Comparative antioxidant and antiplasmodial activities of 11-O-galloylbergenin and bergenin isolated from *Bergenia ligulata*

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Abstract. In the present study, the antioxidant and antiplasmodial activities of bergenin was compared with its natural derivative 11-O-galloylbergenin for the first time. Both compounds were isolated from *Bergenia ligulata*. 11-O-galloylbergenin was found to be very active in in-vitro antioxidant assay as compared to bergenin, which was found to be almost inactive. The EC\textsubscript{50} values of 11-O-galloylbergenin were 7.45±0.2 µg/mL and 5.39±0.28 µg/mL in DPPH antioxidant assay and reducing power assay respectively, while IC\textsubscript{50} value for antiplasmodial assay of both compounds were less than 2.5 µM. Interestingly, in the total antioxidant phosphomolybdate assay, 11-O-galloylbergenin was found more potent (CAHT: 940.04±115.30) as compared to \(\alpha\)-tocopherol (CAHT: 552.4±27.91).

INTRODUCTION

Reactive oxygen species (ROS) in a biological system results in lipid peroxidation, protein peroxidation and denaturation, DNA damage and other deleterious effects like cellular degeneration in the cells. Various pathological conditions and diseases are induced by the accumulation of these reactive species including cardiovascular disease, ageing, cancer, inflammatory diseases and a variety of other disorders (Petrone \textit{et al}., 1980; Halliwell \textit{et al}., 1992; Knight, 1995; Finkel \textit{et al}., 2000). Antioxidant compounds are used to overcome the damages induced by Reactive oxygen species (ROS). Antioxidants both from synthetic as well as natural sources were studied but synthetic ones were found to be carcinogenic as compared to natural antioxidants (Thompson & Moldeus, 1988). Plants are rich sources of antioxidant compounds of diverse chemical nature (Mokbel & Hashinaga, 2006).

Similarly malaria is still the most important parasitic disease in the world, causing 2-3 million deaths each year. The rising resistance of *Plasmodium* spp., especially *Plasmodium falciparum* to known antimalarial such as chloroquine makes the search for new antimalarial drugs increasingly important. Many conventional antiparasitic drugs, e.g. quinine and the artemisinine derivatives, originated from natural sources (Wright & Phillipson, 1990).

Bergenin is a bioactive molecule and reported to have antioxidant (Heitor \textit{et al}., 2008), neuroprotective (Takahashi \textit{et al}., 2003), hypolipidaemic (Jahromi \textit{et al}., 1992), anti-HIV (Piacente \textit{et al}., 1996), antiarrhythmic (Pu \textit{et al}., 2002), anti-inflammatory (Swarnalakshmi \textit{et al}., 1984), antimalarial (Da Silva \textit{et al}., 2004), PTP1B inhibitory activity (Rong \textit{et al}., 2005) and gastro-protective (Goel \textit{et al}., 1997) activities. The exact molecular mechanism is still not clear for these activities. Other important pharmacological activities
include inhibitory effect on platelet aggregation (Lee et al., 2005; Nazir et al., 2007), inhibitory effect on bovine adrenal tyrosine hydroxylase (TH), the rate-limiting enzyme in the biosynthesis of catecholamine (Zhang et al., 2003). Its antioxidant activities are well established and found be as potent antioxidant as ascorbic acid or quercetin (Lee et al., 2005). Its hepatoprotective activity is studied in-vitro and in-vivo in various models such as carbon tetrachloride-intoxicated rats (Lim et al., 2000) and D-galactosamine-induced hepatotoxicity in rats (Lim et al., 2001). The antifungal activities of bergenin against seven pathogenic plant species were reported (Prithiviraj et al., 1997).

Wide spectrum of the pharmacological profile of bergenin still permits the investigations for its new therapeutic targets associated with molecular mechanisms. In the current study we have analyzed the comparative antioxidant and antiplasmodial assays of bergenin and 11-O-galloylbergenin, isolated from Bergenia ligulata. 

MATERIALS & METHODS

General Experimental Procedures

$^{1}$H-NMR, $^{13}$C-NMR were recorded at 400 MHz for $^{1}$H, at 100 MHz for $^{13}$C using TMS as internal standard with Bruker DPX-400 instrument in deuterated solutions. Mass spectra were recorded on Agilent 5973N instrument using EI mode. The aluminum sheets precoated with silica gel 60 F254 (20 x 20 cm, 0.2 mm thick; E-Merck) were used for TLC and silica gel (200-300 mesh) for column chromatography.

For antioxidant activities all the chemicals used were of analytical grade, gallic acid and quecetein were purchased from Acros (USA), 1,1- diphenyl-2-picryl hydrazyl radical (DPPH) from Fluka (Germany), α-tocopherol from E. Merck (Germany), trichloroacetic acid from Riedal-deHaen (Germany) sodium phosphate from Panreac (Spain), ammonium molybdate from ABSCO(UK), Folin-Ciocalteu’s phenol reagent (FCR), sodium carbonate, ascorbic acid, potassium ferricyanide, ferric chloride, sulfuric acid and the other reagents were purchased from Merck (Germany). All other unlabelled chemicals and reagents were of analytical grade. Spectra were recorded on a SP-3000 PLUS Spectrophotometer (Optima, Japan) and the commercial solvents used for extraction were re-distilled.

Plant Material

Plant material (roots) of Bergenia ligulata was collected from the district Hazara Division in the North of Pakistan in July 2010 and identified void Voucher No. 1124 (pup) by Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar, Peshawar, Pakistan.

Extraction & Isolation

Air-dried roots of B. ligulata (6 kg) were chopped and grinded. The grinded material was extracted three times with 20 L commercial ethanol for 72 h and filtrate was evaporated under vacuum, affording 260 g (4.3%). 50 g of this extract was loaded over silica gel column eluted with chloroform-methanol in order of increasing polarity, yielded two compounds; bergenin (1; 0.5 g) and 11-O-galloylbergenin (2; 0.05 g).
**DPPH Radical Scavenging Assays**
The DPPH radical scavenging activities of the isolated compounds were estimated using a slight modified form of the protocol reported by Blois (1958) and Mavi et al. (2004). 1 mM methanolic DPPH radical solution was mixed with 3 ml of ethanolic solution containing the sample (20-100 µg) and control (without sample). Mixture was analyzed after 30 minutes at 517 nm. Decrease in the absorbance given by DPPH solution indicated the increase in DPPH radical scavenging activities. Ascorbic acid, α-tocopherol, quercetin and gallic acid were used as standards. Percentage radical scavenging activity (% RSA) was calculated using equation:

\[
% \text{RSA} = \frac{\text{control absorbance} - \text{sample absorbance}}{\text{control absorbance}} \times 100
\]

The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations. The concentration showing 50% inhibition (EC$_{50}$) was calculated from the % RSA against concentration.

**Total Antioxidant Capacity**
The total antioxidant capacity of the compounds was evaluated by the method of Prieto et al. (1999). An aliquot of 0.3 mL of the sample solution (two replicates) was mixed with 1 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The effective concentration of the sample required to scavenge DPPH radical by 50 µg/mL was obtained and measured absorbance at 695 nm against standard and blank solutions. Total antioxidant capacity was expressed as equivalents of ascorbic acid µmole/mg of the compounds.

**Antiplasmodial Assay**
For antiplasmodial activity the samples were tested in duplicate on one occasion against chloroquine sensitive (CQS) strain of *Plasmodium falciparum* (D10). Continuous in-vitro cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager & Jensen (1976). Quantitative assessment of antiplasmodial activity in-vitro was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler et al. (1993).

The samples were prepared from 2 mg/mL stock solution in 10% DMSO and sonicated to enhance solubility. Chloroquine (CQ) was used as the reference drug in all experiments. Compounds were stored at -20°C until use. Test samples were tested at three concentrations which were 10 µM, 5 µM and 2.5 µM against CQ (0.05 µM, 0.02 µM and 0.01 µM).

**RESULTS AND DISCUSSION**
The isolated compounds bergenin (1) and 11-O-galloylbergenin (2) were characterized by comparing its physical and spectral data with previous literature (Yoshida *et al.*, 1982; Srinivasas *et al.*, 2007). 11-O-Galloylbergenin was investigated first time from this plant. Bergenin and 11-O-galloylbergenin showed 6.44±1.91% and 85.10±0.418% radical scavenging activity at concentration...
of 100 µg/mL, respectively. The EC$_{50}$ values for 11-O-galloylbergenin in DPPH assay was 7.45±0.2 µg/mL. However the standards were quercetin (98.18±0.187%), ascorbic acid (97.60±0.519%), gallic acid (98.03±0.491%) and α-tocopherol (92.14±0.32%) were found relatively better than the 11-O-galloylbergenin (Table 1). The EC$_{50}$ values for standard compounds (Table 2) were 4.19±1.41 µg/mL (quercetin), 6.31±1.03 µg/mL (ascorbic acid), 4.53±0.92 µg/mL (gallic acid) and 32.50±1.58 µg/mL (α-tocopherol).

Similarly antioxidant activity in terms of reducing power was found very high for 11-O-galloylbergenin (2) as compared to bergenin (1). At 25 µg/mL concentration the reducing powers (Table 1) for bergenin, 11-O-galloylbergenin, quercetin, ascorbic acid, gallic acid and α-tocopherol were 0.045±0.003, 1.321±0.035, 3.28±0.031, 1.23±0.024, 21.38±0.069 respectively. The EC$_{50}$ values for 11-O-galloylbergenin were 5.39±0.28 µg/mL against standards (Table 2); 1.88±0.031 µg/mL (quercetin), 3.29±0.031 µg/mL (ascorbic acid), 1.23±0.023 µg/mL (gallic acid) and 21.48±0.071 µg/mL (α-tocopherol).

Table 1. Antioxidant activities

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>^dDPPH assay scavenging power at 25 µg/mL (%)</th>
<th>^bReducing Power at 25 µg/mL (µg/mL)</th>
<th>^cTotal Antioxidant Phosphomolybdate assay as ascorbic acid equivalents (µmole/mg of extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bergenin</td>
<td>6.44±1.910</td>
<td>0.045±0.003</td>
<td>48.28±2.46</td>
</tr>
<tr>
<td>2</td>
<td>11-O-Galloylbergenin</td>
<td>85.10±0.418</td>
<td>1.321±0.035</td>
<td>940.04±115.30</td>
</tr>
<tr>
<td>3</td>
<td>Ascorbic acid</td>
<td>97.60±0.519</td>
<td>3.28±0.045</td>
<td>2469.4±149.7</td>
</tr>
<tr>
<td>4</td>
<td>Gallic acid</td>
<td>98.03±0.491</td>
<td>1.23±0.024</td>
<td>2176.1±173</td>
</tr>
<tr>
<td>5</td>
<td>Quercetin</td>
<td>98.18±0.187</td>
<td>1.86±0.031</td>
<td>2057.51±124.3</td>
</tr>
<tr>
<td>6</td>
<td>α-tocopherol</td>
<td>92.14±0.320</td>
<td>21.38±0.069</td>
<td>552.4±27.91</td>
</tr>
</tbody>
</table>

^a,b,c The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations

Table 2. EC$_{50}$ values$^{a,b}$ (µg/mL) in reducing power and DPPH scavenging assays

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>DPPH Radical scavenging assay (EC$_{50}^{a}$)</th>
<th>Reducing Power (EC$_{50}^{b}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bergenin</td>
<td>&lt;100</td>
<td>&lt; 25</td>
</tr>
<tr>
<td>2</td>
<td>11-O-galloylbergenin</td>
<td>7.45±0.2</td>
<td>5.39±0.28</td>
</tr>
<tr>
<td>3</td>
<td>Ascorbic acid</td>
<td>6.31±1.03</td>
<td>3.29±0.031</td>
</tr>
<tr>
<td>4</td>
<td>Gallic acid</td>
<td>4.53±0.92</td>
<td>1.23±0.023</td>
</tr>
<tr>
<td>5</td>
<td>Quercetin</td>
<td>4.19±1.41</td>
<td>1.88±0.031</td>
</tr>
<tr>
<td>6</td>
<td>α-Tocopherol</td>
<td>32.50±1.58</td>
<td>21.48±0.071</td>
</tr>
</tbody>
</table>

$^{a}$ EC$_{50}$ (µg/mL): effective concentration at which 50% of DPPH radicals are scavenged

$^{b}$ EC$_{50}$ (µg/mL): effective concentration at which the absorbance is 0.4
Compounds 1 and 2 were tested against the CQS D10 strain of *P. falciparum*. Both compounds were displayed a significant activity at low concentration with IC$_{50}$ values less than 2.5 µg/mL against a value of 0.028 µM for CQ as positive control (Table 3).

In-vitro models of antioxidant activities of 11-O-galloylbergenin showed a potent and effective antioxidant especially from CAHT analysis and found that 11-O-galloylbergenin is more active as compared to α-tocopherol. These promising antioxidant activities revealed that compound 2 may be potentially useful for various pathological conditions associated with the devastating effects of reactive oxygen species. Furthermore, 11-O-galloylbergenin showed excellent antioxidant activities as compare to bergenin and its synthetic derivatives (Takahashi et al., 2003).

### References

- Makler, M.T., Raise, J.M., Williams, J.A., Bancroft, J.E., Piper, R.C., Gibbins, B.L. & Hinrichs, D.J. (1993). Parasite lactate dehydrogenase as an assay for *Plas-

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample (µM)</th>
<th>D10 IC$_{50}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Bergenin</td>
<td>2.41 ± 0.4</td>
</tr>
<tr>
<td>2</td>
<td>11-O-galloylbergenin</td>
<td>2.34 ± 0.2</td>
</tr>
<tr>
<td>4</td>
<td>Chloroquine</td>
<td>0.028 ± 0.004</td>
</tr>
</tbody>
</table>
modium falciparum drug sensitivity. 