

Research Article



Phytochemical Screening, Antioxidant Profile and Cytotoxic Activity of Methanol Extract of *Bergenia ligulata*.

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ABSTRACT

Bergenia ligulata is the prominent ayurvedic herb for anticancer activity. The leaf of *Bergenia ligulata* was extracted with methanol and evaluated by phytochemical analysis for the content of innumerable metabolites like primary and secondary. The antioxidant efficacy was assessed through method DPPH free radical scavenging activity. Estimation of total phenolic content, total flavonoid content and tannin content were done to confirm the presence of these photochemical. The cytotoxic effect was determined against the Adrenal gland cancer cell lines (PC12 using MTT assay). It was revealed from the phytochemical analysis of methanol extract of *Bergenia ligulata* that tannin, saponin, cardio glycosides, protein, quinines, phenols and flavonoid were present. A significant antioxidant activity was revealed by the methanol extract 42.8±3.40%. Quantity of total phenol content, total flavonoid content and tannin content were the highest in the 139.8 ±9.06mg of GAE/g, 77±6.40 mg of QE/g, 70.4±6.40mg of TAE/g at 500 µg concentration. At lowest concentration of 3.12 µg/ml of methanol extract of *Bergenia ligulata* cell death was observed with the highest value of 61.1±4.86% respectively. The IC 50 value of methanol extract of *Bergenia ligulata* was found to be 53.925% µg/ml respectively against PC12 cell line. The study suggests that methanol extract of *Bergenia ligulata* has significantly antioxidant property. *Bergenia ligulata* is a good candidate for isolation of antioxidant, total phenol content, total flavonoid content, tannin and anticancer activity that can be a breakthrough for pharmaceutical industry. The antioxidant activity present in *Bergenia ligulata* have strong cytotoxic activity suggests that it can be considered for anti-cancer treatment.

Keywords: Methanol extract, *Bergenia ligulata*, Total phenolic, cytotoxic, Antioxidant activity.

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INTRODUCTION

Nature is a rich source of highly diverse and innovative chemical structures¹. Human's depends on plants for food, clothing, shelter, fuel and medicine². Therapeutic plant used for herbal tea, crude extract, phyto pharmaceutical or herbal mixture or isolated compounds^{3, 4}. Herbalism is a traditional medicinal or folk practice based on the use of plants and plant extracts⁵. *Bergenia ligulata* syn. *Saxifraga ligulata* is widely accepted under this name. The use of various names attributed to it, viz., *Pashanbheda*⁶, *Pashana*, *Zakhmehayat*⁷, *Asmaribheda*, *Ashmabhed*, *Nagabhid*, *Upalbheda*, *Parwatbhed* and *Shilabhed* (dissolving or piercing stones or slabs) etc⁸. This plant belongs to family *saxifragaceae*. It is a chief botanical source of pashanbheda drug used for indigenous system of medicine and incorporated in medical texts and material media⁹.

Bergenin exhibits various biological activities such as antiulcer, anti-hepatotoxic, anti-HIV, anti-arrhythmic; neuroprotective, antifungal, anti-inflammatory, immunomodulatory and burn wound healing effects¹⁰. Owing to such a broad spectrum of biological activities associated with *bergenin*, a number of studies have been devoted to either derivatives the molecule or synthesize its related compounds to optimize it as a lead molecule^{11, 12}. This plant has been used in the Ayurvedic formulations for various ailments¹³.

The aim of the present study was to determine the phytochemical, total phenolic, total flavonoid and tannin contents of methanol leaf extract obtained from *Bergenia ligulata* and to evaluate their antioxidant and anticancer potential. To our knowledge, this is the report for the phenolic composition and bioactivity of methanol extract of *Bergenia ligulata*.

MATERIALS AND METHODS

Standards and reagents

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH), Ascorbic acid, Methanol, Folin- Phenol Reagent (1:1), 20% sodium carbonate, Gallic acid, MEM, Fetal Bovine Serum (FBS), Trypsin, Mmethyl Thiazolyl Diphenyl- Tetrazolium Bromide (MTT), Dimethylsulfoxide (DMSO) and Antibiotics were purchased from Hi media & Sigma Aldrich.



Plant material

The leaf of *Bergenia ligulata* was obtained from Health innovation medicinal plant nursery, Tiruchy, Tamil Nadu, India. Leaf sample was identified and deposited at Department of Medicinal Botany, National Institute of Siddha, Chennai, India. Its voucher number NISMB3692019. The collected raw material was shade, dried and powdered. (Fig. 1)



Figure 1: *Bergenia ligulata*

Bergenia ligulata (Paashaanbhed, Prashanbheda, and other spellings in Ayurveda traditional Indian medicine) is a plant belonging to the family *Saxifragaceae* and the genus *Bergenia*.

Tamil Name: Sirupilai

Extract preparation

25 g of powdered leaf samples was extracted with 250mL pure methanol for 10 h using sox let apparatus. Afterwards the resulting extracts were filtered and solvent was evaporated under reduced pressure at 35°C using rotary vacuum evaporator. At last, the residues were kept in small sterile bottles under refrigerated conditions until used. The yield of evaporated dried extracts was used.

Cell line

The adrenal gland cancer cell lines (PC12) obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in minimal essential media supplemented with 10% FBS, penicillin 20µl (100 U), and streptomycin (1000 µg/ml) and amphotericin B (100 µg/ml) in a humidified atmosphere of 5% CO₂ at 37 °C.

Phytochemical analysis

Phytochemical tests of the methanol extracts of *Bergenia ligulata* (5 gram of dried extract) was carried out qualitatively for the presence of tannin, saponin, flavonoids, alkaloids, proteins, steroid, quinines, terpenoid according to the standard procedure¹⁴.

Antioxidant activity

Aliquot 3.7 ml of absolute methanol in all test tubes along with blank then, add 100µl of absolute methanol to blank. Add 100 µl of Ascorbic acid to tube marked as standard and

100 µl of respective samples to all other tubes marked as tests. Then, finally add 200 µl of DPPH reagent to all the test tubes including blank. Incubate all test tubes at room temperature and dark condition for minimum of 30 minutes. Then, check absorbance of all samples at 517nm. % Antioxidant activity = {(absorbance at blank) – (absorbance at test) / (absorbance at blank)} X 100¹⁵.

Total phenolic content

A total phenolic content of sample was measured by the folin- ciocalteus method¹⁶. Total phenolic content was expressed as milligrams Gallic acid equivalents per gram of methanol extracts (mg GAE/g).

Total flavonoid content

Total flavonoid content was determined using aluminum tri chloride assay¹⁷. The flavonoid content was expressed as milligrams quercetin equivalents per gram of plant extract (mg QE/g).

Tannin activity

Different concentrations of standard (1mg/10 ml) and (sample 1mg/ml) were taken in separate test tubes. Then diluted to 10 ml using distilled water. 0.5 ml of folin reagent was added. 2.5 ml of sodium carbonate was added. Incubate at room temperature for 40 min. Measure O.D at 725 nm¹⁸

In vitro assay for cytotoxic activity (MTT assay)

The anticancer activity of sample against (adrenal gland cancer cell lines) PC12 (determined by the MTT assay¹⁹. Cells (1 × 10⁵/well) were plated in 0.2 ml of medium/well in 96-well plates. Incubate at 5 % CO₂ incubator for 72 hours. Then, add various concentrations of the samples in 0.1% DMSO for 48hrs at 5 % CO₂ incubator. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 20µl/well (5mg/ml) of 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-tetrazolium bromide (MTT) in phosphate-buffered saline solution was added. After 4hrs incubation, 1ml of DMSO was added. Viable cells were determined by the absorbance at 540nm. The effect of the samples on the proliferation of PC12 cells was expressed as the % cell viability, using the following formula:

Calculation

% cell viability = A540 of treated cells / A540 of control cells × 100%

Statistical Analysis

All measurements were performed in triplicate and the results were represented as mean ± SEM. Computer software SPSS version 17.0 analyzer was used for analysis

RESULTS

Phytochemical analysis

The leaf powder of *Bergenia ligulata* was extracted by refluxing with methanol and the extract was used for the

evaluation of phytochemical screening. This extract was subjected to preliminary phytochemical screening for the presence of different chemical groups. The presence of Tannins, saponin, flavonoid, proteins, quinines, cardio glycosides and phenols. (Table1)

Table 1: Preliminary phytochemical screening of methanol extract of *Bergenia ligulata*.

S. No	Contents	Presence / Absent in methanol extract.
1.	Tannin	+
2.	Saponin	+
3.	Flavonoid	+
4.	Alkaloids	-
5.	Proteins	+
6.	Steroid	-
7.	Quinines	+
8.	Terpenoid	-
9.	Cardio glycosides	+
10.	Phenols	+

+ Present - Absent

Antioxidant activity

The antioxidant activity of the methanol extract of *Bergenia ligulata* was evaluated in for its DPPH free radical scavenging activity. The methanol extract of *Bergenia ligulata* showed efficient antiradical scavenging activity was recorded 42.8 ± 3.40 % at the concentration of $500\mu\text{g/ml}$.

Table 2: Antioxidant activity

S. No	Concentration of the extract ($\mu\text{g/ml}$)	Antioxidant activity (%)
1	100	4.2 ± 0.3
2	200	11.4 ± 0.9
3	300	21.4 ± 1.60
4	400	30.0 ± 2.21
5	500	42.8 ± 3.40

Total Phenolic Content

The phenolic activity of methanol extract of *Bergenia ligulata* was found to be 33 ± 2.03 , 59.6 ± 4.20 , 83.4 ± 6.10 , 112.7 ± 8.08 & 139.8 ± 9.06 (mg of GAE/g) at concentration of 100 to $500\mu\text{g}$.

Table 3: Total Phenolic content

S. No	Concentration of the extract ($\mu\text{g/ml}$)	Total Phenolic content (%)
1	100	33 ± 2.03
2	200	59.6 ± 4.20
3	300	83.4 ± 6.10
4	400	112.7 ± 8.08
5	500	139.8 ± 9.06

The highest amount of phenolic content was recorded in 139.8 ± 9.06 mg of GAE in gram. The lowest amount 33 ± 2.03 mg of GAE/g was recorded at the $100\text{-}\mu\text{g}$ concentration.

Total flavonoid content

Methanol extract of *Bergenia ligulata* showed 15.4 ± 0.90 , 28.8 ± 1.70 , 43.2 ± 2.32 , 58.1 ± 4.22 & 77 ± 6.40 mg of QE/g flavonoid content at 100, 200, 300, 400 & 500 μg concentrations of the extract, respectively. The maximum content was 77 ± 6.40 mg of QE/g in $500\mu\text{g}$.

Table 4: Total flavonoid content

S. NO	Concentration of the extract ($\mu\text{g/ml}$)	Total flavonoid content (%)
T	100	15.4 ± 0.90
2	200	28.8 ± 1.70
3	300	43.2 ± 2.32
4	400	58.1 ± 4.22
5	500	77.3 ± 6.40

Tannin content

Methanol extract of *Bergenia ligulata* showed 14.2 ± 0.7 , 25.4 ± 1.9 , 42.6 ± 3.1 , 56.8 ± 4.9 & 70.4 ± 6.40 tannic acid EQ mg/g in different concentration of 100 to $500\mu\text{g}$. It seemed that tannin content was different among the five concentrations and highest tannin amount 70.4 ± 6.40 mg of EQ/g.

Table 5: Tannin content

S. No	Concentration of the extract ($\mu\text{g/ml}$)	Tannin content (%)
1	100	14.2 ± 0.7
2	200	25.4 ± 1.9
3	300	42.6 ± 3.1
4	400	56.8 ± 4.9
5	500	70.4 ± 6.40

Cytotoxic activity

The anticancer activity of samples on PC12 cells was determined by the MTT assay. For cytotoxicity different concentrations of methanol extract was tested against cell line. MTT assay was carried out in 24 well plates. The assay is often used to measure viable cells where MTT (3-(4, 5-dimethyl thiazol-2yl)-2, 5) diphenyl tetrazolium bromide a yellow tetrazole) is reduced to purple formazin in living cells. For determination of IC 50, cells were assessed to proliferation and 1C50 value were calculated and tabulated for cell line. Cancer is considered to be the public health problem in developing countries. Despite the intense efforts to develop treatments, effective agents are still not available. In this regard natural product extracts continue to be the most promising source of new drugs for cancer. The cytotoxic effects of the extracts seem to be inducing cell apoptosis. It also stores partial cellular differentiation and degradation.



Cytotoxic activity of methanol extract of *Bergenia ligulata* on PC12 (Table 6, Fig.2 & 3) the present study attempted to find out anticancer components from methanol extract of *Bergenia ligulata*. Cell lines revealed that the plant studied was moderately cytotoxic to both the cancer cell lines. The cytotoxic effect of the methanol extract was compared with the standard, *Bergenia ligulata* showed 7.4 ± 0.60 , 14.9 ± 1.20 , 35.3 ± 2.38 , 34.3 ± 2.85 , 43.2 ± 3.27 ,

55.2 ± 4.62 , 61.1 ± 4.86 of % cytotoxicity at $200 \mu\text{g/ml}$, $100 \mu\text{g/ml}$, $50 \mu\text{g/ml}$, $25 \mu\text{g/ml}$, $12.5 \mu\text{g/ml}$, $6.25 \mu\text{g/ml}$ & $3.12 \mu\text{g/ml}$ concentration of the extracts respectively, against PC12 cell lines. Results indicate that methanol extract showed IC 50 value $53.925 \mu\text{g/ml}$ for PC 12 cell line. This can be considering as promising result for anticancer drug.

Table 6: Cytotoxic activity of methanol extract of *Bergenia ligulata* on PC12

S. No	Concentration($\mu\text{g/ml}$)	Dilution	Absorbance - 540nm	% cell Viability
1	200	Neat	0.05	7.4 ± 0.60
2	100	1:1	0.10	14.9 ± 1.20
3	50	1:2	0.17	34.3 ± 2.38
4	25	1:4	0.23	35.3 ± 2.85
5	12.5	1:8	0.29	43.2 ± 3.27
6	6.25	1:16	0.37	55.2 ± 4.62
7	3.12	1:32	0.41	61.1 ± 4.86
8	Control	-	0.67	100 ± 0.00

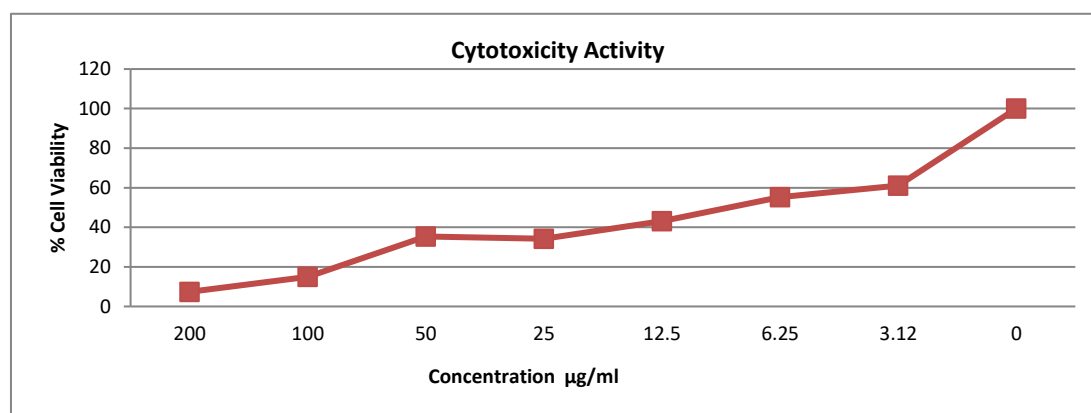


Figure 2: Cytotoxic activity of methanol extract of *Bergenia ligulata* on PC12

DISCUSSION

Phytochemical screening of *Bergenia ligulata* showed the presence of constituents like saponins, flavonoids, phenols, tannin, quinines, and carbohydrates. The result was similar to the phytochemical analysis was reported in earlier studies²⁰. Variety of phytoconstituents as paashaanolactone, catechin, anthraquinone, steroids, phenolics, flavonoids, saponins, tannins and terpenoids, have been reported in methanol extract of *Bergenia ciliata*²¹.

Antioxidants have recently become a topic of increasing interest to health and food science researchers and medical experts²². The methanol extract displayed greater potential in all antioxidant assays. The antioxidant activity of medicinal plants, fruits and vegetables has been reported to be positively correlated to their total phenolic contents due to their ability to scavenge free radicals^{23, 24}. The role of free radicals in many diseases conditions has been well established. Several biochemical reactions in our body generate reactive species and these are capable of

damaging crucial bimolecular. If they are not effectively scavenged by cellular constituents, they lead to disease conditions. Some authors have reported from the extracts of *Bergenia ligulata*, *Bergenia ciliata* and *Bergenia stachii* act as antioxidant candidate mainly by DPPH scavenging activity, OH radical, scavenging activity, superoxide anion radical scavenging activity, ferric reducing antioxidant power assay and metal chelating method²⁵.

Strong DPPH radical scavenging activity of *Bergenia ligulata* methanol extract can be due to higher content of total phenolic compounds. The effect of phenolic compounds on preventing radical scavenging was studied and it is generally assumed the ability of the compounds to act as hydrogen donors^{26, 27}. Similar to our results a strong correlation between the phenolic content, the antioxidant capacity were compared to the previous studies²⁸⁻³¹.

Methanolic extract of *Bergenia ciliata* rhizome had higher phenolic content compared to the aqueous extract³². It was found 38% phenolic content in *Bergenia ciliata* because the presence of aromatic rings, phenolic and

flavonoids are nucleophilic in nature, hence responsible for the chelating properties³³.

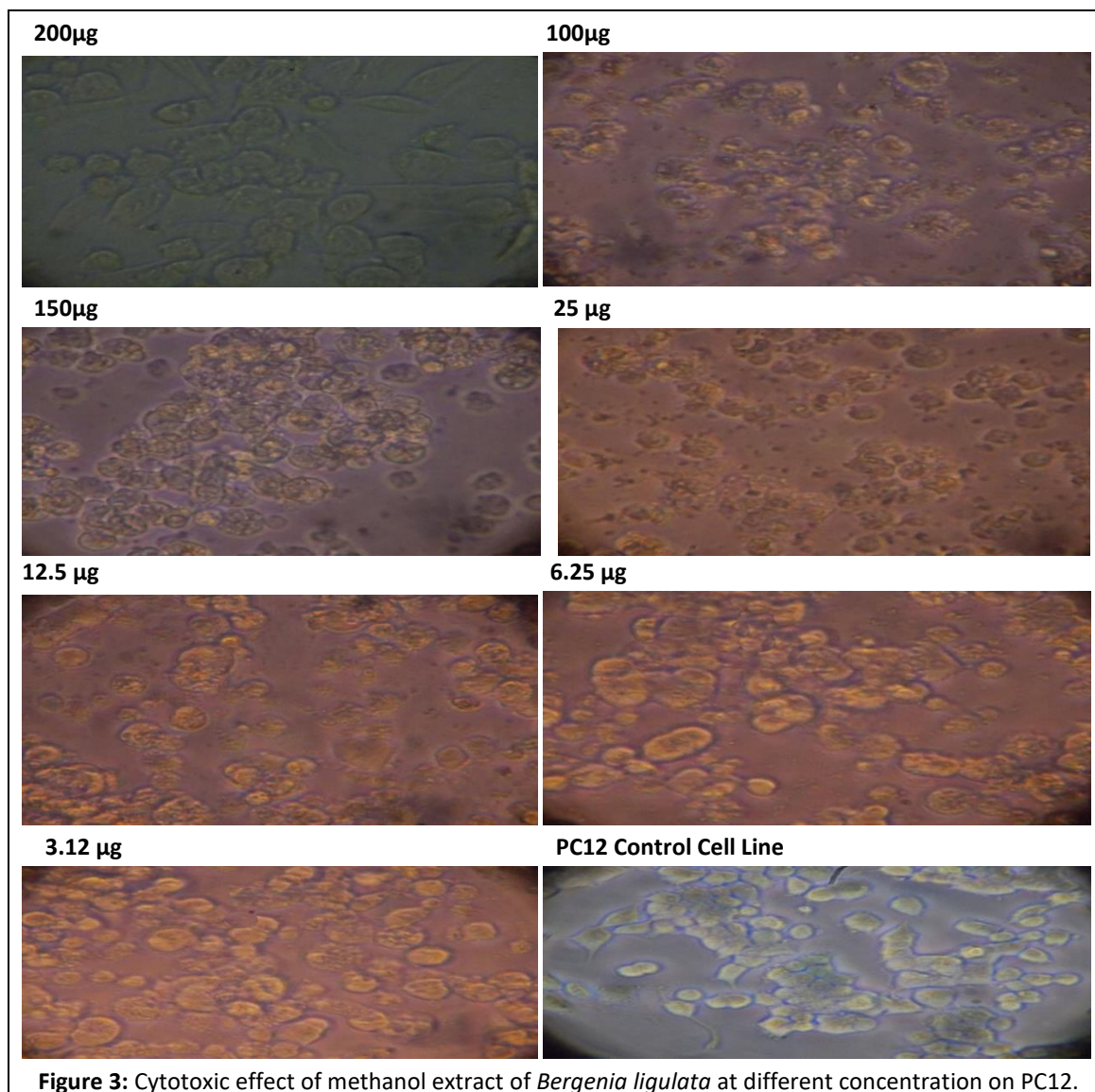


Figure 3: Cytotoxic effect of methanol extract of *Bergenia ligulata* at different concentration on PC12.

Highest flavonoid content in the extract of ethyl acetate in *Bergenia ciliata*³⁴. Flavonoids, carotenoids and triterpenes contain antioxidant activity by scavenging reactive oxygen species which prevent potential damage to cellular components such as DNA, protein and lipids³⁵. Furthermore, an increasing reliance on the use of these medical plants has been traced to the extraction and development of several drugs including chemotherapeutics³⁶. The use of plant products as anticancer agents has a long history. Several drugs currently used as anticancer/chemotherapeutic agents isolated from plant species. The extracts (both methanolic and aqueous) of *Bergenia ciliata* were found to have promising potential towards the development of drugs that might be used to target tumors for chemoprevention/chemotherapy to check neoplastic growth and malignancy. *Bergenia ciliate* bear potent anti-neoplastic activities that may have prospective clinical use as precursor for preventive medicine³⁷.

Many plants have been reported carrying anticancerous potential against different cell lines^{38, 39}. Significant anticancerous activity of methanol extract of *Bergenia ligulata* and against pc12 cell lines in the current study might be attributed to the presence of anticancerous compounds, essential to be identified and isolated for further investigation.

CONCLUSION

In conclusion, the results of this work suggest that the leaf extract of *Bergenia ligulata* appeared as a good source of health promoting phenols, flavonoid, tannin and beneficial effects like antioxidant and cytotoxic activities. Hence, *Bergenia ligulata* extract and their components can be recommended for therapeutic purposes and be used as an alternative medicine.

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