Research Article

Bergenin: Isolation from aqueous extract of *Bergenia ciliata*, antioxidant activity and *in silico* studies

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ABSTRACT

Aim: Isolation of bergenin from *Bergenia ciliata*, evaluation for antioxidant activity, and docking studies. **Materials and Methods:** Column chromatography was performed for the isolation of bergenin from *B. ciliata* aqueous extract and characterized by nuclear magnetic resonance. Antioxidant activity of bergenin was assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and docking studies of bergenin were performed against molecular targets relevant to urinary ailments. **Results:** The phenolic nature of bergenin improves solubility in aqueous solution; isolation of bergenin from aqueous extract was performed by column chromatography and purified bergenin was evaluated for antioxidant activity using DPPH assay. It was showing mild-to-moderate antioxidant action, whereas docking studies of bergenin against pathogens target showing good ligand-binding interaction. **Conclusion:** Isolation of bergenin from aqueous extract was reported 1st time and showing mild-to-moderate antioxidant action. Moreover, docking studies of bergenin against different pathogens show promising ligand-binding interaction.

Keywords: Antioxidant, *Bergenia ciliata*, bergenin, characterization, docking studies, isolation

Introduction

Bergenia ciliate commonly known as Pashanbheda is renowned for numerous pharmacological activities. Rural communities from Himalaya region use *B. ciliata* for the treatment of the number of human ailments.[1] Mixture of polyphenolic compounds mainly bergenin, tannic acid, catechin, and other number of phytochemicals makes *B. ciliata* as one of the most explored plants.[2,3] The plant is a part of the number of ayurvedic formulations and rhizomes of *Bergenia* species are reported to possess antilithiatic activity.[4] Bergenin (Bergenin [BGE]) [Figure 1] also known as cuscutin, one of the major polyphenolic phytoconstituents from Pashanbheda has been reported to exhibit plethora of pharmacological actions such as antidiabetic, antiarrhythmic, antihepatotoxic, and antiplasmodial activities and many more.[5-7] Hence, the present work describes a novel methodology for the isolation of a major polyphenolic, bergenin from *B. ciliata* rhizomes along with antioxidant action and docking studies that support the relevance of BGE for the treatment of urinary complications.

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Materials and Methods

Plant material

Plant material was procured from store, Ayurvet Limited, and authenticated by comparing with reference material present in quality control laboratory.

Chemicals

1,1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Sigma-Aldrich, Bengaluru, India. Organic solvents for column chromatography were purchased from SD Fine Chemicals, Mumbai.

Isolation procedure of bergenin

The powdered rhizomes of *B. ciliata* (1 kg) were successively macerated with petroleum ether and methanol. Petroleum ether-treated material was discarded and methanol fraction was filtered and concentrated using rotary evaporator to get crude extract (130 g). Methanol extract was dispersed in water and extracted with petroleum ether and chloroform. Petroleum ether and chloroform fraction were discarded and water layer was concentrated to get powdered extract (85 g). The water extract was subjected to passage over 230–400 mesh silica gel which was eluted using petroleum ether/ethyl acetate gradient 2–50% affording 15 major pooled fractions (10 g). Pooled fractions were further chromatographed over Sephadex LH20 to afford 100 mg BGE. Mobile phase for thin-layer chromatography: Ethyl acetate: acetic acid: formic acid: water: 8:0.9:0.9:2.

DPPH antioxidant assay

Different concentrations (100, 200, 300, 400, and 500 µg/ml) of sample were taken in different test tubes. Five milliliters of a 0.1 mM methanolic solution of DPPH were added to these test tubes and shaken vigorously. Similar conditions were given to the standard ascorbic acid for antioxidant action. A control without the test compound, but with an equivalent amount of methanol, was maintained. The test tubes were allowed to stand at room temperature for 30 min.[8] The absorbance of the samples was measured at 517 nm. DPPH radical scavenging activity was calculated by the following equation:

$$\text{DPPH antioxidant activity (\%)} = (A_c - A_s) \times 100/A_c$$

Where, A_c is the absorbance control and A_s is the absorbance in the presence of samples.

Docking studies

The prominent molecular targets relevant to the urinary system were sorted; their structures were obtained from the Protein Data Bank (https://www.rcsb.org) and docking studies were performed with DockThor (https://dockthor.lncc.br/v2/) using default parameters. The docking grid was set to cover the binding site of known ligands. The least energy dock poses were identified and visualized in Mcule (https://mcule.com/).

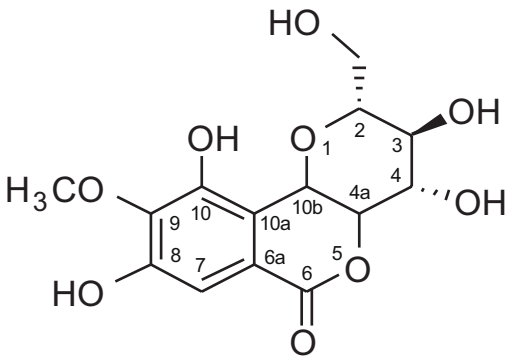

Figure 1: Structure of bergenin

Statistical analysis

All quantitative measurements were expressed as mean ± SD ($n = 3$). Analysis was performed using one-way analysis of variance and the group means were compared by Turkey's multiple comparison *post hoc* tests.

Results and Discussion

Characterization and docking studies

Solubility of BGE in aqueous extract could be attributed to its phenolic nature, thus making it more polar. The isolated BGE was characterized by nuclear magnetic resonance [Table 1] and antioxidant activity of isolated compound was performed against DPPH using ascorbic acid as standard [Table 2]. It was showing mild-to-moderate free radical scavenging action at different concentrations. The hydroxyl

Table 1: ^{1}H (400 MHz, CD_3OD) and ^{13}C-nuclear magnetic resonance (100 MHz, CD_3OD) spectral data of BGE (chemical shifts (δ) are in ppm)

Proton	δ_H	δ_c
2	3.59_m	72.5
3	3.21 dd	71.0
4	3.65 dd	73.9
4a	3.90 dd	79.6
6		163.5
6a		118.2
7	6.97 s	109.6
8	9.70 OH arom	151.0
9		141.1
10	8.20 OH aliph	146.5
10a		116.7
10b	5.5 d	75.5
11	3.75 m	61.1
OCH_3	3.82	60.1

BGE: Bergenin

Table 2: Antioxidant activity of BGE against 1,1-diphenyl-2-picrylhydrazyl

Concentration (µg/ml)	Bergenin (% inhibition)	Ascorbic acid (% inhibition)
100	6.123±0.1858	93.043±0.9274
200	12.533±0.3512	94.230±0.1179
300	19.440±0.4530	94.707±0.4153
400	25.193±0.3535	95.707±0.7755
500	29.323±0.7280	96.307±0.1007

BGE: Bergenin

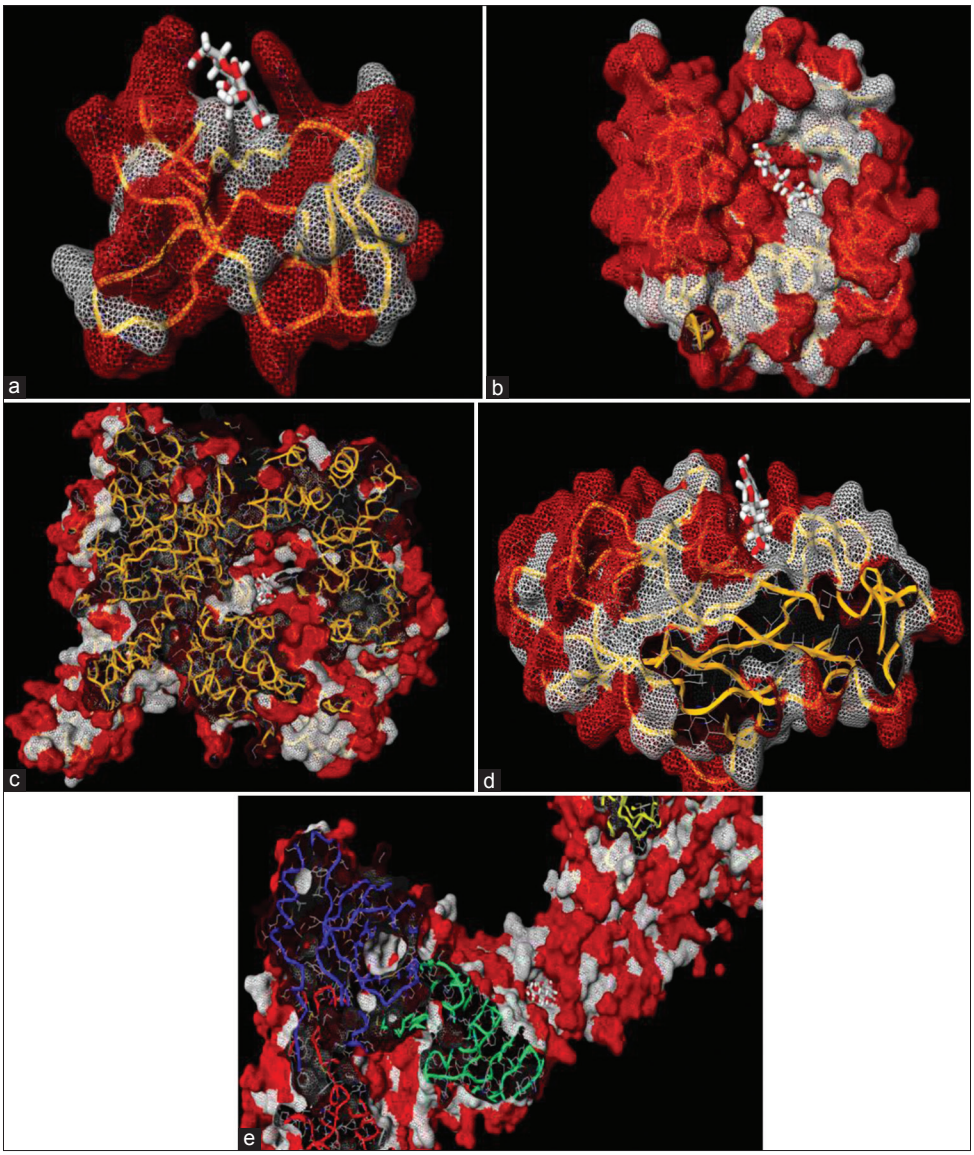

Figure 2: Host targets for buffer ground electrolyte (a) purine nucleoside phosphorylase, (b) carbonic anhydrase XIV, (c) ADAM17, (d) xanthine oxidoreductase, (e) Type 1 fimbrin D-mannose-specific adhesion

group present in BGE might be stabilizing free radical by forming conjugate and delocalization of charge across aromatic ring. Docking studies of BGE against host targets also show promising results for urinary support.

Docking studies

The binding interaction of the isolated compound against host targets was also investigated using *in silico* approach. Purine nucleoside phosphorylase (a), carbonic anhydrase XIV (CA14) (b), ADAM17 (ADAM17) (c), and xanthine oxidoreductase (XOR) (d) appeared prominent host targets for BGE. Type 1 fimbrin D-mannose-specific adhesion (fimH) (e), having an essential role in the host attachment and biofilm formation by uropathogenic E. coli, appeared a prominent pathogen target for BGE [Figure 2]. The compound was showing good ligand-binding interaction against pathogen targets.

Conclusion

Isolation process of BGE, one of the potent litholytic agents from *B. ciliate* rhizomes, is reported in the number of publications. However, isolation of BGE from aqueous phase is documented 1st time. *B. ciliata* is one of the major herbs of the number of commercial water-based syrups used as litholytic agent. The present study acclaimed mild antioxidant activity of BGE. Docking studies confirmed the utilization of BGE against urinary diseases.

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