Tropical Biomedicine 31(1): 143–148 (2014)

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

Uddin, G.[1*], Sadat, A.[1] and Siddiqui, B.S.[2]
[1]Institute of Chemical Sciences, University of Peshawar, Peshawar, 25120, Pakistan
[2]HEJ Research Institute of Chemistry, University of Karachi, Karachi, Pakistan
[*]Corresponding author email: ghiasuddin@upesh.edu.pk
Received 27 May 2013; received in revised form 19 September 2013; accepted 10 October 2013

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_{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_{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 α-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 *et al.*, 1980; Halliwell *et al.*, 1992; Knight, 1995; Finkel *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 antiparasitiec 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 *et al.*, 2008), neuroprotective (Takahashi *et al.*, 2003), hypolipidaemic (Jahromi *et al.*, 1992), anti-HIV (Piacente *et al.*, 1996), antiarrhythmic (Pu *et al.*, 2002), anti-inflammatory (Swarnalakshmi *et al.*, 1984), antimalarial (Da Silva *et al.*, 2004), PTP1B inhibitory activity (Rong *et al.*, 2005) and gastro-protective (Goel *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.

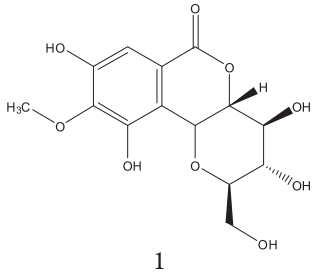
For antioxidant activities all the chemicals used were of analytical grade, gallic acid and quecetein were purchased from Acros (USA), 1,1- diphenyl-2-picryl hydrazyl radical (DPPH) from Fluka (Germany), α-tocopherol from E. Merck (Germany), trichloroacetic acid from Riedal-deHaen (Germany) sodium phosphate 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

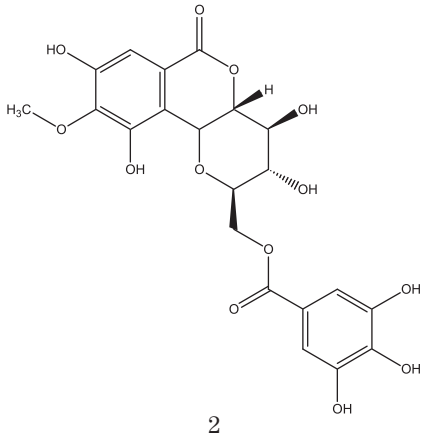
Plant material (roots) of *Bergenia ligulata* was collected from the district Hazara Divison in the North of Pakistan in July 2010 and identified void Voucher No. 1124 (pup) by Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar, Peshawar, Pakistan.

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).



1



2

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.

Determination of Reducing Power

The reducing power of the compounds was determined by the method of Oyaizu (1986). Briefly, the assay medium contained various concentrations (20 to 100 µg/mL) of the compounds in 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. 2.5 mL of trichloroacetic acid (10% w/v) was added after incubation at 50°C for 30 min and the mixture was then centrifuged at 1,750 × g for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride and the absorbance of the resultant solutions were read at 700 nm. Quercetin, ascorbic acid, gallic acid and α-tocopherol used as standards. Higher absorbance indicates higher reducing power. The assays were carried out in triplicate and the results are expressed as mean values ± standard deviations. The concentration providing 0.4 of absorbance (EC_{50}) was calculated from the absorbance at 700 nm.

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, 1.86±0.031, 3.28±0.045, 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).

Total antioxidant phosphomolybdate assay as ascorbic acid equivalents (CAHT: µmole/mg of test compound), calculated for bergenin, 11-*O*-galloylbergenin, quercetin, ascorbic acid, gallic acid and α-tocopherol were 48.28±2.46, 940.04±115.30, 2057.51± 124.3, 2469.4±149.7, 2176.1±173 and 552.4± 27.91 respectively (Table 2).

Table 1. Antioxidant activities

S. No.	Sample	[a]DPPH assay % Radical scavenging assay (at100 µg/ml)	[b]Reducing Power (at 25µg/ml)	[c]Total Antioxidant Phosphomolybdate assay as ascorbic acid equivalents (CAHT) (µmole/mg of extract)
1	Bergenin	6.44 ± 1.910	0.045 ± 0.003	48.28 ± 2.46
2	11-*O*-Galloylbergenin	85.10 ± 0.418	1.321 ± 0.035	940.04 ± 115.30
3	Ascorbic acid	97.60 ± 0.519	3.28 ± 0.045	2469.4 ± 149.7
4	Gallic acid	98.03 ± 0.491	1.23 ± 0.024	2176.1 ± 173
5	Quercetin	98.18 ± 0.187	1.86 ± 0.031	2057.51 ± 124.3
6	α-tocopherol	92.14 ± 0.320	21.38 ± 0.069	552.4 ± 27.91

[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

S. No.	Sample	DPPH Radical scavenging assay (EC_{50}[a])	Reducing Power (EC_{50}[b])
1	Bergenin	<100	< 25
2	11-*O*-galloylbergenin	7.45 ± 0.2	5.39 ± 0.28
3	Ascorbic acid	6.31 ± 1.03	3.29 ± 0.031
4	Gallic acid	4.53 ± 0.92	1.23 ± 0.023
5	Quercetin	4.19 ± 1.41	1.88 ± 0.031
6	α-Tocopherol	32.50 ± 1.58	21.48 ± 0.071

[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

Table 3. Antimalarial activity of compound **1** and **2** against *P. falciparum* (D10)

S. No.	Sample (µM)	D10 IC_{50}
1	Bergenin	2.41 ± 0.4
2	11-*O*-galloylbergenin	2.34 ± 0.2
4	Chloroquine	0.028 ± 0.004
5	Artemisinin	0.026 ± 0.005

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).

Acknowledgments. The authors are very thankful to Higher Education Commission of Pakistan for granting Indigenous Scholarship and to Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar, Pakistan and Abdul Majid, Lecturer, Department of Botany, Hazara University, Pakistan for identification of plant.

REFERENCES

Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature* **181**: 1199-1201.

Da Silva, T.B.C., Alves, V.L., Mendonça, L.V.H., Conserva, L.M., Da Rocha, E.M.M., Andrade, E.H.A. & Lemos, R.P.L. (2004). Chemical constituents and preliminary antimalarial activity of *Humiria balsamifera*. *Pharmaceutical Biology* **42**: 94-97.

Finkel, T. & Holbrook, N.J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature* **408**: 239.

Goel, R.K., Maiti, R.N., Manickam, M. & Ray, A.B. (1997). Antiulcer activity of naturally occurring pyrano-coumarin and isocoumarins. *Indian Journal of Experimental Biology* **35**: 1080-1083.

Halliwell, B., Gutteridge, J.M.C. & Cross, C.E. (1992). Free radicals, antioxidants and human disease: where are we now. *Journal of Laboratory and Clinical Medicine* **119**: 598.

Heitor, A., Izandina, L.,Gilmar, P., Piló-Veloso, D. & Antônio-Flávio, A. (2008). Antioxidant activity of (+)-bergenin – a phytoconstituent isolated from the bark of *Sacoglottis uchi* Huber (Humireaceae). *Organic and Biomolecular Chemistry* **6**: 2713-2718.

Jahromi, M.A.F., Chansouri, J.P.N. & Ray, A.B. (1992). Hypolipidaemic activity in rats of Bergenin. *Phytotherapy Research* **6**: 180.

Knight, J.A. (1995). Diseases related to oxygen-derived free radicals. *Annals of Clinical and Laboratory Sciences* **25**: 111.

Lee, Y.Y., Jang, D.S., Jin, J.L. & Yun-Choi, H.S. (2005). Anti-platelet aggregating and anti-oxidative activities of *11-O-(4'-O-methylgalloyl)*-bergenin, a new compound isolated from Crassula cv Himaturi. *Planta Medica* **71**: 776.

Lim, H.K., Kim, H.S., Choi, H.S., Oh, S. & Choi, J. (2000). Hepatoprotective effects of bergenin, a major constituent of *Mallotus japonicus*, on carbon tetrachloride-intoxicated rats. *Journal of Ethnopharmacology* **72**: 469-474.

Lim, H.K., Kim, H.S., Choi, H.S., Choi, J., Kim, S.H. & Chang, M.J. (2001). Effects of bergenin, the major constituent of *Mallotus japonicus* against D-galactosamine-induced hepatotoxicity in rats. *Pharmacology* **63**: 71-75.

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-*

modium falciparum drug sensitivity. *Journal of American Society of Tropical Medicine and Hygiene* **48**: 739-741.

Mavi, A., Terzi, Z., Ozgen, U., Yildirim, A. & Coskun. (2004). Antioxidant properties of some medicinal plants. *Biological and Pharmaceutical Bulletin* **27**: 702-705.

Mokbel, M.S. & Hashinaga, F. (2006). Evaluation of the antioxidant activity of extracts from buntan (Citrus grandis Osbeck) fruit tissues. *Food Chemistry* **94**: 529.

Nazir, N., Koul, S., Qurishi, M.A., Taneja, S.C., Ahmad, S.F., Bani, S. & Qazi, G.N. (2007). Immunomodulatory effect of bergenin and norbergenin against adjuvant-induced arthritis – A flow cytometric study. *Journal of Ethnopharmacology* **112**: 401-405.

Oyaizu, M. (1986). Studies on product of browning reaction prepared from glucose amine. *Japanese Journal of Nutrition* **44**: 307-315.

Petrone, W.F., English, D.K., Wong, K. & Mccord, J.M. (1980). Free radicals and inflammation: superoxide-dependent activation of a neutrophil chemotactic factor in plasma. *Proceedings of the Natural Academy of Sciences* **77**: 1159-1163.

Piacente, S., Pizza, C., De-Tommasi, N. & Mahmood, N. (1996). Constituents of *Ardisia japonica* and their *in-vitro* anti-HIV activity. *Journal of Natural Products* **59**: 565-569.

Prieto, P., Pineda, M. & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochemistry* **269**: 337-341.

Prithiviraj, B., Singh, U.P., Manickam, M., Srivastava, J.S. & Ray, A.B. (1997). Antifungal activity of bergenin, a constituent of *Fluggea microcarpa*. *Plant Pathology* **46**: 244-248.

Pu, H.L., Huang, X., Zhao, J.H. & Hing, A. (2002). Synthesis and biological evaluation of bergenin analogues as mushroom tyrosinase inhibitors. *Planta Medica* **68**: 372-374.

Rong, Z., Bogang, L. & Guolin, Z. (2005). Chemical study on *Cyathula officinalis* Kuan. *Journal of Asian Natural Products Research* **7**(3): 245-252.

Swarnalakshmi, T., Sethuraman, M.G., Sulochana, N. & Arivudainambi, R. (1984). Antimicrobial activity of bergenin from *Endopleura uchi*. *Current Science* **53**: 917.

Srinivasas, R., Chandrasekar, M.J.N., Nanjan, M.J. & Suresh, B. (2007). Antioxidant activity of *Caesalpinia digyna* root. *Journal of Ethnopharmacology* **113**: 284-291.

Thompson, D. & Moldeus, P. (1988). Cytotoxicity of butylated hydroxyanisole and butylated hydroxytoluene in isolated rat hepatocytes. *Biochemical Pharmacology* **37**: 2201.

Trager, W. & Jensen, J.B. (1976). Human malaria parasites in continuous culture. *Science* **193**: 673-675.

Takahashi, H., Kosaka, M., Watanabe, Y., Nakade, K. & Fukuyama, Y. (2003). Synthesis and neuroprotective activity of bergenin derivatives with antioxidant activity. *Bioorganic and Medicinal Chemistry* **11**: 1781-1788.

Wright, C.W. & Phillipson, J.D. (1990). Natural products and the development of selective antiprotozoal drugs. *Phytotherapy Research* **4**: 127-139.

Yoshida, T., Seno, K., Takama, Y. & Okuda, T. (1982). Bergenin derivatives from *Mallotus japonicus*. *Phytochemistry* **21**: 1180-1182.

Zhang, Y.H., Fang, L.H., Lee, M.K. & Ku, B.S. (2003). *In vitro* inhibitory effects of bergenin and norbegenin on bovine adrenal tyrosine hydroxylase. *Phytotherapy Research* **17**: 967-969.