Radical Scavenging Activity of Gallic Acid from *Woodfordia Fruticosa* Flowers

Lok Ranjan Bhatt, Chen Nam Yook¹, Hwa Jung Choi², Seung Hwa Baek³

*Department of Bionanochemistry, Wonkwang University, 1: Department of Food Nutrition, Wonkwang Health Sciences, 2: Natural Medicine Research Centre, Korea Research Institute of Bioscience & Biotechnology, 3: Department of Herbal Resources, Professional Graduate School of Oriental Medicine Wonkwang University*

*Woodfordia fruticosa* Kurz (Lythraceae) is used in the treatment of various ailments in traditional medicines. DPPH activity guided fractionation and purification process was used to identify the free radical-scavenging components from the flowers of this plant. The methanolic extract of the plant was first fractionated into four extracts; namely, *n*-hexane, chloroform, ethyl acetate and water fractions. Among them, the ethyl acetate fraction was found to be the most effective and was further subjected to activity guided-fractionation and isolation procedures. After successive column chromatography on silica gel and Sephadex LH-20, gallic acid, which is responsible for the radical scavenging activity, was isolated and its structure was elucidated by spectral methods (¹H NMR, ¹³C NMR) and by comparison with literature.

**Key words:** *Woodfordia fruticosa*, antioxidants, gallic acid, DPPH, HPLC

---

**Introduction**

*Woodfordia fruticosa* Kurz (family: Lythraceae) is widely distributed throughout Nepal below 1,500 m and also in a majority of the south eastern and far eastern Asian countries and in tropical Africa.¹ The plant is commonly known as *Dhaunga/Dhauyo* and is used in the treatment of various ailments. Flowers are used in haemorrhage, dysentery, menorrhagia, stomach troubles, leucorrhoea² disorders of the mucous membrane, liver diseases and safe stimulant in pregnancy³. Leaf decoction is orally administered in malarial fever; pounded bark is used to cure wounds⁴. Besides other constituents, a number of phenolic compounds have been reported from the flowers⁵,⁶ and leaves⁷,⁸ of this plant.

Free radicals and related species have attracted a great deal of attention in recent years as these species have been implicated in aging and a number of human diseases. Moreover these species are responsible for oxidation of lipid in foods; thereby forming off-flavours and undesirable chemical compounds which may be detrimental to health. Natural antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retard lipid oxidative rancidity in food, cosmetics and pharmaceuticals⁹. Among the various natural antioxidants, phenolic compounds are reported to be active, quenching oxygen-derived free radicals by donating hydrogen atom or an electron to the free radical. During the initial screening of selected Nepalese medicinal plant extracts (data not shown) against DPPH radical, crude extract from *W. fruticosa* flowers showed the strongest DPPH radical scavenging activity. Hence, activity-guided fractionation of this plant was conducted using DPPH antioxidant assay.

**Materials and Methods**

1. General procedures

   NMR spectra were recorded on a JEOL JNM-ECP 500 FT NMR spectrometer with TMS as internal standard. UV spectrum was recorded on a UV-vis Diode Array Spectrophotometer, Hewlett Packard. TLC was carried out on precoated Silica gel F254 (Merck, art. 5715) plates and Spots were detected under UV (254 and 366 nm). Column chromatography was performed using Silica gel 60 (Merck, 40 - 63 and 63 - 200 µm) and Sephadex LH-20 (Sigma, Amersham Biosciences, Ltd, 25 - 100 µm). HPLC analysis was carried out, using Hitachi L-6200 liquid chromatograph (Tokyo, Japan) equipped with C-18 column (Bondapak, Waters, 300 x 95 µm), injection valve furnished with a 20-µl loop and Peak Simple software, model 202/203, SRI instruments, USA, UV detector
M 481 was used as a detector (280 nm). Isocratic elution was carried out at a flow rate of 1 L/min with acetonitrile: water (90:10) and methanol: water (15:85) as a mobile phase. Gradient elution was carried out as follow. Solvent A: 0.05 M H3PO4 + 0.05 M KH2PO4, Solvent B: Acetonitrile, 0 min- 5% B, 5 min- 5% B, 20 min-15% B, 30 min 15% B, 40 min- 30% B, 50 min 50% B, 60 min- 100 % B, flow rate of 1 mL/min.

2. Plant material

Woodyfordia fruticosa flowers, in their natural habitat, were collected and identified by myself from Dhading district, Central Nepal in March 2005 and authenticated by Dr Lokendra R. Sharma and Mr. Puran Pd. Kurni, Department of Plant Resources, Ministry of Forest & Soil Conservation. The voucher specimen was deposited in Natural Product Research Laboratory, Wonkwang University, Korea.

3. Extraction and isolation procedure

Extraction of the dried flowers with MeOH and H2O and fractionation of the former extract with solvent - solvent extraction were done as described previously7. Dried and powdered flowers (133 g) were extracted three times (3 hours) with boiling methanol (3 x 700 mL) and then with boiling water (3 x 700 mL). Supernatant was concentrated by using rotary evaporator to give methanol extract (52 g) and supernatant from water extract after filtration was lyophilized in vacuo to give a water extract (27 g). The crude methanol extract was dissolved in water and successively partitioned with hexane, chloroform and ethyl acetate, thus obtaining the hexane, chloroform ethyl acetate (EtOAc) extracts and aqueous residue. Among these, EtOAc extract showed the strongest antioxidant activity by DPPH assay and was further fractionated by repeated column chromatography.

4. Determination of total phenolic content

The total phenolic content of the crude extract was determined following the Folin-Ciocalteu method. The reaction mixture containing 200 µL of appropriate concentration of sample, 750 µL of the Folin-Ciocalteu reagent (1:10) was mixed thoroughly. After one minute, 2 mL of 7.5% sodium carbonate solution was added. The final mixture was diluted to 7 mL with deionized water. After 2 hrs incubation in dark at room temperature the absorbance was measured at 765 nm against the blank (solution contained all the reaction reagents except the sample). Gallic acid (0-500 mg/L) was used for calibration of a standard curve. Total phenolic content was determined as gallic acid equivalents (GAE) and values were expressed as mg of acid/g of plant material (in GAE).

5. DPPH radical-scavenging activity

The potential AA of extracts, fractions and pure compounds was determined on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical. Following Yen and Chen59 with slight modifications. In brief, 500 µL of various concentrations of the samples in methanol was added to 500 µL of methanol solution of DPPH (0.3 mM). The mixture was shaken vigorously and left to stand for 30 in the dark, and the absorbance was then measured at 517 nm against a blank. Extract concentration providing 50% inhibition (IC50) was calculated form the graph plotting inhibition percentage against extract concentration.

6. Nitric oxide scavenging activity

Nitric oxide (NO) scavenging effect of the extract was measured following Marcocci et al11. In brief, 10 mM sodium nitroprusside solution in phosphate buffered saline (PBS), pH 7.4 was prepared immediately before the experiment. Sodium nitroprusside (final concentration 5 mM) in PBS was mixed with samples, diluted in PBS and incubated at 25°C for 150 minutes. After incubation, samples (0.5 mL) were removed and diluted with 0.5 ml of Greiss reagent (1% sulfanilamide, 2% H3PO4 and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) and the absorbance was read at 546 nm. The inhibition of nitric oxide generation was estimated by comparing the absorbance values of control with that of treatments. The same reaction mixture without the sample or standard but the equivalent amount of PBS served as the control.

7. Metal chelating activity

The ferrous ions chelating activity was estimated as described by Dinis et al35. Briefly, different concentrations (0.1-1.0 mg/mL) of extracts in 0.4 L methanol were added to a 50 µL solution of FeCl2 (2 mM). The reaction was initiated by the addition of 5 mM ferrozine (200 µL) and total volume was adjusted to 4 with methanol. Then the mixture was shaken vigorously and left standing at room temperature for 10 Absorbance of the solution was then measured spectrophotometrically at 562 nm. The percentage of inhibition of ferrozine-Fe2+ complex formation was calculated using the formula:

Metal chelating effect (%) = ([A0-A1]/A0]×100,

Where A0 is the absorbance of the control, and A1 is the absorbance in the presence of the sample and standards. The control contains FeCl2 and ferrozine, complex formation molecules.
Results and Discussion

In our previous screening of Nepalese medicinal plant extracts against DPPH radical, flower extracts of *W. fruticosus* showed strongest activity. Hence, the extract was further evaluated for its total phenolic content, nitric oxide scavenging and metal chelating activity. It possessed high phenolic content and enabled to scavenge NO radicals but showed poor metal chelating capacity (Table 1). The results showed that the extract could be better act as free radical scavenger rather than metal chelator. Sequential extraction of the flower of *W. fruticosus* with various solvents was carried out and obtained fractions were tested against DPPH radical. Among different solvent fractions, the ethyl acetate extract demonstrated highest antioxidant activity (Table 2).

| Table 1. Total phenolic content, NO scavenging and metal chelating activities of *W. fruticosus* flower methanol extract |
|----------------|------------------------|---------------------|
|                | \( C_\text{AE} \) (mg/g GAE) | \( \text{mg/g GAE} \) |
| TPC            | 1600 ± 15.5             | 555.18 ± 3.09       |
| Metal chelating activity | 41.20 ± 4.50           |                    |

Table 2. DPPH radical scavenging activity of crude extracts and gallic acid from *W. fruticosus* flower

<table>
<thead>
<tr>
<th>Extracts</th>
<th>( C_\text{AE} ) (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>175.98 ± 8.34</td>
</tr>
<tr>
<td>Chloroform</td>
<td>51.55 ± 2.19</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>11.92 ± 0.20</td>
</tr>
<tr>
<td>Water</td>
<td>14.90 ± 0.20</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>4.60 ± 0.23</td>
</tr>
<tr>
<td>BHA</td>
<td>26.18 ± 0.57</td>
</tr>
</tbody>
</table>

Hence, it was selected for the isolation of active compound responsible for the antioxidant activity. Silica gel column chromatography of crude EA extract (eluted with 95% chloroform/Methanol to 100% Methanol) yielded three fractions (WFCA 1 - WFCA 3). TLC analysis was performed on silica gel using chloroform: methanol: water (6:4:1) as the mobile phase. Compounds were visualized after spraying the plates with a methanol solution of diphenylboric acid-B-ethylamino ester followed by ethanol solution of polyethylene glycol and ferric chloride solution. Further purification of WFCA 3 with Sephadex LH-20 (60 g) column, developing with aqueous methanol (60 % Methanol to 100 % Methanol) yielded nine fractions (WFCA 3-1 - WFCA 3-9), wherein active fraction, WFCA 3-2 was further purified using Sephadex LH-20 column chromatography, eluted with Methanol: H₂O (80:20) and yielded four subfractions. Subfraction three showed a single spot on TLC, Rf value = 0.60 (Chloroform: Methanol: water; 6:4:1) and after evaporation yielded white amorphous solid (42 mg). The purity of the isolated compound was confirmed by HPLC, which showed single peak in the HPLC chromatogram (RT: 3.42 min) (Fig. 1, 2).

![HPLC chromatogram of the ethyl acetate extract of *W. fruticosus* flower](image)

**Fig. 1.** HPLC chromatogram of the ethyl acetate extract of *W. fruticosus* flower. HPLC conditions: Solvent A: 0.05 M H₃PO₄ + 0.05 M KH₂PO₄, Solvent B: Acetonitrile, 0 min: 5 % B, 5 min: 5 % B, 20 min: 15 % B, 30 min 15 % B, 40 min: 30 % B, 50 min 50 % B, 60 min: 100 % B, flow rate of 1 mL/min. QA: Gallic acid.

![HPLC chromatogram of gallic acid (GA) isolated from *W. fruticosus* flower](image)

**Fig. 2.** HPLC chromatogram of gallic acid (GA) isolated from *W. fruticosus* flower. HPLC conditions: Solvent A: 0.05 M H₃PO₄ + 0.05 M KH₂PO₄, Solvent B: Acetonitrile, 0 min: 5 % B, 5 min: 5 % B, 20 min: 15 % B, 30 min 15 % B, 40 min: 30 % B, 50 min 50 % B, 60 min: 100 % B, flow rate of 1 mL/min.

Moreover, the compound was subjected to spectroscopic analysis to elucidate the structure. The compound has only singlet signal (δ 7.14) in the ¹H NMR spectrum and seven carbon signals in the low field in the ¹³C NMR spectrum. Based on these data, the compound was identified as gallic acid (C₇H₆O₅). The data are in agreement with the previous reports. Previously, gallic acid was reported from leaves and stems of *W. fruticosus* but there is no previous report of this compound from flowers of this plant.

Gallic acid, isolated from EA extract of *W. fruticosus* flower showed very strong DPPH radical scavenging activity that was quite higher than EA extract and reference antioxidant (BHA). Previous authors also reported very strong DPPH radical scavenging activity of gallic acid. According to these authors, the inductive effect of the three hydroxyl groups in gallic acid is an important factor that enhance its activity.
Moreover, gallic acid and phenolic compounds possessing a similar structure as gallic acid are reported to demonstrate high activity towards DPPH • free radical.10

Acknowledgements

This research was supported by Wonkwang Health Sciences College in 2008, Korea.

References