SHORT COMMUNICATION

Structure activity relationship of bergenin, p-hydroxybenzoyl bergenin, 11-O-galloylbergenin as potent antioxidant and urease inhibitor isolated from Bergenia ligulata

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Ethanol extract of the aerial parts of Bergenia ligulata was subjected to solvent–solvent separation followed by various chromatographic techniques that lead to isolation of bergenine (1), p-hydroxybenzoyl bergenin (2), 11-O-galloylbergenin (3) and methyl gallate (4) as major constituents. Ethyl acetate fraction showed a dose-dependent urease inhibitory pattern with IC50 value of 54 \( \mu \)g/mL. Structures of compounds 1 and 3 were established by XRD and 2, 4 by NMR. All these compounds were subjected to DPPH scavenging activity, reducing power assay and urease inhibitory activity. The EC50 7.45 \( \pm \) 0.2 \( \mu \)g/mL and 5.39 \( \pm \) 0.28 \( \mu \)g/mL values in terms of antioxidant and reducing power, respectively, were less for 3. Compounds 1–3 showed moderate to significant urease inhibitory potential with IC50 57.1 \( \pm \) 0.7, IC50 48.4 \( \pm \) 0.3 and 38.6 \( \pm \) 1.5. Antioxidant activities and urease inhibitory potential were investigated and compound 3 was found to be the most active.

Keywords: reactive oxygen species; antioxidant assay; Jack bean urease; peptic ulcers

1. Introduction

Accumulation of reactive oxygen species (ROS) in a living cell results in the lipid peroxidation, causes oxidative damage to proteins and denaturation of DNA and also can cause diseases such as cancer, diabetes, aging and cardiovascular dysfunction (Halliwell et al. 1992, Knight 1995; Finkel & Holbrook 2000). Antioxidant compounds are used to protect and overcome the damages induced by ROS. Plants are rich sources of antioxidant compounds of diverse chemical nature with minimal side effects or no side effect at all (Mokbel & Hashinaga 2006).

Urease has diverse functions and its inhibition has received special attention over the past few years and many urease inhibitors have been reported such as hydroxamic acid derivatives (Amtul et al. 2002), hydroxyurea (Uesato et al. 2002), hydroxamic acids (Odake et al. 1994),...
phosphorodiamidates (Faraci et al. 1995), imidazoles such as rabeprazole (Pope et al. 1998), lansoprazole (Park et al. 1996), omeprazole (Nagata et al. 1993), quinines (Kuhler et al. 1995), thiol-compounds (Bundy & Bremner 1973), plaunotol and its thiourea derivatives (Todd & Hausinger 1989).

Bergenin (1) is a bioactive molecule and reported to have neuroprotective (Kogen et al. 1999), antitussive (Takahashi 2003), hypolipidaemic (Piegen 1980), anti-HIV, antiarrhythmic (Jahromi et al. 1992), antiinflammatory (Piacente et al. 1996), PTP1B inhibitory (Pu et al. 2002) and gastroprotective (Swarnalakshmi et al. 1984) activities. The exact molecular mechanism is still not clear for these activities. Other important pharmacological activities include an inhibitory effect on platelet aggregation (Li et al. 2005) and an inhibitory effect on bovine adrenal tyrosine hydroxylase, the rate-limiting enzyme in the biosynthesis of catecholamine (Goel et al. 1997). Its antioxidant activities are well established, and interestingly in one study it was found to be a potent antioxidant as ascorbic acid or quercetin (Nazir et al. 2007). Its hepatoprotective activity is studied in vitro and in vivo in various models such as carbon tetrachloride-intoxicated rats (Lee et al. 2005) and D-galactosamine-induced hepatotoxicity in rats (Zhang et al. 2003). The antifungal activities of bergenin against seven pathogenic plant species were reported (Lim et al. 2000). However, for 11-O-galloylbergenin (2), only the anti-inflammatory and analgesic activities have been reported.

Wide spectrum of the pharmacological profile of bergenin (1) still permits the investigations for its new therapeutic targets and the associated molecular mechanisms. In the current study, the structure–activity relation with respect to antioxidant and urease inhibitory potential of bergenin (1), para-hydroxybergenin (2) and 11-O-galloylbergenin (3) isolated from Bergenia ligulata was studied.

2. Results and discussion
The bergenin (1) and 11-O-Galloylbergenin (3) were transparent crystals and characterised by Single Crystal X-ray Diffraction (Figure S1) and also 2 and 3 were hitherto unreported form the genus Bergenia and 3 was isolated for the first time in a crystalline form as a natural product. Compounds 2 and 4 (Figure S2) were isolated as an amorphous powder and their structures were established by 1HN M R, 13C NMR and by comparing their spectral data with those of the reported compounds from the literature (Yoshida et al. 1982).

2.1. Antioxidant SAR
In DPPH radical scavenging assay compound 1, 2, 3 and 4 showed EC_{50} value of >100, 21.16 ± 0.4, 7.45 ± 0.2 and 4.53 ± 0.92 µg/mL (Table S2), respectively. The EC_{50} values for 11-O-galloylbergenin (2) in DPPH assay was least amongst the bergenin derivatives, i.e. 7.45 ± 0.2 µg/mL (Table S2). The EC_{50} values for standard compounds were 4.19 ± 1.41 µg/mL (quercetin) and 6.31 ± 1.03 µg/mL (ascorbic acid).

Similarly, antioxidant activity in terms of reducing power was found significant for 11-O-galloylbergenin (3) as compared p-hydroxybenzoyl bergenin (2) and bergenin (1). At 25 µg/mL concentration, the reducing powers for 1, 2, 3 and 4 were 0.045 ± 0.003, 0.824 ± 0.0007, 1.321 ± 0.035 and 1.23 ± 0.024 µ/g/mL, respectively (Table S1). The EC_{50} values for 11-O-galloylbergenin was 5.39 ± 0.28 µg/mL less than bergenin EC_{50} >25 and higher from methyl gallate EC_{50} 1.23 ± 0.023 (Table S2).

From all the in vitro models of antioxidant activities, it is evident that 11-O-galloylbergenin (3) is a potent and effective antioxidant. Furthermore, it is also established that benzoyl moiety along with three hydroxyl groups (two meta and one para) has important structural features for the scavenging of free radicals that are appreciably involved in interaction with the free radicals.
and the inhibitory potential decreases if OH groups are removed from the benzoyl moiety as \( p \)-hydroxybenzoyl bergenin has \( EC_{50} \) of 64.16 ± 0.071 and 0.824 ± 0.0007 \( \mu g/mL \) in terms of DPPH radical scavenging and reducing power, respectively, higher than 11-\( O \)-galloylbergenin (3).

2.2. Urease Inhibitory Activity SAR

The bergenin and its natural derivatives (2, 3) displayed moderate to significant urease inhibitory potential and 11-\( O \)-galloylbergenin is the most potent urease inhibitor with \( IC_{50} \) value of 23.1 ± 0.7 \( \mu M \) as compared to Thiourea (Table S3). The methyl gallate did not display any significant inhibitory effect, i.e. \( IC_{50} > 100 \mu M \), but it significantly increases the inhibitory potential of bergenin when attached to it through ester linkage, i.e. \( IC_{50} \) value of bergenin is 37.2 ± 1.5 \( \mu M \) while that of 11-\( O \)-galloylbergenin \( IC_{50} \) is 23.1 ± 0.7 \( \mu M \). Furthermore, \( IC_{50} \) value of \( p \)-hydroxybenzoyl bergenin is 32.4 ± 0.4 \( \mu M \) suggesting that benzoyl function along with the three hydroxyl groups are effectively involved in interaction with enzyme. Based on this study, it is therefore suggested that substitution of another galloyl moiety in compound 3 will sufficiently increase the inhibitory potential.

The bergenin and its natural derivatives (3 and 4) proved to have no significant cytotoxicity allowing them to be used as a lead compound for the synthesis of new therapeutic agent, which could be more effective and specific for the treatment of various kinds of ulcers with no deleterious side effects (Takahashi et al. 2003).

3. Conclusion

It is concluded from the present study that bergenine and its natural derivatives possess urease inhibitory potential and that substitution of a galloyl moiety remarkably increases the inhibitory potential.

Supplementary material

Experimental details relating to this paper are available online, alongside Figures S1 and S2 and Tables S1–S17.

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