Antioxidant activity of *Gardenia jasminoides* Ellis fruit extracts

Trishna Debnath, Pyo-Jam Park, Narayan Chandra Deb Nath, Nadira Binte Samada, Hee Won Park, Beong Ou Lim

The objective of this study was to characterise the antioxidant properties of both water and ethanol extracts from the fruit of *Gardenia jasminoides* Ellis (GJE). The IC$_{50}$ values for DPPH (1,1-diphenyl-2-picryl-hydrazyl), ABTS [2,2’-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)diammonium salt], hydroxyl and superoxide radical-scavenging activities were 0.14, 0.21, 1.08 and 1.43 mg/ml for the water-based extract, and 0.36, 0.39, 1.56 and 1.99 mg/ml for the ethanol-based extract, respectively. The extracts also showed strong reducing power, nitrite-scavenging activity, inhibition of lipoic acid oxidation, superoxide dismutase-like (SOD-like) activity and catalase activity. However, the water extract had a higher antioxidant activity than the ethanol extract. In addition, the antioxidant activities were highly correlated with the observed phenolic and flavonoid contents. Therefore, our study strongly suggests that extracts derived from the fruit of *Gardenia jasminoides* could be an excellent source of antioxidants as dietary supplements.

1. Introduction

Redox reactions that occur continuously and naturally are crucial for controlling the metabolic system in the living body. Free radicals or reactive oxygen species (ROS) are introduced into the body as products of normal metabolic functions or from the environment. Superoxide anion radicals, hydroxyl free radicals and non-free radicals or reactive species and defend the cell from oxidative stress by donating hydrogens or electrons to reactive radicals or other reactive species and defend the cell from oxidative stress by donating hydrogens or electrons to reactive radicals or species (Rice-Evans, Miller, & Paganga, 1997).

Polyphenolic compounds, derived from plants, have potent antioxidant properties (Hu, Shen, & Wang, 2009). Because of their high stability and ability to delocalize odd electrons, polyphenols are highly active antioxidants and they behave as organic ligands by chelating transition metal ions (Rice-Evans et al., 1997). Moreover, α-tocopherol and ascorbate compounds are considered to be less effective antioxidants in vitro than are polyphenols. Synthetic antioxidant compounds, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), are harmful and therefore risky to use due to some possible side effects (Hu et al., 2009). For this reason, plant antioxidant compounds have received considerable attention as natural antioxidants.

*Gardenia jasminoides* Ellis (GJE) is a flowering plant belonging to the Gardenia genus and Rubiaceae family. It has traditionally been used as a folk medicine in many Asian countries. The fruit of GJE has been used to treat inflammation, jaundice, headache, oedema, fever, hepatic disorders and hypertension (Lelono, Tachibana, & Itoh, 2009). For example, Hsu et al. (1999) have found that crocetin, a major component of Gardenia fruits, inhibits lipid peroxidation. Also, it was shown that extracts derived from the Gardenia fruit contain two well-known compounds, geniposide and genipin, that are strong anti-inflammatory agents (Koo, Lim, Jung, & Park, 2006). To our knowledge, there are no reports that detail the antioxidant activities of water and ethanol extracts produced from the dried fruits of GJE. Therefore, our goal was to evaluate the antioxidant properties of GJE fruit extracts by analysing the total phenolic and flavonoid contents, synthetic free radical (such as DPPH and...
ABTS) -scavenging activity, hydroxyl and superoxide free radical-scavenging activities, nitrite-scavenging activity, inhibition of linoleic acid oxidation, and the reducing power (Fe³⁺–Fe²⁺) and superoxide dismutase-like (SOD-like) as well as catalase activities.

2. Materials and methods

2.1. Materials and chemicals

Folin–Ciocalteu reagent (FC reagent), sodium nitrite, gallic acid, trichloroacetic acid (TCA), ascorbic acid (AA), DPPH, potassium persulphate, ABTS, linoleic acid, α-tocopherol, anhydrous sodium phosphate (dibasic), anhydrous sodium phosphate (monobasic), ferrous chloride, ammonium thiocyanate, ethylenediaminetetra-acetic acid (EDTA), 5,5'-dimethyl pyrroline-1-oxide (DMPO) and pyrogallol, were purchased from Sigma Chemical Co. (St. Louis, Missouri, USA). Ferric chloride and sodium hydroxide were obtained from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). The catalase assay kit was purchased from Cayman Chemical Company (Ann Arbor, MI, USA). All chemicals were used without any further purification.

2.2. Preparation of extracts

Dried fruits of GJE were ground to a powder (Chou, Kuo, & Lin, 2009). 100 g of the powder (equivalent to 1000 g of fresh weight) was extracted with 700 ml of de-ionised water (DW) at 85 °C for 3 h (water extraction) or 500 ml of 70% ethanol at room temperature (RT) for 24 h (ethanol extraction; Lelono et al., 2009). Each extraction was performed three times. After filtration, the extracts were evaporated under reduced pressure at 40 °C in a vacuum rotary evaporator. Afterwards, the concentrates were dried and weighed to determine total extractable compounds. Finally, the extracts were stored in a refrigerator at 4 °C until used. Yields (% w/w) were calculated [Yield (%) = (total extracted sample weight/total dry sample weight) × 100]. The stock samples were prepared by dissolving the extracted samples in DW to perform different assays.

2.3. Determination of total phenolic content

The total phenolic content of the extracts was determined by using the FC (Folin–Ciocalteu) method (Marinova, Ribarova, & Atanassova, 2005). Briefly, 10 mg of each extract was dissolved in 1 ml of DW and different concentrations of gallic acid (0–1 mg/ml) were prepared in ethanol. 40 μl of each solution of sample or a standard reagent was mixed with 20 μl of 1 N FC reagent. Next, the mixture was incubated for 3–5 min at RT, followed by addition of 20% sodium carbonate solution and incubated at RT for 30 min. Finally, the absorbance was measured at 700 nm wavelength, with a UV–visible spectrophotometer. Total phenolic content was determined from the standard calibration curve. Results were expressed as percentage of gallic acid equivalents (GAE) per 100 g dry mass.

2.4. Determination of total flavonoid content

Total flavonoid content was determined by aluminium chloride colorimetric assay (Marinova et al., 2005). The concentrations of the sample and standard (catechins) solutions were 10 mg/ml and 25, 20, 15, 10, 5 μg/ml, respectively. 25 μl of each sample or standard reagent was mixed with 125 μl of DW, followed by addition of 8 μl of 5% sodium nitrate solution. The mixture was incubated at RT for 5 min. Ten percent aluminium chloride solution was mixed with 15 μl of the above mixture. Finally, the absorbance was measured at 517 nm. Total flavonoid content was expressed as a percentage of the catechins equivalent (CE) per 100 g dry mass obtained from the standard calibration curve.

2.5. DPPH radical-scavenging activity

DPPH radical-scavenging activities of ethanol and water extracts were determined by the method of Hu et al. (2009). Different concentrations (0.12–2.00 mg/ml) of sample and BHT (as positive control) were prepared. 100 μl of each sample/standard solution was mixed with 100 μl of DPPH solution. Then, the mixture was incubated at RT for 30 min. Absorbance of the solution was measured at 517 nm. The control (instead of sample and standard) was prepared according to same procedure. DPPH radical-scavenging activity was calculated by using the following equation:

\[ \text{DPPH radical-scavenging activity} (\%) = \left( \frac{A - B}{C} \right) \times 100 \]

where, \( A = \) absorbance of DPPH + sample/standard, \( B = \) absorbance of sample/standard + methanol, \( C = \) absorbance of DPPH + DW/methanol and \( D = \) absorbance of methanol + DW.

2.6. ABTS radical-scavenging activity

ABTS was dissolved in DW at a final concentration of 7 mM and mixed with a potassium persulphate solution at a final concentration of 2.45 mM. The reaction mixture was left to settle at RT for 12–16 h in the dark before use (Jeong et al., 2009). For each experiment, freshly prepared ABTS solution was diluted with 0.01 M phosphate buffer saline (PBS, pH 7.4) to adjust its absorbance to within 0.70 ± 0.02 at 734 nm wavelength. Then 0.1 ml of various concentrations of the sample (0.12–2.00 mg/ml) and BHT (0.025–0.125 mg/ml) was mixed with 0.9 ml of ABTS solution. Finally, the absorbances were measured at 734 nm after incubation at RT for 5 min. The scavenging activity of ABTS free radical was calculated by using the following equation:

\[ \text{ABTS scavenging activity} (\%) = \left( \frac{C - D}{A - B} \right) \times 100 \]

where, \( A = \) absorbance of ABTS solution + sample/standard, \( B = \) absorbance of potassium persulphate + sample/standard, \( C = \) absorbance of ABTS solution + DW/methanol and \( D = \) potassium persulphate + DW/methanol.

2.7. Inhibition of linoleic acid oxidation

The inhibition of lipid peroxidation was determined by the method of Lee et al. (2009) with a little modification; 2.5 ml of each test sample (1 mg/ml) was mixed with 1 ml of 0.1 M sodium phosphate buffer (pH 7.0), followed by addition of 1 ml of 50 mM linoleic acid solution dissolved in 99% ethanol. After addition of 0.5 ml of DW, the mixture was incubated at 40 °C in a water bath in the dark. Then 50 μl of the above mixture was mixed with 2.5 ml of 75% ethanol and 50 μl of 30% ammonium thiocyanate. After 3 min, 50 μl of 20 mM ferrous chloride was added to the mixture, followed by incubation for 3 min at RT. The positive and negative controls were subjected to the same procedures as the test samples except that, for the negative control, only the DW was added and α-tocopherol (0.5 and 1 mg/ml) was used as a positive control. The absorbance was measured at 500 nm wavelength, every day, until the absorbance of the control reached its maximum value. We continued the experiment for up to 6 days. The scavenging activity was calculated by using the following equation:

\[ \text{Scavenging activity (\%) = 100 - [(B/A) \times 100]} \]
where, $B$ is the absorbance of the control reaction index $x = \frac{B}{t}$ (at maximum hour) and $A$ is the absorbance of sample reaction index $x = \frac{A}{t}$.

2.8. Reducing power

Reducing power activities of ethanol and water extracts were determined according to the method of Hu et al. (2009) with a little modification; 1 ml of each sample or standard reagent (BHT as positive control) at various concentrations (0.125–2.00 mg/ml) was mixed with 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 ml of potassium ferricyanide (10 mg/ml) solution. Then the mixture was incubated at 50 °C in a water bath for 30 min and mixed with 2.5 ml of TCA (100 mg/ml) and centrifuged at 3000 rpm for 10 min. 2.5 ml of the upper solution was transferred and mixed with 2.5 ml of DW, followed by addition of 1 ml of FeCl3 solution (1 mg/ml). The absorbance was measured at 700 nm wavelength. The effective concentration of each sample at 0.5 absorbance (EC50) was also evaluated.

2.9. Nitrite-scavenging activity

Nitrite scavenging activity was performed as reported elsewhere (Choi, Park, & Choi, 1989; Lee et al., 2009). Briefly, 1 ml of each sample or standard reagent at various concentrations, from 0.125–2.00 mg/ml, was mixed with 1 ml of NaNO2 (1 mM); 0.1 N each sample or standard reagent at various concentrations, from EDTA in DW; 0.8 mM DMPO in PBS of pH 7.4) in 20 ml of each solution (0.8 mM riboflavin in DW; 1.6 mM EDTA at pH 8.5, containing 10 mM EDTA). After 10 min, 1 ml of 1 N HCl was added to the above mixture to stop the reactions. Ascorbic acid was used (0.125–2 mg/ml) as a positive control. The absorbance was taken at 420 nm. SOD-like activity was calculated by using the following equation:

\[
\text{Scavenging activity} (\%) = 100 - \left(\frac{(A - B)}{C} \times 100\right),
\]

where, $A =$ Absorbance of sample/standard without reagent, $B =$ Absorbance of sample/standard with reagent, and $C =$ Absorbance of the control.

2.10. Hydroxyl and superoxide radical-scavenging activities

The hydroxyl and superoxide radical scavenging activities of each extract were measured by using an electron spin resonance (ESR) spectrometer (Jeol, Tokyo, Japan). For this purpose, 0.3 M DMPO and 10 mM H2O2 were dissolved in phosphate buffer solution (pH 7.4), and 10 mM aqueous solutions of FeSO4 was mixed with 20 μl of sample or standard solution at various concentrations. The mixture was incubated for 2.5 min at RT. Hydroxyl radicals were produced by an iron-catalysed Haber–Weiss reaction (known as Fenton-driven Haber–Weiss reaction) and the hydroxyl radicals rapidly reacted with nitrene spin-trap DMPO (Rosen & Rauckman, 1984). An ESR spectrometer was used to detect the resultant DMPO-OH adducts. The superoxide radical-scavenging activity was evaluated by using the UV-irradiated riboflavin/EDTA system (Guo et al., 1999). The reaction mixture was prepared by adding 20 μl of each solution (0.8 mM riboflavin in DW; 1.6 mM EDTA in DW; 0.8 mM DMPO in PBS of pH 7.4) in 20 μl of each extract-standard solution of various concentrations. The mixture was irradiated for 1 min under a UV lamp at 365 nm. Hydroxyl and superoxide radical-scavenging activities were determined by using the following equation:

\[
\text{Scavenging activity} (\%) = \left(\frac{C - A}{C}\right) \times 100,
\]

where, $A$ is the peak height of the sample and $C$ is the peak height of the control.

2.11. Superoxide dismutase like (SOD-like)-scavenging activity assay

The SOD-like scavenging activities of water and ethanol extracts were determined, using the method described by Marklund and Marklund (1974) with slight modification. Briefly, 200 μl of different sample solutions (0.125–2.0 mg/ml) was mixed with 200 μl of pyrogallol solution (7.2 mM in water) and 3 ml of 50 mM Tris–HCl buffer, at pH 8.5, containing 10 mM EDTA. After 10 min, 1 ml of 1 N HCl was added to the above mixture to stop the reactions. Ascorbic acid was used (0.125–2 mg/ml) as a positive control. The absorbance was taken at 420 nm. SOD-like activity was calculated by using the following equation:

\[
\text{Scavenging activity} (\%) = 100 - \left(\frac{(A - B)}{C} \times 100\right),
\]

where, $A =$ Absorbance of sample/standard with reagent, $B =$ Absorbance of sample/standard without reagent and $C =$ Absorbance of the control.

2.12. Catalase activity assay

Cayman's catalase assay kit was used to investigate the enzymatic activity in the peroxidation function of CAT (catalase) and the assay was performed according to the manufacturer's instructions. Briefly, 100 μl of assay buffer and 30 μl of methanol were mixed. The sample solution, at various concentrations from 0.25 to 2.0 mg/ml, was mixed with the above mixture. For standard curve calibration, 20 μl of different formaldehyde solutions was also added in the mixture separately to make final concentrations of formaldehyde of 0, 5, 15, 30, 45, 60 and 75 μM. Next, 20 μl of H2O2 was added to each solution to start the reaction and solutions were incubated on a shaker for 20 min at RT. 30 μl of potassium hydroxide and purpald was added to each well and the whole was incubated for 10 min at RT, followed by addition of 10 μl potassium periodate. After incubation for 5 min, the absorbance was measured at 540 nm. The positive control (bovine liver catalase) was also prepared according to the manufacturer's instructions. Catalase activity was expressed as nmol/min/ml.

2.13. Statistical analysis

All experiments were performed in triplicate and all data were expressed with mean standard deviations (SD). The correlation coefficient ($r^2$) between the parameters tested was established by regression analysis. Statistical analyses were performed with GraphPad InStat. The observed differences were analysed for statistical significance by one-way analysis of variance with Tukey's multiple comparison as a post hoc test. Differences of $P < 0.05$ were considered as significant.

3. Results and discussion

The yields were found to be 5.9% and 5.04% (w/w) for the water-based and ethanol-based GJE extracts, respectively, therefore the extractions performed with water resulted in the higher total yield. Phenolics are secondary metabolites which are major components of plants and can scavenge free radicals. The free radical-scavenging activity is one of the main functions of phenolic phytochemicals (Dorman, Pettoketo, Hiltunen, & Tikkanen, 2003). As described above, the total phenolic content of the GJE extracts was determined from a regression equation of the calibration curve ($y = -0.5181x -0.5933$) and expressed in gallic acid equivalents (GAE). These values were 53.5 and 44.8 mg GAE per 100 g of dry mass for the water and ethanol extracts, respectively (Table 1).

Flavonoids are a group of polyphenolic compounds with various chemical structures and characteristics. They occur in fruits,
and reducing power activity (EC50) of the extracts of

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Ellis (GJE) fruit.

Table 2

<table>
<thead>
<tr>
<th>Extract</th>
<th>Total phenolics a (mg GAE/100 g of dry mass)</th>
<th>Total flavonoids a (mg CE/100 g of dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water extract</td>
<td>53.5 ± 1.17</td>
<td>25.7 ± 2.02</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>44.8 ± 0.38</td>
<td>20.7 ± 0.34</td>
</tr>
</tbody>
</table>

GAE, gallic acid equivalents; CE, catechin equivalents.

* Each value is expressed as mean ± standard deviation (n = 3).

vegetables and nuts as well as in tea. They are also an integral part of human diets. It is well-known that flavonoids exhibit many types of biological activities, including anti-inflammatory, antiviral, antibacterial activities and they also act as antioxidants (Middleton, Kandaswami, & Theoharides, 2000). Moreover, flavonoids are metal chelating agents and inhibitors of lipid peroxidation (Cook & Samman, 1996). Here, the total flavonoid content was determined from the regression equation of a calibration curve (y = 0.294x + 0.2849) and expressed in catechin equivalents (CE). The flavonoid contents of the water and ethanol GJE extracts were 25.7 and 20.7 mg CE/100 g dry mass, respectively (Table 1), indicating high correlation between total phenolics and flavonoids ($r^2 = 0.9754$ and 0.9985 for the water and ethanol extracts, respectively). The water extract showed more phenolic and flavonoid contents than did the ethanol extract. The phenolic and flavonoid contents of both GJE extraction methods were more robust than those of some common fresh and dry fruits, including Phoenix dactylifera, as determined by Biglari, Alkarkhi, and Easa (2008).

The synthetic nitrogen-centred DPPH free radical is not biologically relevant but is widely used to determine antioxidant activity. The unpaired electron of the DPPH free radical has a strong absorption maximum at 517 nm and is purple and/or deep violet in colour. When it is mixed with an antioxidant, the antioxidant compound donates an electron to DPPH and its colour changes to light yellow. The water and ethanol extracts scavenged DPPH free radicals in a concentration-dependent manner. The scavenging activity ranged from 48.4% to 93.0% and 39.0% to 87.2% for the water and ethanol extracts, respectively, for GJE extract concentrations from 0.125 to 2.0 mg/ml. The concentration at which 50% of the DPPH radicals were scavenged, ($IC_{50}$), were 0.14 and 0.36 mg/ml for the water and ethanol extracts, respectively (Table 2). Furthermore, antioxidant capacity of the water extract was nearly equal to that of BHT (94.7%) at 2.0 mg/ml (P > 0.05; Table 2). However, the ethanol-based GJE extract was significantly less effective than was BHT (P < 0.05; Table 2) but showed stronger scavenging activity than those of some common fruits studied by Yan, Teng, and Jhi (2006). In addition, the fruit extract of Gardenia jasminoides shootlets and calli was observed by Sayd et al. (2010).

Excessive production of free radicals can produce a lipid peroxidation chain reaction and lipid peroxidation, which are responsible for pathological disorders (Cook & Samman, 1996). Linoleic acid is an unsaturated fatty acid that produces peroxide by autoxidation. Here, hydrogen peroxide converts ferrous to ferric ion by oxidation. Then, the red-coloured ferric thiocyanate is produced by the reaction of ferric ion and ammonium thiocyanate. We monitored the inhibition of peroxide formation by the water and ethanol extracts for up to 6 days. The absorbance data of linoleic acid peroxidation, after addition of the test samples, are plotted in Fig. 1. Linoleic acid auto-oxidation in the control reaction was strong from the first day of analysis, reached its maximum on the fourth day, and started to drop from the fifth day. The inhibition of peroxide formation by the water and ethanol extracts were 72.4% and 54.5%, respectively, on the sixth day. The inhibition activity of both extracts was not significantly different (P > 0.05) from the activity of $\alpha$-tocopherol (at 0.5 and 1.0 mg/ml). In addition, the water and ethanol extracts showed significantly stronger activity than the control (P < 0.05) on the sixth day. These results prove that both extracts of gardenia fruits are able to donate hydrogens and are also capable of terminating the lipid peroxidation chain reaction. It has been reported by Cook and Samman

Table 2

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH radical a</th>
<th>ABTS radical a</th>
<th>Nitrate a</th>
<th>Reducing power a</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$IC_{50}$ (mg/ml)</td>
<td>Scavenging activity (%)</td>
<td>$IC_{50}$ (mg/ml)</td>
<td>Scavenging activity (%)</td>
</tr>
<tr>
<td>DW extract</td>
<td>0.14 ± 0.01</td>
<td>93.0 ± 2</td>
<td>0.21 ± 0.01</td>
<td>96.4 ± 0.5</td>
</tr>
<tr>
<td>Ethanol extract</td>
<td>0.36 ± 0.02</td>
<td>87.2 ± 1.4</td>
<td>0.39 ± 0.02</td>
<td>94.3 ± 0.5</td>
</tr>
<tr>
<td>BHT</td>
<td>0.13 ± 0.06</td>
<td>94.7 ± 1.0</td>
<td>0.050 ± 0.005</td>
<td>95.2 ± 0.6 d</td>
</tr>
</tbody>
</table>

* Each value is expressed as mean ± standard deviation (n = 3).

* $IC_{50}$ (mg/ml): the concentration at which 50% is inhibited.

* $EC_{50}$ (mg/ml): effective concentration at which the absorbance is 0.5.

* Scavenging activity of BHT at 0.125 mg/ml.
(1996) that the flavonoids can inhibit lipid peroxidation. The strong inhibitory activity of GJE fruit extract might be due to its high flavonoid content. These flavonoids could increase the stability of lipid-containing foods (Cook and Samman, 1996). The Fe³⁺-Fe²⁺ reducing power of a compound is used to determine the electron donating capacity of antioxidants and is one of the main characteristics of phenolic compounds (Dorman et al., 2003). We observed a dose-dependent Fe³⁺ reducing power of the GJE extracts, as indicated by higher absorbance. The reducing power of the water and ethanol extracts increased from 0.13 to 0.55 and 0.09 to 0.50, respectively, at 0.125 to 2 mg/ml. The observed EC₅₀ of the water extract (1.73 mg/ml) was significantly higher (P < 0.05) than that of the ethanol extract (1.91 mg/ml; Table 2). The reducing power of the positive control, BHT, was more pronounced than that of both extracts with an EC₅₀ of 0.126 mg/ml (P < 0.05) shown in Table 2. Our data suggest that the extracts have a capacity to chelate transition metals. The correlation between reducing power and antioxidant activity was determined by the linoleic acid system (r² = 0.998 and 1, for water and ethanol extract, respectively), DPPH radical-scavenging system (r² = 1 and 0.995, for water and ethanol extracts, respectively) and ABTS radical-scavenging system (r² = 0.996 and 0.997, for water and ethanol extract, respectively). It was reported by Chung, Chen, Hsu, Chang, and Chou (2005) that the antioxidant properties are concomitant with the development of reducing power activity; our results are well consistent with the previous report.

Nitrite and amine compounds are present in protein-containing foods and both leafy and root vegetables; they can produce nitrosamine, and are responsible for cell damage and cancer (Lee et al., 2009). The nitrite-scavenging activity of the water and ethanol extract was increased in a dose-dependent manner, using a concentration ranging from 0.125 to 2.0 mg/ml. The water extract showed 23.0–68.5% scavenging activity whereas the ethanol extract showed 22.0–56.8% scavenging activity. The IC₅₀ values were 1.09 and 1.58 mg/ml, respectively, for the water and ethanol extracts, indicating that the scavenging activity of the water extract was significantly more potent than that of the ethanol extract (P < 0.05; Table 2). Previously, Choi et al. (1989) reported that phenolic compounds, that occur naturally in plants, may inhibit the formation of nitrosamine. Therefore, the high phenolic content of the GJE extracts might be responsible for the increased nitrite-scavenging activity. The scavenging activity of BHT was significantly stronger than those of extracts (P < 0.05; Table 2).

(a) Hydroxyl radical-scavenging activity of the water and ethanol extracts of Gardenia jasminoides Ellis (GJE) fruit at various concentrations by using an electron spin resonance (ESR) spectrometer. Ascorbic acid (AA) was used as a positive control. The superscript denotes a significant difference (a P < 0.05). Statistical comparisons were made with different concentrations of the water and ethanol extracts versus the AA (0.005 mg/ml). Each value is expressed as the mean ± SD (n = 3). (b) ESR spectra of the extracts and control (without sample/standard). The highest peak of the spectrum represents the relative amounts of the DMPO-OH adduct. Water and ethanol extracts inhibited hydroxyl radicals in a concentration-dependent manner (Fig. 2). The scavenging activities were 67.9% and 61.2% at 2 mg/ml with IC₅₀ values of 1.08 and 1.56 mg/ml for water and ethanol extract, respectively (Fig. 2). The superoxide radical scavenging activity of...

![Fig. 1](image.png)

![Fig. 2](image.png)
both extracts were determined and found to be 56.5 ± 0.1% (IC$_{50}$ of 1.43 ± 0.01 mg/ml) and 50.1 ± 0.04% (IC$_{50}$ of 1.99 ± 0.04 mg/ml) at 2 mg/ml of water and ethanol GJE extracts, respectively (Fig. 3). In both analyses, ascorbic acid (AA) was more effective (IC$_{50}$ values were 0.0017 mg/ml and 0.058 mg/ml for hydroxyl and superoxide radical, respectively) than were the extracts.

All living bodies have a complex antioxidant defence system that includes various antioxidant enzymes, such as superoxide dismutase and catalase. A rapid and facile method for the assay of SOD-like activity, based on the ability to inhibit the auto-oxidation of pyrogallol, is widely used to predict antioxidant capability. Both GJE extracts were found to have SOD-like activities, although the water extract showed significantly higher ($P < 0.05$) activity (67.1%) than the ethanol extract (54.1%) at 2 mg/ml. However, the activity of AA was more effective (95.0% at 2 mg/ml) than those of both extracts ($P < 0.05$; Table 3).

Aerobic metabolism produces superoxide anion as a byproduct and superoxide dismutase breaks it up into H$_2$O and H$_2$O$_2$ and then H$_2$O$_2$ is converted to H$_2$O and O$_2$ by catalase. Therefore, the catalase activity of extracts is very important. The catalase assay is based on the reaction of the enzyme with methanol in the presence of an optimum concentration of hydrogen peroxide (H$_2$O$_2$). At the end of the reaction, formaldehyde is formed and is measured by a colorimetric method, using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as the chromogen. Purpald specifically forms a bicyclic heterocycle with aldehydes which, upon oxidation, changes from colourless to a purple colour. The catalase activity of water-based GJE extract was significantly higher (0.94 nmol/min/ml) than that of the ethanol-based GJE extract (0.91 nmol/min/ml; Table 3). However, both extracts had catalase activity that was significantly lower ($P < 0.05$) than that of the positive control (0.96 nmol/min/ml).

The medicinal property of plants is closely related to their antioxidant capacity. Therefore, the antioxidant activity is a widely used parameter to determine the bioavailability of foodstuffs as medicinal plants. In addition, one dimensional analysis is not sufficient for the complete assessment of these properties (Frankel & Meyer, 2000). The antioxidant properties of plant extracts should be performed using different methods to ensure the effectiveness of such materials. Therefore, we evaluated the antioxidant activities by using nine different methods and using two solvents of different polarities for the extraction.

In conclusion, we have demonstrated that both GJE extraction methods result in high total phenolic and flavonoid contents. The extracts showed the highest scavenging activities against DPPH, ABTS, hydroxyl and superoxide radicals, had reducing power activity and also showed nitrite-scavenging activity. They inhibited lipid peroxidation and showed potent SOD-like and catalase enzymatic activities. In addition, we have observed a significant and linear relationship between the antioxidant activity and total phenolic and flavonoid contents. In every case, the water-based extract exhibited higher antioxidant activities than did the ethanol-based extract. Therefore, we believe that the water extraction method is a promising technique to isolate natural antioxidants from the fruit of GJE. However, the antioxidant activity was evaluated using in vitro experiments only and future in vivo experiments will lead to a more complete assessment of the antioxidant properties of GJE.

### Acknowledgement

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### References


