressed antioxidant activity (Kleszczynska et al 1999). Hence, we chromatographically fractionated the aqueous extract of rose flowers and examined the fractions for antioxidant activity.

## Materials and Methods

### Reagents

CM32-cellulose cation exchanger was obtained from Whatman. Sephadex G-75 was obtained from Amersham Biosciences (Piscataway, New Jersey). DNA (Type I, calf

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**Acknowledgment and funding:** This project was supported by National Natural Science Foundation (30270039) and Tianjin Natural Science Foundation (023803211). We thank Miss Fion Yung for her skilled secretarial assistance.

thymus), bleomycin sulfate, butylated hydroxyanisole (BHA), phenazin methosulfate (PMS), $\beta$-nicotinamide adenine dinucleotide (reduced form, NADH), nitroblue tetrazolium (NBT) and thiobarbituric acid (TBA) were obtained from Sigma (St Louis, MO). 2,2′-Azo-bis-(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako (Osaka, Japan). All other chemicals were of the highest quality available.

### Animals

Male Sprague-Dawley rats, 150–200 g, were used in this study. The rats were housed under normal laboratory conditions ($21 \pm 2\,^{\circ}C$, 12-h light–dark cycle) with free access to standard rodent chow and water. The experiments using animal tissues were conducted in accordance with the guidelines and with the approval of the Animal Research Ethics Committee, The Chinese University of Hong Kong.

### Cation exchange chromatography on CM32-cellulose

Dried rose (*Rosa rugosa* Thunb.) flowers (100 g) from Mainland China were cut up into small pieces, soaked overnight in distilled water at room temperature and then boiled under reflux for 2 h. The extract was centrifuged at 5000 rev $min^{-1}$ for 10 min. The supernatant was collected and lyophilized.

The lyophilized powder (50 mg) was dissolved in 10 mM ammonium acetate ($NH_4OAc$) buffer (pH 4.5) and centrifuged to remove insoluble material. The solution was chromatographed on a column of CM-cellulose ($2.5 \times 35$ cm) equilibrated and eluted with $NH_4OAc$ buffer (10 mM, pH 4.5). After elution with 250 mL of the buffer, the column was washed sequentially with 250 mL of 200 mM $NH_4OAc$ buffer (pH 4.5) and 250 mL of 400 mM $NH_4OAc$ buffer (pH 8.0). The flow rate was 50 mL $h^{-1}$ and the fraction size was 2.5 mL. The carbohydrate content of the fractions was estimated with the anthrone–$H_2SO_4$ method (Kawagishi et al 1990). The absorbance at 280 nm was also measured. The fractions were monitored for antioxidant activity as described below. The active fractions were lyophilized and subjected to the next isolation step.

### Gel filtration on Sephadex G-75

The active fraction obtained from CM32-cellulose was applied to a Sephadex G-75 column ($2.5 \times 100$ cm) in 50 mM $Na_2HPO_4$-$NaH_2PO_4$ buffer (pH 7.0) and was eluted with the same buffer at a flow rate of 24 mL $h^{-1}$. Fractions of 4 mL were collected. The eluate was monitored with a UV detector at 280 nm. The elution volume of dextran blue 2000 (Amersham Biosciences) was used as void volume. The active fractions were lyophilized.

### HPLC analysis

After the aforementioned two-step chromatography, the active fractions were subjected to reverse-phase HPLC, which was conducted using an ODS $C_{18}$ column ($19 \times 300$ mm) and an LC-A4 HPLC. The buffer used was 60% methanol–40% $Na_2HPO_4$-$KH_2PO_4$ (0.025 M, pH 6.5). The flow rate was 30 mL $h^{-1}$.

### FTIR spectroscopy

A 2-mg sample was ground in a mortar. KBr powder (200 mg), which had been ground to approximately 2 $\mu$m and dried in an oven at 110–150 °C for 48 h, was then added and ground evenly together with the sample. The mixture was compressed in vacuum to form a semi-transparent tablet with a diameter of 5 mm or 13 mm. The tablet was placed in a sample window of an FTIR spectrometer (Magna-560). A KBr tablet was placed in the reference window. Spectroscopy was then performed.

### NMR spectroscopy

A 10-mg sample was dissolved in 250–300 $\mu$L $D_2O$. After filtration the solution was transferred to a sample tube (5 mm in diameter) and placed in a sample window of a UNITY plus-400 NMR spectrometer. Spectroscopic measurements were then performed.

Five-millimetre gradient reverse $^{1}H/^{13}C$ double probes were used. The working frequencies for $^{1}H$ and $^{13}C$ were, respectively, 400.13 MHz and 100.614 MHz. The measurements were made at room temperature.

### UV scanning analysis

The samples were dissolved in buffer and scanned from 190 nm to 800 nm using a DU-7 ultraviolet scanner (Beckman).

### Analysis of elements

The sample powder was directly introduced into a Perkin–Elmer elemental analyser, and the percent content (w/w) of each element in the sample was determined. The mole value of each element was calculated in relation to mole ratio of carbon using the following formula:

$$A\ (mol) = [6 \times C(MW) \times A\%]/[C\% \times A(MW)] \qquad (1)$$

Where A is the element detected except C, and C stands for carbon.

### Assay of protein content

Protein content was assayed using the method of Lowry et al (1951).

### Assays of lipid peroxidation using brain and kidney homogenates

For the in-vitro studies, the brains and kidneys of normal rats were dissected and homogenized in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 14 000 rev $min^{-1}$ for 15 min. One-millilitre samples of the supernatant were incubated with the test samples in the presence of

10 $\mu$M $FeSO_4$ and 0.1 mM ascorbic acid at 37 °C for 1 h. The reaction was stopped by addition of 0.1 mL trichloroacetic acid (28% w/v) and 1.5 mL thiobarbituric acid (TBA, 1% w/v) in succession, and the solution was then heated at 100 °C for 15 min. After centrifugation to remove precipitated protein, the absorption of the malondialdehyde (MDA)–TBA complex was measured at OD 532 nm (Liu et al 1997). Butylated hydroxyanisole (BHA) was used as a positive control. The inhibition ratio (%) was calculated using the following formula:

$$\text{Inhibition ratio (\%)} = (A - A1)/A \times 100\% \quad (2)$$

Where A was the absorbance of the control, and A1 was the absorbance of the test sample.

### Assay for erythrocyte haemolysis

Blood was obtained from male adult Sprague-Dawley rats by cardiac puncture and collected in heparinized tubes. Erythrocytes were separated from plasma and the buffy coat and washed three times with 10 volumes of 0.15 M NaCl. During the last wash, the erythrocytes were centrifuged at 2500 rev $min^{-1}$ for 10 min to obtain a constantly packed cell preparation (Miki et al 1987).

Erythrocyte haemolysis was mediated by peroxyl radicals in this assay system (Sugiyama et al 1993). A 10% suspension of erythrocytes in pH 7.4 phosphate-buffered saline (PBS) was added to the same volume of 200 mM AAPH solution (in PBS) containing samples to be tested at different concentrations. The reaction mixture was shaken gently during incubation at 37 °C for 2 h. The reaction mixture was then removed, diluted with 8 volumes of PBS and centrifuged at 2500 rev $min^{-1}$ for 10 min. The absorbance A of the supernatant was read at 540 nm. Similarly, the reaction mixture was treated with 8 volumes of distilled water to achieve haemolysis, and the absorbance B of the supernatant, obtained after centrifugation, was measured at 540 nm. The percentage haemolysis was calculated by $(1 - A/B) \times 100\%$.

L-Ascorbic acid was used as a positive control.

### Assays for free radical scavenging activity

*Superoxide radical generation (Liu et al 1997)*
Superoxide radicals are generated in a phenazin methosulfate (PMS)–$\beta$-nicotinamide adenine dinucleotide (reduced form, NADH) system by oxidation of NADH and assayed by the reduction of nitroblue tetrazolium (NBT). In this experiment, the superoxide radicals were generated in 3 mL of Tris-HCl buffer (16 mM, pH 8.0), which contained 78 $\mu$M NADH, 50 $\mu$M NBT, 10 $\mu$M PMS and samples to be tested at different concentrations. The colour reaction between superoxide radicals and NBT was detected at OD 560 nm. L-Ascorbic acid was used as a control. The inhibition ratio (%) was calculated using equation 2.

*Hydroxyl radical generation (Chen et al 2002)*
The hydroxyl radicals were generated in an L-ascorbic acid–$CuSO_4$ system by reduction of $Cu^{2+}$ and were assayed by the oxidation of cytochrome C. In this experiment, the hydroxyl radicals were generated in 1 mL of 0.15 mM sodium phosphate buffer (pH 7.4), containing 100 $\mu$M L-ascorbic acid, 100 $\mu$M $CuSO_4$, 12 $\mu$M cytochrome C and the samples to be tested at different concentrations. The mixture was incubated at 25 °C for 90 min. The change in transmittance caused by the colour change of cytochrome C was measured at 550 nm. Thiourea was used as a control. The inhibition of hydroxyl radical generation by thiourea was taken as 100%. The inhibition ratio (%) was calculated using the following formula:

$$\text{Inhibition ratio (\%)} = [(T - T2)/(T - T1)] \times 100\% \quad (3)$$

where T is the transmittance of the hydroxyl radical (OH·) generation system, and T1 and T2 are the transmittances of the control (no OH· generation) and test systems, respectively.

### Bleomycin-dependent DNA damage

The assay was carried out according to the method of Aeschbach et al (1994) and Chan & Tang (1996) with minor modifications. The reaction mixture (0.5 mL) contained DNA (0.5 mg $mL^{-1}$), bleomycin sulfate (0.05 mg $mL^{-1}$), $MgCl_2$ (5 mM), $FeCl_3$ (50 $\mu$M) and samples to be tested at different concentrations. L-Ascorbic acid was used as a positive control. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by addition of 0.05 mL EDTA (0.1 M). Colour was developed by adding 0.5 mL thiobarbituric acid (TBA) (1% w/v) and 0.5 mL HCl (25% v/v) followed by heating at 80 °C for 10 min. After centrifugation, the extent of DNA damage was measured by increase in absorbance at 532 nm.

## Results

The aqueous extract of rose flowers was potent in inhibiting erythrocyte haemolysis (87.5% and 86.2% inhibition at 500 and 100 $\mu$g $mL^{-1}$, respectively), lipid peroxidation in brain homogenate (92.4% and 91.9% inhibition at 500 and 100 $\mu$g $mL^{-1}$, respectively), superoxide radical production (90.5% and 88.4% inhibition at 500 and 100 $\mu$g $mL^{-1}$, respectively) and hydroxyl radical formation (79.3% and 59.8% inhibition at 500 and 100 $\mu$g $mL^{-1}$, respectively).

Chromatography of the aqueous extract of rose flowers on CM-cellulose resulted in three fractions (absorbance peaks), F1, F2 and F3, which were eluted by 10 mM $NH_4OAc$ buffer (pH 4.5) (Figure 1). All three fractions possessed antioxidant activity. No absorbance peaks with antioxidant activity were eluted with 200 mM $NH_4OAc$ (pH 4.5) or 400 mM $NH_4OAc$ (pH 8.0). Fractions F1, F2 and F3 were all strongly adsorbed on DEAE-cellulose (data not shown). The UV absorption spectra of Fl, F2 and F3 revealed that the biggest absorption peaks occurred in the vicinity of 260–280 nm. The active fractions Fl and F2 coincided with the polysaccharide peaks, suggesting that the antioxidant activity in Fl and F2 was due to polysaccharide (data not shown).

Gel filtration of fraction Fl on Sephadex G75 yielded two fractions (absorbance peaks), Fl-a (small) and Fl-b

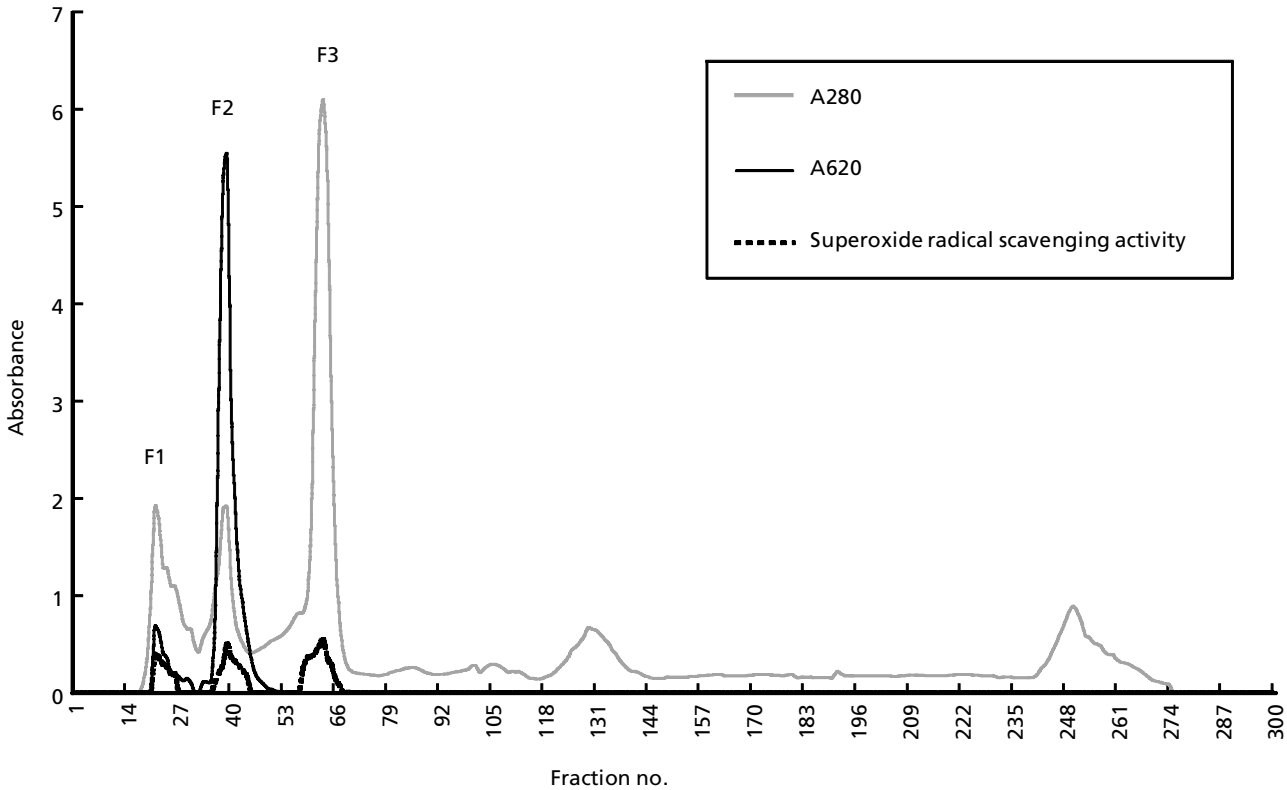


**Figure 1** Elution of F21 from CM32-cellulose column.

(large) with antioxidant activity residing in Fl-a. Both F2 and F3 gave rise to a single fraction (absorbance peak) with antioxidant activity after chromatography on Sephadex G75. Fl-a was eluted in the void volume and thus had a molecular weight $\geq 70$ kDa. F2 and F3 had a molecular weight below 2.5 kDa and 1.4 kDa, respectively. The carbohydrate contents of Fl-a, F2 and F3 were, respectively, 23.12%, 84.74% and 0%, The protein contents of F1-a, F2 and F3 were, respectively, 8.57%, 0% and 0%. F3 yield a single peak when subjected to HPLC. F2 and Fl-a yielded a major absorbance peak and, in addition, two to three minor absorbance peaks. The results show that F3 is a single fraction.

In the $^{1}$H NMR spectrum of F3, a single peak appeared at $\delta = 7.1$ ppm, indicating the presence of -CH= in a heterocyclic ring or an aromatic ring (Figure 2A). $^{13}$C-$^{1}$H HMQC (Figure 3A) obtained using 2D-NMR showed that this structure corresponded to the single peak ($\delta = 111.771$ ppm) in the $^{13}$C NMR spectrum (Figure 2B). $^{13}$C-$^{1}$H HMBC (Figure 3B) indicated that the C atoms at chemical shifts $\delta = 129.237$ ppm, $\delta = 138.210$ ppm, $\delta = 146.548$ ppm and $\delta = 176.388$ ppm were related to the C atom at $\delta = 111.771$ ppm (i.e. to the -CH= structure). These four C atoms were adjacent to, or separated by one C atom from, the C atom in -CH=. HMQC revealed that these four C atoms were all quaternary carbon atoms, and that F3 was highly unsaturated.

The single peak at $\delta = 146.548$ ppm in $^{13}$C NMR indicated the presence of a -C=C-O- structure (Figure 2B). Because F3 was water-soluble and only $D_2O$ can be used as solvent in $^{1}$H NMR, active hydrogen in -OH is exchanged by deuterium. The signal due to proton did not appear but moved to the solvent peak ($\delta = 4.8$ ppm). F3 showed an absorption peak in FTIR at 3429.3 cm$^{-1}$ formed by OH radicals (Figure 4). It can be deduced that the -C=C-O- structure is -C=C-OH. Free water in the sample absorbs at 3710 cm$^{-1}$ and can easily be distinguished. Meanwhile, the -C=C-O- structure was abundant in F3.

When a drop of freshly prepared 1% $FeC1_3$ solution was added to a solution of F3, the latter turned purple, indicating the presence of phenolic structures. F3 showed strong absorption at 260 nm, indicating the existence of conjugated double bonds. The strong absorption at 260–300 nm suggests the presence of three or more conjugated double bonds. Flavonoids show an absorption peak near 260 nm and also near 300 nm. F3 did not show an absorption peak near 300 nm, indicating that it is not a flavonoid. The $^{1}$H NMR and FTIR spectra of vitamin C are different from those of F3, indicating that they are separate substances.

Based on the above analyses, F3 is probably a derivative of gallic acid. A-C=C-O- structure is present in F3 as indicated by the single peak at $\delta = 176.388$ ppm in $^{13}$C NMR. The $^{13}$C NMR and 2D NMR spectra of F3 contained signals that match the structure of gallic acid. It can be deduced the F3 is a compound related to gallic acid. Results of elemental analysis indicate that the simplest C:H:O ratio in F3 is 12:19:14. The extent of

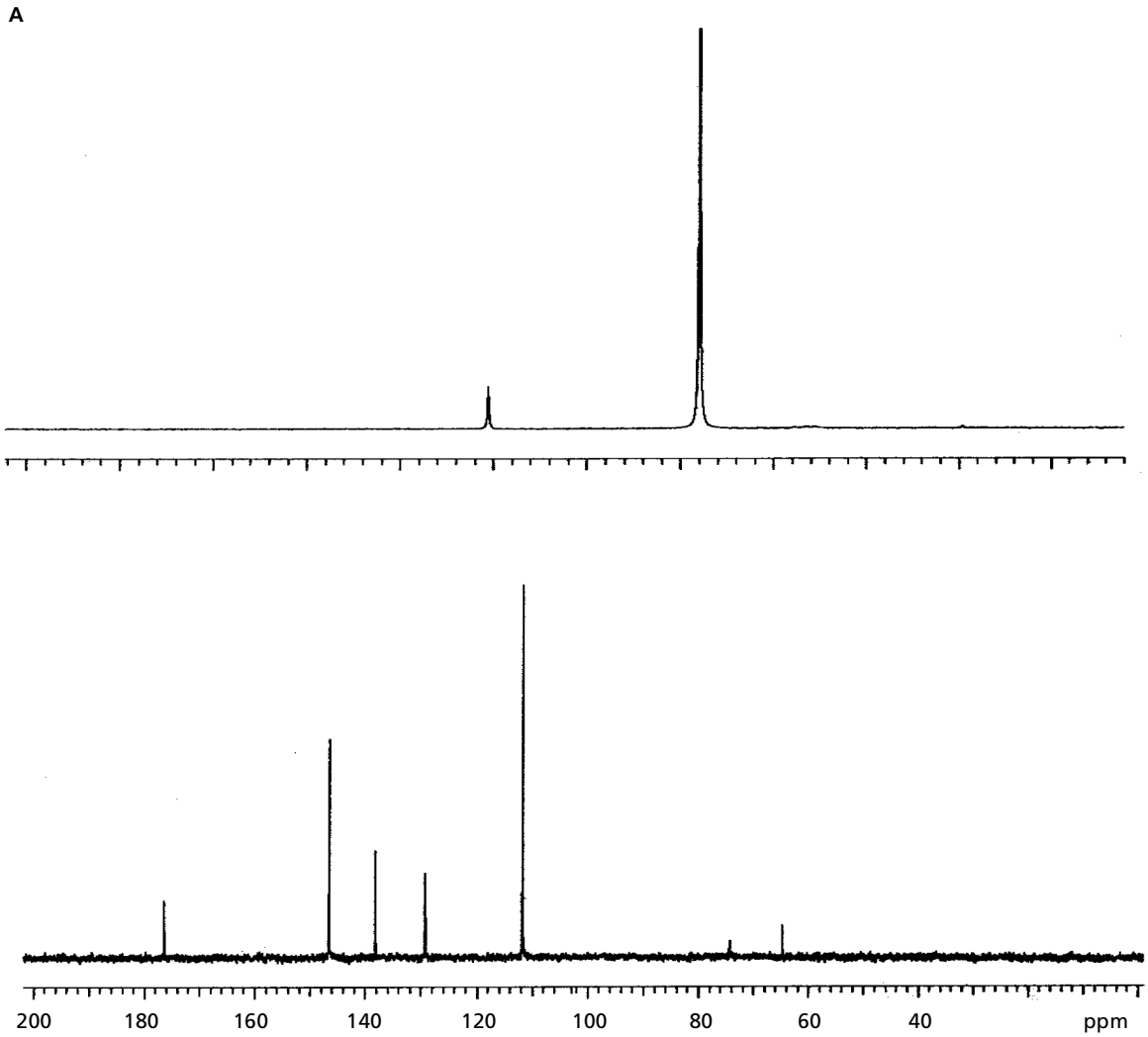


**Figure 2** 1-D NMR spectra of F3. A. $^{1}H$ NMR spectrum. B. $^{13}C$ NMR spectrum.

unsaturation is $(13 \times 2 + 2 - 19)/2 = 5$, correlating well with that of gallic acid.

F3 and vitamin C exhibited the highest potency in inhibiting haemolysis, followed by the crude extract (F21). Over 90% inhibition was observed at 50 $\mu g\,mL^{-1}$ with both F3 and vitamin C. At the same concentration, F21 caused 81.52% inhibition. At 250 and 500 $\mu g\,mL^{-1}$, F21 brought about 84.73% and 87.95%, inhibition, respectively. Fl-a and F2 manifested the second lowest and the lowest inhibitory potency, respectively. Neither Fl-a nor F2 exhibited a conspicuous inhibitory effect on haemolysis at 50 $\mu g\,mL^{-1}$ (14.29% and 10.41% inhibition, respectively), and they displayed an effect similar to that of F3 and vitamin C only at 500 $\mu g\,mL^{-1}$ (94.04% and 85.27% inhibition, respectively). The data show that F3 was the major antioxidant principle in rose flowers. It exhibited 92.32% inhibition at 50 $\mu g\,mL^{-1}$.

F3 and the crude extract (F21) manifested a potency similar to that of BHA in inhibiting lipid peroxidation in rat brain homogenate, as well as in rat kidney homogenate. F2 and Fl-a exhibited an inhibitory potency lower than that of the crude extract (Table 1).

F3 and the crude extract F21, were the most potent in scavenging superoxide radicals (Table 2). F2 and Fl-a showed lower potency.

Again, F3 was the most active in scavenging hydroxyl radicals (Table 3). The activity of F2 and Fl-a was lower than that of the crude extract, which was the second most potent.

The crude extract, Fl-a, F2 and F3 had very little effect on DNA damage. The extent of DNA damage, as reflected by OD532, was $0.014 \pm 0.001$ for control, $0.080 \pm 0.003$ for F1-a (500 $\mu g\,mL^{-1}$), $0.073 \pm 0.002$ for F2 (500 $\mu g\,mL^{-1}$) and $0.042 \pm 0.006$ for F3 (500 $\mu g\,mL^{-1}$).

## Discussion

The results of this investigation disclosed that the major antioxidant principle in rose flowers is a derivative of gallic acid (MW < 2.5 kDa, 0% protein and 0% carbohydrate) and that a polysaccharide (MW < 2.5 kDa, 84.74% carbohydrate, 0% protein) and a polysaccharide–peptide

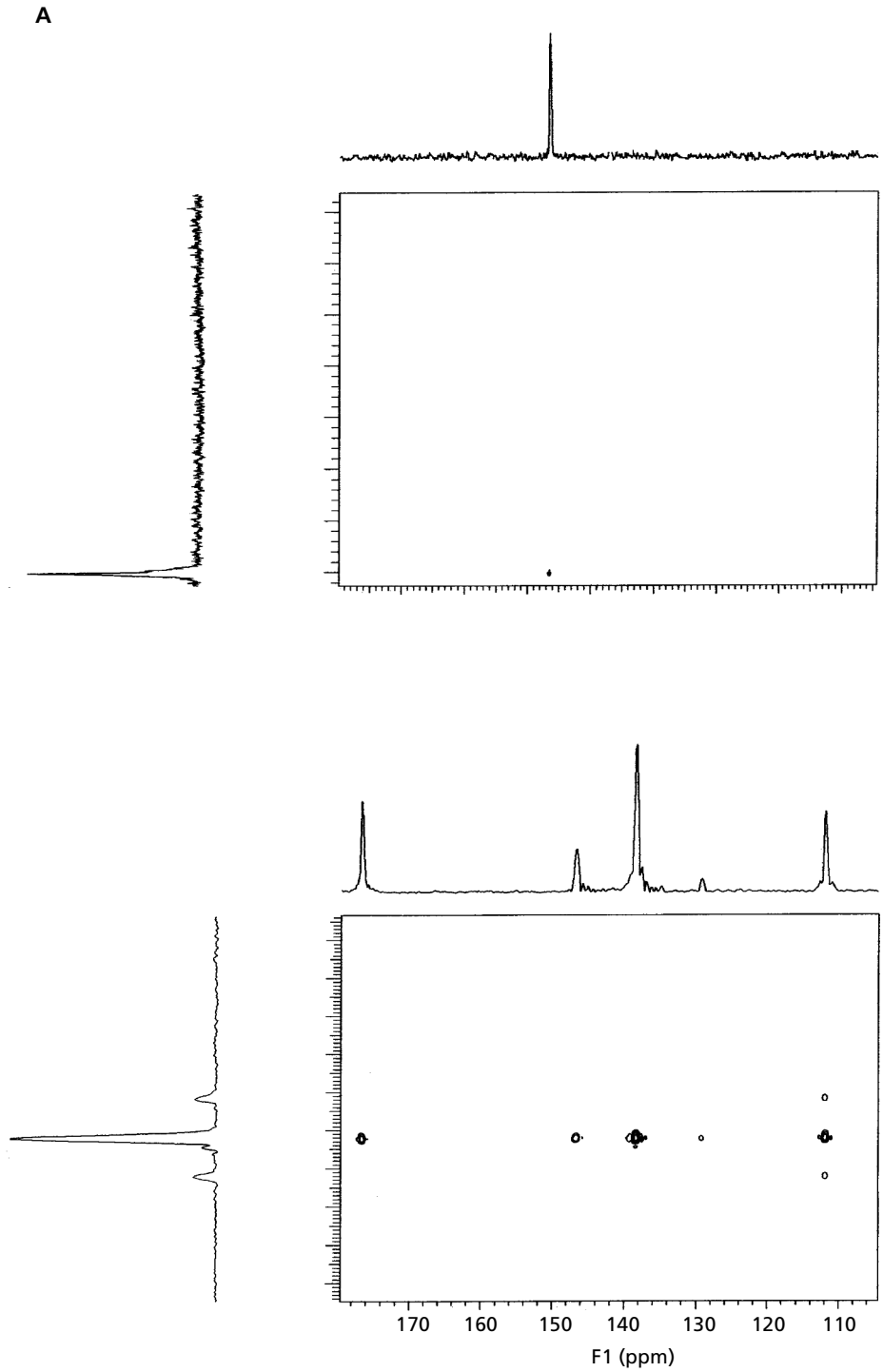


**Figure 3** 2-D NMR spectra of F3. A. $^{13}C$-$^{1}H$ heteronuclear multiple quantum coherence spectrum (HMQC) of F3. B. $^{13}C$-$^{1}H$ heteronuclear multiple bond correlation spectrum (HMBC) of F3.

(MW $\geq$ 70 kDa, 8.57% protein, 23.12% carbohydrate) are also components with some antioxidant activity. Although the literature pertaining to the antioxidant activity of gallic acid and its derivatives is voluminous (Ho et al 1992; McPhail et al 1999), this study constitutes the first demonstration that a gallic acid derivative is the major antioxidant in rose flowers. The spectroscopic data of this component in rose flowers showed an abundance of aromatic hydroxyl groups and aromatic carboxyl groups, which closely correlate with the structure of gallic acid (3,4,5-trihydroxybenzoic acid). Proteins and carbohydrates were absent in the preparation of this gallic acid derivative. The presence of a

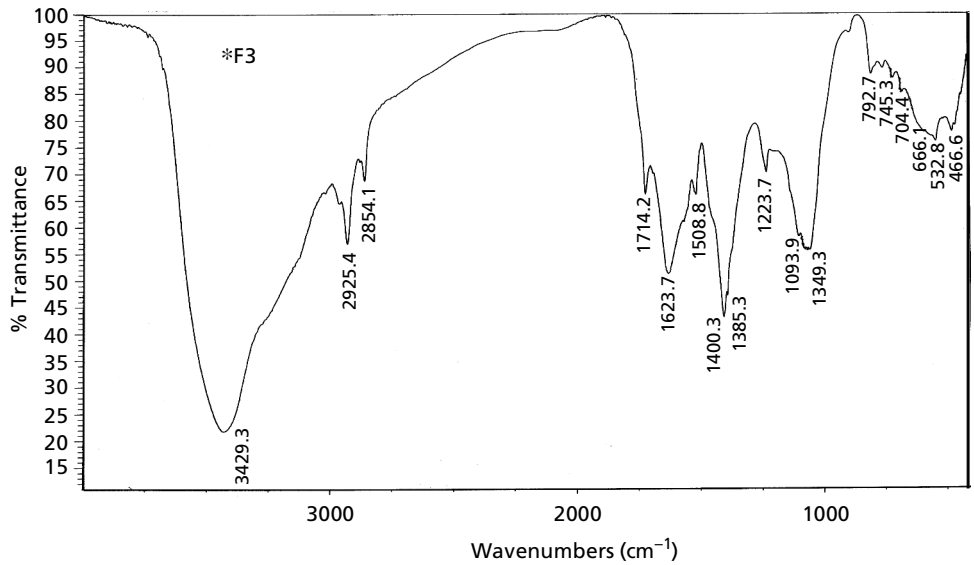


**Figure 4** Fourier transform infrared spectrum (FTIR) of F3.

**Table 1** Effects of chromatographic fractions derived from rose flower extract on lipid peroxidation in rat brain and kidney homogenates.

| Sample | Concn ($\mu$g mL$^{-1}$) | Brain MDA formation (OD532 nm) | Inhibition (%) | Kidney MDA formation (OD532 nm) | Inhibition (%) |
|---|---|---|---|---|---|
| Control | — | $0.902 \pm 0.004$ | 0 | $0.680 \pm 0.015$ | 0 |
| Butylated hydroxyanisole (BHA) | 50 | $0.066 \pm 0.006^{a}$ | 92.68 | $0.079 \pm 0.008^{a}$ | 88.38 |
| | 250 | $0.064 \pm 0.009^{a}$ | 92.90 | $0.050 \pm 0.007^{a}$ | 92.65 |
| | 500 | $0.056 \pm 0.002^{a}$ | 93.79 | $0.048 \pm 0.002^{a}$ | 92.94 |
| F21 (polysaccharide–peptide) | 50 | $0.075 \pm 0.004^{a}$ | 91.69 | $0.079 \pm 0.001^{a}$ | 88.38 |
| | 250 | $0.072 \pm 0.003^{a}$ | 92.02 | $0.070 \pm 0.005^{a}$ | 89.71 |
| | 500 | $0.069 \pm 0.001^{a}$ | 92.35 | $0.070 \pm 0.003^{a}$ | 89.71 |
| F1-a (polysaccharide–peptide) | 50 | $0.823 \pm 0.029^{c}$ | 8.76 | $0.634 \pm 0.006^{b}$ | 6.76 |
| | 250 | $0.486 \pm 0.014^{c}$ | 46.12 | $0.496 \pm 0.005^{b}$ | 27.06 |
| | 500 | $0.091 \pm 0.006^{c}$ | 89.91 | $0.420 \pm 0.010^{b}$ | 38.24 |
| F2 (polysaccharide) | 50 | $0.874 \pm 0.054^{c}$ | 3.10 | $0.655 \pm 0.019^{b}$ | 3.68 |
| | 250 | $0.792 \pm 0.011^{d}$ | 12.20 | $0.469 \pm 0.008^{b}$ | 31.03 |
| | 500 | $0.727 \pm 0.008^{d}$ | 19.40 | $0.351 \pm 0.012^{b}$ | 48.38 |
| F3 (gallic acid derivative) | 50 | $0.207 \pm 0.005^{b}$ | 77.05 | $0.083 \pm 0.001^{a}$ | 87.79 |
| | 250 | $0.071 \pm 0.001^{a}$ | 92.13 | $0.081 \pm 0.005^{a}$ | 88.09 |
| | 500 | $0.063 \pm 0.004^{a}$ | 93.02 | $0.078 \pm 0.002^{a}$ | 88.53 |

The values are means $\pm$ s.d., n = 3. Data bearing different superscripts are significantly different. Data for BHA, F21 and F3 are significantly different from the other data sets as confirmed using the Kruskal–Wallis test.

single sharp peak in HPLC analysis attests to the purity of the preparation. It is noteworthy that the preparation of gallic acid derivative displayed, in most of the assays for antioxidant activity, an antioxidant potency slightly higher than that of the crude extract of rose flowers, indicating that the unfractionated crude extract was already quite potent as an antioxidant preparation.

Guo et al (1996) reported that the free radical scavenging activity and lipid peroxidation inhibitory effect of EGCG ((−)-epigallocatechin gallate) and ECG ((−)-epicatechin gallate) are stronger than those of EC ((−)epicatechin) and EGC((−)epigallocatechin). EGCG and ECG just carry one more gallic acid structure than EC and EGC. This provided a clue to the structure–activity relationship of the antioxidant activity of gallic acid derivatives.

Ng et al (2000) tested a variety of flavonoids, lignans, an alkaloid, a bisbenzyl, coumarins and terpenes isolated from Chinese herbs for antioxidant activity as reflected in the ability to inhibit lipid peroxidation in rat brain and kidney homogenates and rat erythrocyte haemolysis. The compounds with antioxidant effects, with two exceptions, had at least one free aromatic hydroxyl group in their structure.

**Table 2** Effects of chromatographic fractions derived from rose flower extract on superoxide radical generation.

| Sample | Concn ($\mu g\,mL^{-1}$) | Superoxide radical generation (OD 560 nm) | Inhibition (%) |
|---|---|---|---|
| Control (buffer) | — | $0.598 \pm 0.006$ | 0 |
| Vitamin C | 500 | $0.091 \pm 0.007$ | 84.71 |
| F21 (polysaccharide–peptide) | 50 | $0.118 \pm 0.006^{a}$ | 80.27 |
| | 100 | $0.069 \pm 0.002^{a}$ | 88.46 |
| | 500 | $0.057 \pm 0.002^{a}$ | 90.47 |
| F1-a (polysaccharide–peptide) | 50 | $0.553 \pm 0.012^{b}$ | 7.53 |
| | 100 | $0.446 \pm 0.009^{b}$ | 25.42 |
| | 500 | $0.232 \pm 0.005^{b}$ | 61.20 |
| F2 (polysaccharide) | 50 | $0.536 \pm 0.008^{b}$ | 10.37 |
| | 100 | $0.429 \pm 0.011^{b}$ | 28.26 |
| | 500 | $0.211 \pm 0.005^{b}$ | 64.72 |
| F3 (gallic acid derivative) | 50 | $0.075 \pm 0.002^{a}$ | 87.46 |
| | 100 | $0.061 \pm 0.004^{a}$ | 89.80 |
| | 500 | $0.043 \pm 0.002^{a}$ | 92.81 |

The values are means $\pm$ s.d., n = 3. Data bearing different superscripts are significantly different. Data for F21 and F3 are significantly different from the other data sets as confirmed using the Kruskal–Wallis test.

**Table 3** Effect of chromatographic fractions derived from rose flower extract on hydroxyl radical generation.

| Samples | Concn ($\mu g\,mL^{-1}$) | Hydroxyl radical generation (T550 nm) | Inhibition (%) |
|---|---|---|---|
| Control | $CuSO_4$ | $53.80 \pm 0.57$ | |
| OH· | Buffer | $70.23 \pm 1.98$ | |
| Thiourea | 500 | $53.10 \pm 0.14$ | 100.00 |
| F21 (polysaccharide–peptide) | 50 | $65.53 \pm 1.83^{b}$ | 28.61 |
| | 100 | $62.43 \pm 0.97^{b}$ | 47.47 |
| | 500 | $57.17 \pm 1.03^{b}$ | 79.49 |
| F1-a (polysaccharide–peptide) | 50 | $69.63 \pm 1.41^{c}$ | 3.65 |
| | 100 | $67.60 \pm 0.91^{c}$ | 16.01 |
| | 500 | $63.80 \pm 0.74^{c}$ | 39.14 |
| F2 (polysaccharide) | 50 | $67.63 \pm 1.53^{c}$ | 15.82 |
| | 100 | $66.83 \pm 0.56^{c}$ | 20.69 |
| | 500 | $61.50 \pm 1.12^{c}$ | 53.13 |
| F3 (gallic acid derivative) | 50 | $61.10 \pm 0.64^{a}$ | 55.57 |
| | 100 | $56.90 \pm 0.93^{a}$ | 81.13 |
| | 500 | $51.73 \pm 1.27^{a}$ | 100.00 |

The values are means $\pm$ s.d., n = 3. Data bearing different superscripts are significantly different. Data for F21 are significantly different from those for F3 as confirmed using the Kruskal–Wallis test.

In this study, the gallic acid derivative possessed aromatic hydroxyl groups, and it is suggested that this structure plays an important role in its antioxidant activity.

Apart from the gallic acid derivative, the rose flower extract contained two other components with antioxidant activity, although their antioxidant activity was lower than that of the crude extract due to the presence of the highly active gallic acid derivative. One of the components, represented by fraction F2, is a polysaccharide. Protein was absent from this polysaccharide. The other component, represented by fraction Fl-a, may be a polysaccharide–peptide complex in view of the presence of protein in this preparation. Very few reports on the antioxidant activity of polysaccharides and polysaccharide–peptides complexes are available in the literature (Shin et al 2001; Ruperez et al 2002).

In sum, this study has revealed the presence, in rose flowers, of different compounds with different antioxidant potencies. This observation is important in view of the fact that the rose flower is used as a traditional Chinese herbal medicine.

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