



Physiochemical analysis, antioxidant activity and phenolic content determination of *Bergenia ciliata* (Haw.) Sternb. Leaves

^{1,2} Prashant Kumar Singh, ³ Mohammad Mukim, ⁴ Shalini Tripathi, ^{2*} AKS Rawat

¹ Institute of Pharmaceutical Sciences, University of Lucknow, Uttar Pradesh, India

² Maharishi University of Information Technology

³ Kota College of Pharmacy, Kota, Rajasthan, India

⁴ Rameshwaram Institute of Technology & Management, Lucknow, Uttar Pradesh, India

Corresponding Author: AKS Rawat

Email: rawataks@rediffmail.com

Abstract

Many medicinal plants contain active compounds that play a crucial role in treating various diseases. People often utilize plants and herbal remedies to enhance their well-being and tap into their beneficial properties. This study aimed to investigate the physiochemical properties of a particular plant and assess the effects of different solvents (ethanol, methanol, and water) on its phenolic profile and ability to scavenge free radicals. The researchers followed standard procedures for preliminary phytochemical and physiochemical analyses. They employed the Folin-Ciocalteu method and a colorimetric approach to quantify the phytochemical content. The free radical scavenging potential was evaluated using the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic) acid (ABTS) assays. The results indicated that ethanol was the most effective solvent for extracting polyphenols. The quantitative phytochemical analysis revealed that the ethanol extract exhibited the highest phenolic content (73.87 mg/g GAE) and flavonoid content (86.90 mg/g QE), while the aqueous extract had the lowest phenolic content (30.33 mg/g GAE) and flavonoid content (22.32 mg/g QE). Additionally, the ethanolic extract of *B. ciliata* demonstrated the highest scavenging potential against DPPH (33.03 µg/mL) and ABTS (24.69 µg/mL). These findings suggest that the ethanolic extract of *B. ciliata* leaves possesses significant antioxidant properties and has the potential to serve as a valuable source of organic antioxidants for the development of functional foods.

Keywords: *Bergenia ciliata*, TPC, TFC, antioxidant, phytochemical analysis, etc.

Introduction

The utilization of natural remedies is of great importance in the marketing and advancement of new drugs [1]. Nearly 25% of conventional medicines are composed of phytocompounds extracted from medicinal plants [2]. The World Health

Organization (WHO) reports that around 80% of individuals in developing countries depend on plants for their basic healthcare needs [3]. Extensive research has been conducted on the antioxidant properties of medicinally valuable botanicals [4].

Previous research has demonstrated that flavonoids, a variety of carotenoids, phenolics, vitamins (C & E), and flavonoids are all primarily responsible for their antioxidant properties [5-7]. As a result of electron transport processes during aerobic respiration, reactive oxygen species (ROS) are constantly produced in the human body and play important roles in homeostasis and cell signaling. The overproduction of reactive oxygen species (ROS) is frequently linked to oxidative stress, which causes oxidative damage to DNA, lipids, and proteins in cells [8].

Antioxidants play a crucial role in preventing oxidative damage and chronic illnesses by combating free radicals. However, the synthetic antioxidant butylated hydroxytoluene (BHT) has faced regulatory measures due to its potential association with cancer and liver damage [9]. Consequently, research has focused on identifying natural antioxidant alternatives with fewer side effects [10]. The pathogenesis of radicals, specifically reactive oxygen species (ROS), is linked to various diseases such as cancer, cardiovascular disorders, neurodegenerative conditions, high blood pressure, diabetes, and premature aging [11]. ROS are generated by factors like radiation, chemicals, toxins, consumption of deep-fried and spicy foods, and physical stress. These factors can lead to the production of abnormal proteins by the immune system and depletion of antioxidants [12].

Antioxidant enzymes naturally present in the body, including catalase, superoxide dismutase, and glutathione peroxidase, contribute to eliminating free radicals and maintaining normal cellular processes [13]. However, during times of increased oxidative stress, the body's antioxidant defenses may become insufficient to maintain optimal cellular function. In such cases, dietary intake of antioxidants may be necessary [14].

B. ciliata, commonly known as hairy Bergenia, is a perennial herb that belongs to the Saxifragaceae family. It is native to the temperate Himalayan region, found at elevations ranging from 800 to 3000 meters. The distribution of *B. ciliata* spans from Afghanistan to Southeast Tibet, including countries such as Bhutan, India, Nepal, and Pakistan. In Bhutan, it can be found in districts like Deothang, Phuntsoling, Mongar, and Ha. In India, it is reported in regions like the Lushai hills, West Bengal, Arunachal Pradesh, Meghalaya, Kumaon Himalayas, Sikkim (Gangtok, Kyongnosla), and Almora district in Uttarakhand, among others. In Nepal, it occurs in districts like Mawanpur, Karpalanchok, and Dolakha. In Pakistan, it is distributed in northern parts, including the FATA region of Khyber Pukhtunkhwa province, Poonch valley, Swat, Abbottabad, Galliyat, and Chitral [15-18]

B. ciliata has a long history of traditional medicinal use in the Himalayan region. Local communities have utilized this plant for treating various ailments. The rhizome of *B. ciliata*, in particular, is highly valued and has been used to address pulmonary infections, leucorrhea, piles, bladder and kidney stones, among other conditions. In Ayurveda, it is commonly employed as a tonic, astringent, antiscorbutic, laxative, and for treating spleen enlargement, dysuria, and ulcers. In West Bengal, the rhizome juice is used as an anti-tussive remedy for cough and cold. *B. ciliata* is renowned for its medicinal properties and has gained popularity in traditional medicine for a variety of health conditions. It is commonly utilized to alleviate symptoms associated with cough, cold, fever, pulmonary infections, heart diseases, ophthalmic issues, hemorrhoids, and stomach disorders. The plant's therapeutic potential has led to its widespread use in treating these ailments [19-21].

The purpose of this study was to evaluate the antioxidant activity, physiochemical properties, preliminary phytochemical screening, TPC, TFC, and water, ethanol, and methanol extracts of *B. ciliata* leaves.

Materials and Methods

Collection and Authentication of Plant Material

The *B. ciliata* leaves were gathered from the surrounding area of Mandi, Himachal Pradesh. Dr. Pankaj Sharma of the Himalayan Pradesh State Biodiversity Board in Shimla, India, identified, confirmed, and certified the plant (HIMCOSTE/HPSBB/279).

Physiochemical Analysis

Various physiochemical parameters like moisture content, ash value, foreign matter, fluorescence analysis, and extractive value were determined as per the standard procedure [22].

Preliminary phytochemical screening

Phytochemical testing was performed on a number of *B. ciliata* leaf extracts [22]. Alkaloids, flavonoids, glycosides, tannins, proteins, saponins, fats, and steroids were identified through a series of identification tests.

Preparation of Extracts

The first step in drying the collected leaves was to wash them in water to get rid of any dirt or foreign objects. Then, after being ground into a coarse powder, the dried leaves were run through sieve No. 14. A thimble-shaped Soxhlet apparatus tube was used to extract the *B. ciliata* leaves that had been dried and powdered (20 g) using a variety of solvents, including ethanol, methanol, and water (300 mL), at 60–65 °C for 3–4 hours. All three extracts were hotly filtered before being evaporated and kept at low temperature in the refrigerator for further investigation.

Total Phenolic Content (TPC) Total Flavonoid Content (TFC)

TPC and TFC of *B. ciliata* leaf extracts were determined by the previously described method [23]. The TPC was expressed as mg of gallic acid equivalent (GAE) while TFC was expressed as mg of quercetin equivalents (QE) per 100 g of extract.

DPPH Radical-Scavenging Assay

DPPH radical scavenging potential of each extract were determined by previously described method [24]. The mixture of various plant extract concentrations and standard ascorbic acid was added to the DPPH radical solution (50 M) separately. The EE, ME, and AE extract had concentrations ranging from 20 to 100 g/mL. The reaction mixtures were left in the dark for thirty minutes after being vigorously shaken. 2 mL of DPPH solution and 2 mL of methanol were combined to make the control solution. The absorbance of the control solution and each reaction mixture was measured at 517 nm

The percentage inhibition was calculated by the following formula:

$$\% \text{ Inhibition} = \frac{AC_{517 \text{ nm}} - AS_{517 \text{ nm}}}{AC_{517 \text{ nm}}} \times 100 \quad (1)$$

Where: AS: Absorbance of the Sample; AC: Absorbance of Control. The IC₅₀ value

was calculated by plotting the graph between the percent inhibition and various concentrations of plant extracts and ascorbic acid.

ABTS Radical-Scavenging Assay

To evaluate the reducing capacity of *B. ciliata* extracts, the ABTS assay was conducted, as mentioned earlier [25]. EE, ME, and AE were prepared at concentrations ranging from 20 to 100 µg/mL. In each experiment, 0.2 mL of the samples at different concentrations were mixed with 1 mL of distilled dimethyl sulfoxide (DMSO). Subsequently, 0.16 mL of ABTS solution was added to reach a final volume of 1.36 mL. After a 20-minute incubation period, the absorbance of the solution was measured at 734 nm using a UV spectrophotometer.

$$\% \text{ Scavenging} = \frac{A_0 - A_1}{A_0} \times 100 \quad (2)$$

Where A₀: absorbance of the control, A₁: absorbance of the sample.

Physicochemical evaluation

Table 1 provides a summary of the physicochemical parameter results. The total ash value (2.34%) was found to be higher than the value of the sulphated ash (2.29%). The water-soluble ash value was 0.36 %, while the acid insoluble ash value was 0.53 %. Further research revealed that the medication has a moisture content of 7.56 %, no foaming or swelling index, and less than 1 % foreign organic content. Ethanol, chloroform, and aqueous had extractive values of 49.34 %, 13.10 % and 7.56 % respectively presented in Table 1. Preliminary phytochemical screening The extracts presence of alkaloids, carbohydrates, flavonoids, phenolic compounds, tannins, and glycosides was confirmed by a number of phytochemical analysis tests, as shown in Table 2. When compared to the extracts made with ethanol and chloroform, the aqueous extract was found to be negative for the presence of alkaloids.

Total Phenolic Content (TPC)

The TPC method was chosen to assess the phenol content of plant extracts. These phenolic compounds can potentially act as antioxidants thanks to their redox properties [26, 27]. To begin, the phenolic content of each extract was determined using the Folin- Ciocalteu reagent. In terms of gallic acid equivalents (GAE) per gramme of dry extract weight, the results are shown in Table 1. According to the findings, the EE had a higher TPC than the ME and AE, with approximately 73.87 ± 0.20 mg GAE/g, 46.83 ± 0.13 mg GAE/g, and 30.33 ± 0.33 mg GAE/g, respectively. Based on the gallic acid calibration curve ($y = 0.0061x + 0.4027$, $R^2 = 0.9786$), the linear equation that follows was used to calculate TPC.

Table 1: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC)

Extracts	TPC (mg/g GAE)	TFC (mg/g QE)
EE	73.87 ± 0.20 ^a	86.90 ± 0.25 ^a
ME	46.83 ± 0.13 ^b	44.77 ± 0.32 ^b
AE	30.33 ± 0.33 ^c	22.32 ± 0.20 ^c

Each value represents the mean and standard error mean of three replicates. EE stands for ethanol extract, ME for methanolic extract, and AE for aqueous extract. Different letters denote statistical significance, which was established at $p < 0.05$.

The higher level of phenolic in the EE may indicate greater bioactivity, namely, antimicrobial and antioxidant properties. Numerous studies have looked into the relationship between phenolic content and antioxidant activity. Velioglu and others [28, 29] reported that certain plant products' antioxidant activity was strongly correlated with the TPC. Sengul *et al.* also stated that phenolic compounds play a role in the defense mechanisms of plants to prevent the formation of ROS, enhancing survival and guarding against molecular harm as well as attacks from microbes, insects, and herbivores [30].

Total Flavonoid Content (TFC)

Flavonoids, as secondary metabolites, possess antioxidant activity that is influenced by the presence and arrangement of free hydroxyl (OH) groups [31]. In this study, the flavonoid content of several plant extracts was quantified using a colorimetric system with aluminum chloride. The results, expressed as quercetin equivalents (QE) per gram of dry extract weight, are presented in Table 1.

According to the findings, the EE had a higher TFC than the ME and AE, with approximately 86.90 ± 0.25 mg QE/g, 44.77 ± 0.32 mg QE/g, and 22.32 ± 0.20 mg QE/g, respectively. Based on the calibration curve of quercetin ($y = 0.0051x + 0.4525$, $R^2 = 0.9888$), the linear equation that follows was used to calculate TFC.

Ethanol is frequently used to extract natural plant compounds, such as the abundance of bioactive phytoconstituents in *B. ciliata* leaves. The widespread therapeutic use of this folkloric botanical could be explained by EE.

Among the many phenolic compounds found in plants, flavonoids make up a significant group. Fruits and vegetables benefit greatly from their color and flavor [32]. Flavonoids have the ability to scavenge radicals due to the presence of hydroxyl groups. The same idea that was used for TPC was used for TFC determination, where a color change was caused by the presence of a flavonoid-aluminum complex [7]. According to Table 1, Leaves of *B. ciliata* TPC and TFC values for EE were the highest, indicating that of the extracts under study, EE contains the most phenolic compounds. In contrast to other natural sources, *B. ciliata* is therefore a promising source of phenolic compounds.

Among the many phenolic compounds found in plants, flavonoids make up a significant group. The color and flavor of fruits and vegetables contribute significantly to their overall appeal [32]. Flavonoids, owing to the presence of hydroxyl groups, possess the ability to scavenge radicals effectively. The same principle applied for determining total phenolic content (TPC) was utilized for assessing total flavonoid content (TFC), where the presence of a flavonoid-aluminum complex caused a color change [7]. According to Table 4, *B. ciliata* leaf EE exhibited the highest values for both TPC and TFC, indicating that EE contains the highest abundance of phenolic compounds among the studied extracts. This highlights the potential of *B. ciliata* as a promising source of phenolic compounds compared to other natural sources.

Antioxidant Activity

DPPH Radical Scavenging Activity

The DPPH radical scavenging activity is based on the one-electron reduction, which mimics the free radical reducing activity of antioxidants. As a positive control, ascorbic acid (AA) was utilized. The DPPH radical inhibition percentages of standard and crude extracts from *B. ciliata* at various concentrations are summarized in Table 2. Among the extracts, EE exhibited the lowest IC₅₀ (concentration required for 50%

inhibition) value of 33.03 $\mu\text{g/mL}$, followed by ME and AE with IC₅₀ values of 66.92 and 81.82 $\mu\text{g/mL}$, respectively (Table 3). Ascorbic acid displayed an IC₅₀ value of 14.82 $\mu\text{g/mL}$. The lower IC₅₀ value of EE indicates its higher capacity to scavenge free radicals compared to ME and AE. The significant antioxidant activity observed in EE showed a positive correlation with the total phenolic content (TPC). Previous studies have reported a strong association between antioxidant capacity and both total flavonoid content (TFC) and TPC [33,34]. *B. ciliata* EE exhibited a dose-dependent ability to scavenge DPPH radicals, indicating that its antioxidant activity increased with increasing concentrations.

Table 2: DPPH radical scavenging activity of *B. ciliata* leaf extracts.

Concentration ($\mu\text{g/ml}$)	AA	EE	ME	AE
20	50.5	45.45	33.33	25.08
40	56.73	51.34	40.06	32.99
60	63.13	60.6	49.15	41.41
80	71.38	68.68	53.7	49.83
100	75.08	73.73	61.61	56.9

AA – Ascorbic acid; EE -Ethanollic Extract; CE -Chloroform Extract; AE -Aqueous Extract. Different letters denote statistical significance, which was established at $p < 0.05$. (each concentration was evaluated independently)

Table 3: *B. ciliata* extracts IC₅₀ values in DPPH and ABTS assay

Crude Extracts	DPPH Scavenging Assay ($\mu\text{g/mL}$)	ABTS Scavenging Assay ($\mu\text{g/mL}$)
AA	14.82	16.92
EE	33.03	24.69
ME	66.92	51.73
AE	81.82	80.19

Statistical significance was determined at $p < 0.05$ and is indicated with different letters.

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assays

B. ciliate EE revealed a dose-dependent ABTS radical scavenging potential (Table 4).

Table 4: ABTS radical scavenging activity of *B. ciliata* leaves extracts.

Concentration ($\mu\text{g/ml}$)	AA	EE	ME	AE
20	50.66	46.77	34.4	25.5
40	56.45	54	40.53	31.06
60	64.03	61	45.32	37.19
80	68.59	66.81	49.22	41.42
100	74.27	72.04	55.34	50.11

AA – Ascorbic acid; EE -Ethanollic Extract; ME -Methanollic Extract. Statistical significance was determined at $p < 0.05$ and is indicated with different letters (each concentration was evaluated independently).

At 20 mg/mL, ascorbic acid inhibited the EE, ME, and AE in proportions of 46.77 percent, 34.4 %, and 25.5 percent, respectively. Ascorbic acid had an IC₅₀ value of 16.92 $\mu\text{g/mL}$, while its EE, ME, and AE values were 24.69 $\mu\text{g/mL}$, 51.73 $\mu\text{g/mL}$, and 80/19 $\mu\text{g/mL}$, respectively (Table 4).

Antioxidants play a crucial role in safeguarding against oxidative damage and combating diseases associated with oxidative stress by neutralizing and disrupting free radical chains. In contrast to natural antioxidants, synthetic antioxidants like BHT are considered controlled substances and are associated with potential liver damage and carcinogenesis [7, 36]. Consequently, extensive research is needed to explore natural sources and identify alternative antioxidants that are both safe and effective, aiming to mitigate the adverse effects [37]. Various naturally occurring substances derived from plants, including lignan secoisolariciresinol [38], have demonstrated promising potential as substitutes for synthetic antioxidants, which may pose risks. Phenolic compounds, due to their redox properties, exhibit efficient metal chelation, hydrogen donation, reduction capabilities, and singlet oxygen inhibition, making them effective agents in combating oxidative stress.

The ABTS assay demonstrated a remarkably high scavenging capacity specifically in *B. ciliata* EE, indicating its strong ability to neutralize ABTS⁺ radicals. This study observed that increasing the concentration of plant extracts resulted in higher DPPH radical scavenging activity, implying a greater ability to transfer a hydrogen atom and produce a lighter solution corresponding to the number of electrons acquired [39]. The ability of *B. ciliata* to transfer hydrogen atoms suggests its ability to scavenge DPPH radicals by converting them into the corresponding hydrazine.

However, it was noted that the DPPH radical scavenging activity of the extracts surpassed their ABTS⁺ scavenging ability. The stereoselectivity of radicals and their solubility in different solvent systems can influence an extract's capacity to interact with and reduce various radicals. Additionally, some substances with ABTS⁺ scavenging activity were found to lack DPPH scavenging potential [39]. The antioxidant assays ABTS and DPPH provide insights into an extract's ability to scavenge free radicals, although their mechanisms differ. ABTS operates primarily through a strict hydrogen atom transfer (HAT) mechanism, while DPPH may exhibit antioxidant activity through both HAT and electron transfer (ET) mechanisms [36]. Based on the current findings, it can be inferred that *B. ciliata* contains antioxidants primarily acting through a HAT mechanism, while the slightly higher, albeit not significantly higher, antioxidant activity observed using DPPH could indicate the presence of antioxidants primarily acting through an ET mechanism.

This study represents the most comprehensive analysis conducted thus far on the phytochemical composition of the notable medicinal plant, *B. ciliata*. The aim was to explore the potential mechanism underlying its antioxidant activity through the utilization of two distinct radical scavenging assays, while considering the relative phytochemical composition of each extract. The well-established association between phenolic compounds and antioxidant activity was further examined in this research. Furthermore, it is widely acknowledged that several variables, including the phytochemical composition, which is closely linked to the chosen extraction method [40-42], can significantly influence antioxidant activity. To address this, we investigated the antioxidant potential and phytochemical profile of *B. ciliata* extract using various extraction solvents. Among the tested solvents, ethanol extraction yielded the most favorable results in terms of both antioxidant potential and phytochemical diversity of the sample [43, 44].

Conclusions

The present study revealed that *B. ciliata* leaf extracts possess notable antioxidant and antimicrobial properties, primarily due to the abundance of phytochemicals such as phenolics and flavonoids. The quantification of total phenolic content (TPC) and total flavonoid content (TFC) in each *B. ciliata* extract (EE, CE, and AE) demonstrated that the choice of extraction solvent significantly influenced the content and composition of bioactive compounds. Among the extracts, *B. ciliata* EE exhibited the highest TPC and TFC levels, indicating its richness in these bioactive compounds. Furthermore, this research shed light on the potential antioxidant mechanisms of *B. ciliata*, particularly its ability to scavenge free radicals. The biological activity of the various *B. ciliata* extracts seems to be closely associated with their phenolic acid and flavonoid content. Therefore, considering the remarkable antioxidant properties of *B. ciliata* leaf extracts, promoting their consumption could potentially play a significant role in preventing various health disorders associated with excessive free radical production, such as carcinoma, cardiovascular diseases, and premature aging. To explore the development of promising food or cosmetic preservatives, further in-depth studies are necessary to isolate and characterize individual bioactive compounds present in *B. ciliata* extracts. This detailed investigation will provide valuable insights into the specific compounds responsible for the observed antioxidant and antimicrobial activities, facilitating the formulation of effective products in the future.

Conflict of Interest

The authors declare no conflict of interest.

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