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Ethnomedicinal Uses and Phytochemical Concentration of *Bergenia ciliata* (Haw.) Sternb. from Three Districts in Bhutan

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Abstract

Bergenia ciliata (Haw.) Sternb., which is commonly known as elephant ears, is a perennial herb in the Saxifragaceae family. It is traditionally used in ethnomedicine to treat kidney stones, lung disorders, and gastrointestinal illnesses due to its rich secondary metabolites. This study documented twelve medicinal uses of *B. ciliata* through semi-structured interviews of 67 informants, using a snowball sampling technique across three study sites. Phytochemical analysis showed a significant difference ($p \leq 0.001$) in tannic, phenolic, and flavonoid content across the study sites. Tannin content was highest in samples collected from Chapcha (0.77 ± 0.08 µg/ml), phenolic from Chendebji (2.85 ± 0.19 µg/ml), and flavonoid from Patshaling (340 ± 94.82 µg/ml). There were no significant differences in the concentrations of catechin, gallic acid, and bergenin. The highest catechin content was found in samples from Chendebji (29.85 ± 4.09 %), the gallic acid concentration was highest from Patshaling (17.67 ± 7.88 %), and Chapcha samples showed the highest bergenin concentration (196.0 ± 19.63 mg/kg). In general, the concentration of most of the phytochemicals was found lower than from other studies in the region which could be due to the differences in the extraction method, stage of maturity of the rhizomes, and the harvesting time apart from the environmental impact. This study suggests that further research on the phytochemical analysis of *Bergenia* is necessary, as it has not been previously explored in Bhutan. Additionally, documenting its uses from other regions of Bhutan is equally important.

Keywords: Ethnomedicine, phytochemicals, secondary metabolites, traditional knowledge

Introduction

Plants serve a variety of purposes globally, with medicinal uses being particularly prevalent (Petrovska, 2012). Herbal remedies are gaining popularity in developed nations, while in remote areas of developing countries, medicinal plants are crucial for immediate healthcare (Thakur et al.,2023). The World Health Organization (WHO) estimates that around 40% of the modern pharmaceutical industry relies on medicinal plants, and over 80% of the global population, especially in developing countries, depend on plant-based traditional medicine for primary healthcare (Yeshi et al.,2017).

In Bhutan, indigenous medicine is deeply embedded in cultural traditions due to the abundance of medicinal plants (Kunwar et al.,2013). Bhutan is home to approximately 5,603 higher plant species, with 600 species

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identified for their medicinal benefits and 300 species used in traditional medicine (Wangchuk et al.,2016). The National Institute for Traditional Medicine (NITM) uses more than 18 tonnes of medicinal plants, most of which come from the wild (Lakey & Dorji, 2016). These plants are vital for healthcare and contribute significantly to the country's Gross National Happiness (Wangchuk & Tobgay, 2015). However, there has been a decline in medicinal plant populations due to unsustainable harvesting, increased international demand, and habitat loss (Lakey & Dorji, 2016). Preserving these plants is crucial as they offer valuable pharmacological benefits due to their rich phenolic content (Gyeltshen et al.,2024).

Bergenia ciliata (Haw.) Sternb., which is known as "Jamtshoshamtha" in Dzongkha and "Pakhanbed" in Lhotshomkha, is a significant medicinal plant in Bhutan, belonging to the Saxifragaceae family. Two species, *B. ciliata* and *B. purpurascens* (Hook. Fil. & Thomson) Engl., are identified in Bhutan (Gyeltshen et al.,2024; Bhutan Biodiversity Portal, 2024). Traditionally, *B. ciliata* has been used in Ayurvedic medicine to treat kidney stones, respiratory infections, gastrointestinal disorders, and skin diseases, among other ailments (Koul et al.,2020; Rai & Rai, 2024). Rich in secondary metabolites such as phenols, flavonoids, and tannins, *B. ciliata* determines diverse pharmacological activities, including anti-inflammatory, antibacterial, antioxidant, anti-cancer, and anti-diabetic properties (Latief et al.,2022; Deeba et al.,2024). These bioactive compounds emphasize their value in both traditional and modern medicine, making them a key resource for medicinal uses (Yousaf et al.,2018).

In Bhutan, local communities have been using this medicinal herb to treat a variety of human illnesses, including fever, stomach aches, and postpartum healing. However, the absence of documented traditional knowledge and practices around the plant, despite its widespread ethnomedical use, is a challenge to the preservation of this valuable cultural and medicinal heritage. In many nations with abundant botanical resources and applications in ethnomedicine, traditional knowledge about medicinal plants and their uses is currently fading at a rapid pace and losing its inherent value (Wangchuk et al.,2011). The reasons for the loss include the migration of people from rural to urban areas, industrialization, biodiversity loss, loss of natural habitats, and lifestyle changes. Therefore, documentation is essential to preserve this traditional knowledge for continuity. Moreover, the environment plays a significant role in modulating phytochemical concentrations by interacting with genetic traits (Saha & Verma, 2013). Given its widespread use, therapeutic potential, and sensitivity to genetic and environmental variables, assessing the phytochemical composition and concentrations of *Bergenia ciliata* across different regions is critical. Therefore, the objectives of the study were to (i) document the traditional knowledge of the medicinal uses of *B. ciliata* and (ii) evaluate the phytochemical concentrations of the plant found growing in the wild in different areas in Bhutan.

Materials and Methods

Study sites

The study was conducted across three different *gewogs* (administrative blocks): Chapcha in Chhukha (27.1934° N, 89.5380° E, elevation 1467–2171 m asl), Patshaling Moed and Patshaling Toed (Beteni) in Tsirang (26.96995° N, 90.16085° E, elevation 1435–1873 m asl), and Chendebji *chiwog* (group of villages) under Tangsebji gewog in Trongsa (27.5003° N, 90.2905° E, elevation 1634–2187 m asl). These locations were selected based on the natural presence of *Bergenia ciliata* in the wild and its significance in traditional medicinal practices within the local communities.

Sampling technique for informant identification

To get administrative approval and locate key informants who had traditional knowledge of

Bergenia as a medicinal plant, an official visit was first made to the local government offices in each study region. A snowball sampling technique was then used to find more informants, with local government representatives recommending one or two informants from each location. Twenty individuals from Chapcha in Chhukha (59% males, 41% females), 25 from Patshaling in Tsirang (39% males, 61% females), and 22 from Chendebji in Trongsa (67% males, 33% females) were interviewed. The age range of the informants was 20 to 69 years old, with the majority of the respondents being 41 to 50 years old. Face-to-face interview was carried out in April and May 2023 using a semi-structured questionnaire. Data were collected on traditional uses, plant parts used, and preparation and use methods.

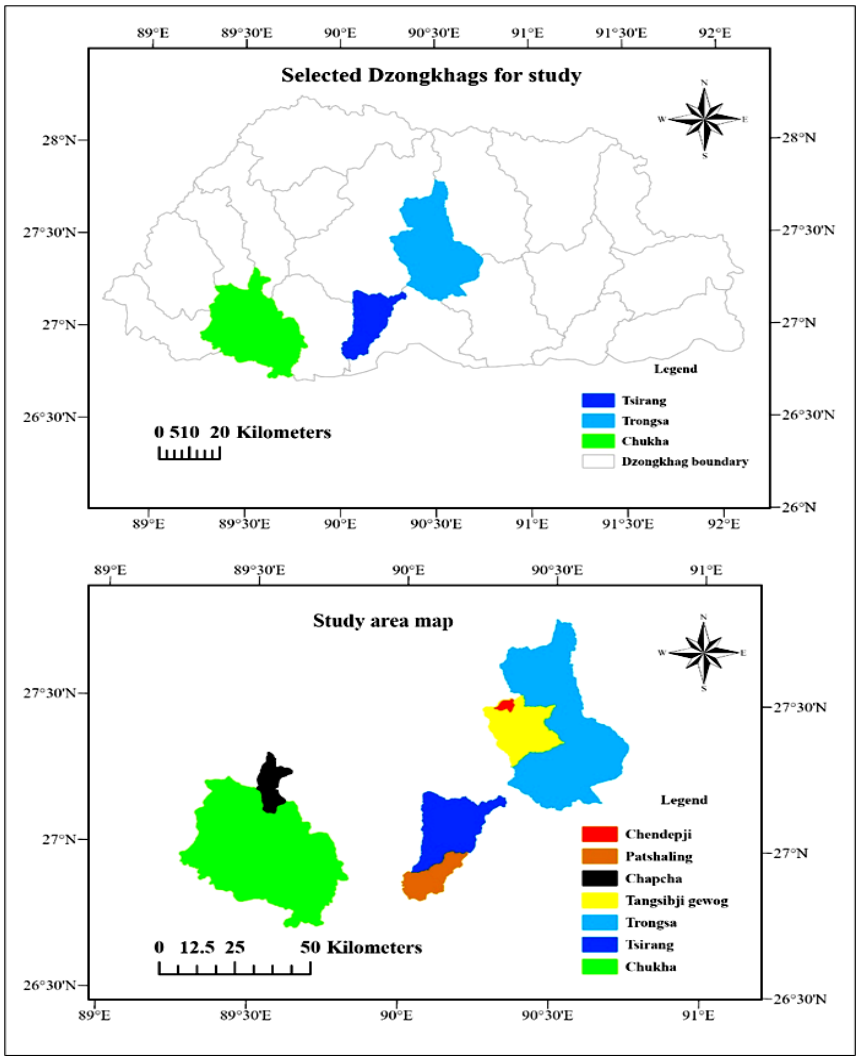

Figure 1: Study area showing the sampling sites

Characteristics of the collection sites

Rhizomes of *Bergenia ciliata* were collected from different areas as shown in Table 1. In Chapcha, the plants were mostly located in rocky, steep areas. Similarly, in Patshaling and Chendebji, *Bergenia* plants were also found on sloped terrain, though the slopes were less rocky and steep than that of Chapcha. To assess the growing conditions, weather data from the nearest weather stations were obtained from the National Centre for Hydrology and Meteorology (Figure 2) illustrating the average temperatures over the past 6 years. In Chapcha, the average mean temperature ranged from 16°C to 18°C, in Patshaling, the average mean temperature varied from 19° C to 20°C. In Chendebji, the average mean temperature ranged from 7°C to 9°C. According to Khare (2007), *Bergenia* thrives in humid, temperate climates, where temperatures generally range between 21°C to 27°C. Average annual rainfall across all sites was low and fluctuated between 150 mm to 180 mm in Chapcha, 130 mm to 140 mm in Patshaling, and 100 mm to 210 mm in Chendebji (Figure 2). Malik (2024) states that *B. ciliata* typically grows in rocky areas at 1200 to 2700 m asl, with an average precipitation range of 690 mm to 1,150 mm.

Sample collection, preparation, and extraction

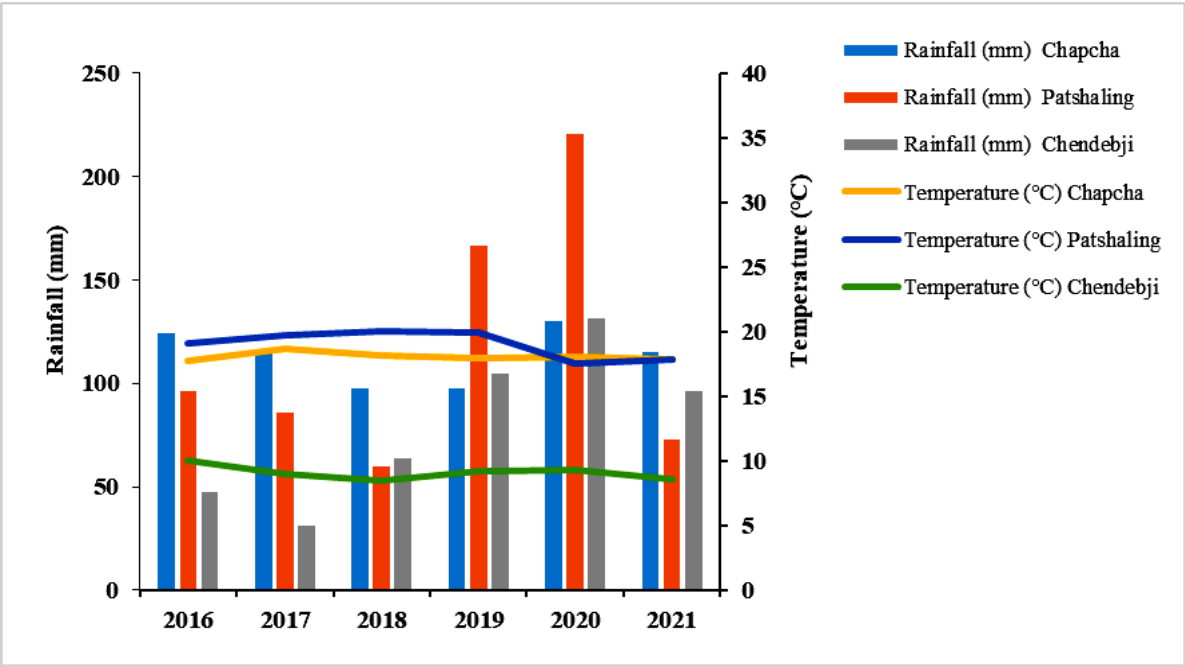

Figure 2: Average temperature and average rainfall of three locations

The growth of *Bergenia ciliata* in rocky and steep slope areas made it difficult to implement precise sampling methods. Therefore, plant samples were collected from accessible areas. Ten plants were collected from each site and the plant's rhizomes were cut into smaller pieces and sun-dried for a week. The dried samples were then ground into fine powder using a mortar and pestle. For solvent extraction, 99.99% ethanol was used. The powdered samples were dissolved in ethanol and refrigerated for 24 hours. Subsequently, the mixtures were agitated in a rotary shaker (REMI RS-18) for 24 hours to ensure thorough mixing. The resulting extracts were filtered through the Whatman filter paper (Grade 1, sheet size of 460 mm x 570 mm). The filtered samples were then ready for phytochemical concentration analysis. The samples for each coordinate site were replicated three times, therefore, there were 30 samples from each study site to determine the total tannin using the Folin-Denis's assay, total phenolic content using the Folin-Ciocaltue method (Bajracharya & Maharjan, 2013), and total flavonoid content using the aluminum chloride method (Shraim et al.,2021). All analyses were conducted using a double-beam UV/Vis spectrophotometer (Model: K8001S, Brand name: Shanghai Yoke Instrument Co., Ltd. SNYH01212002001) in 415 nm. The remaining extracted solution was stored in cold storage and subsequently used to produce solid extracts. The solution was evaporated at 40°C using a rotary evaporator (LRE-B10) at 17 RPM (Revolutions Per Minute). The dried extract was collected, weighed using an analytical balance, and stored in cold storage for further phytochemical analysis.

Key phytochemicals analysis

High-Performance Liquid Chromatography with a Diode Array Detector (HPLC-DAD), is an advanced analytical technique used for separating, identifying, and quantifying compounds in complex mixtures. The HPLC system separates compounds based on their interactions with the stationary and mobile phases, while the DAD records absorbance across a range of wavelengths, providing spectral data for each compound (Arunachalam et al.,2015). This combination enables precise identification and quantification of substances with characteristic UV-Vis absorption profiles (Sarkar et al.,2014).

Ten samples were combined into five groups by pairing two samples from nearby

coordinates. As a result, five samples from each location were prepared for analysis of three components bergenin, catechin, and gallic acid. In total, 45 samples (5 samples × 3 compounds × 3 locations) were sent for analysis to Enviro-check Laboratory in Kolkata, India, which is accredited by the National Accreditation Board for Testing and Calibration Laboratories (NABL), India. It is crucial to use a standard of known purity and quality to ensure accurate and reliable results. In the initial phase, a polar solvent like ethanol/ methanol was selected because it helps in dissolving of the specific phytochemicals in *Bergenia ciliata*, such as gallic acid, catechin, and bergenin. Because the extraction process breaks down cell walls, solvents can enter plant tissues and dissolve substances. Shaking helps in enhancing molecular mobility. To prepare the samples for further analyses, the following procedure was conducted.

First, 0.1 g of the sample was weighed and placed into a 50 ml extraction tube. Next, 25 ml of methanol (MeOH) was added to the tube

Table 1: Sample collection location

Location	Sample No.	Coordinates		Elevation (m)
		Latitude	Longitude	
Chapcha	1	26° 28' 51.77" N,	89° 37' 5.01" E,	1467
	2	27° 0' 54.00" N,	89° 29' 3.01" E,	1567
	3	27° 2' 5.13" N,	89° 23' 43.10" E,	1569
	4	27° 6' 12.18" N,	89° 26' 5.00" E,	1725
	5	27° 19' 9.18" N,	89° 53' 40.54" E,	1769
	6	26° 50' 20.11" N,	89° 29' 30.19" E,	1879
	7	26° 53' 0.28" N,	89° 23' 21.00" E,	2098
	8	27° 18' 92.23" N,	89° 52' 65.58" E,	2001
	9	26° 57' 50.11" N,	89° 22' 12.12" E,	2034
	10	26˚57´50.11" N	89˚22´12.12" E	2171
Patshaling	1	26° 94' 20.28" N,	90° 19' 36.35" E,	1435
	2	27° 4' 5.35" N,	90° 9' 25.87" E,	1457
	3	27° 18' 41.32" N,	89° 54' 83.68" E,	1545
	4	27° 3' 0.28" N,	90° 5' 45.35" E,	1563
	5	26° 91' 43.76" N,	90° 15' 43.89" E,	1657
	6	26° 94' 41.55" N,	90° 19' 33.72" E,	1675
	7	27° 18' 92.25" N,	89° 52' 65.58" E,	1765
	8	26° 94' 41.56" N,	90° 19' 33.72" E,	1768
	9	27° 19' 19.18" N,	89° 53' 40.54" E,	1834
	10	27° 2' 41.26" N,	90° 7' 1.19" E,	1873
Chendebji	1	27° 33' 54.11" N,	90° 27' 41.51" E,	1634
	2	27° 31' 19.00" N,	90° 27' 47.20" E,	1670
	3	27° 31' 91.70" N,	90° 27' 49.01" E,	1765
	4	27° 30' 38.41" N,	90° 29' 36.16" E,	1767
	5	27° 31' 9.19" N,	90° 27' 51.45" E,	1855
	6	27° 48' 67.00" N,	90° 49' 8.01" E,	1874
	7	27° 3' 9.28" N,	90° 5' 45.35" E,	2156
	8	27° 31' 17.00" N,	90° 27' 47.20" E,	2168
	9	27° 31' 61.70" N,	90° 27' 49.01" E,	2178
	10	26° 94' 20.28" N,	90° 19' 36.35" E,	2187

and thoroughly mixed using a vortex. An aliquot of 4 ml was then transferred into a vial. For the cleaning process, 50 mg of Primary Secondary Amine (PSA) and 50 mg of activated charcoal were added to the vial. The mixture was subsequently centrifuged at 5000 RPM. Finally, the solution was subjected to injection.

For the quantification of catechin ($C_{15}H_{14}O_6 \cdot H_2O$), gallic acid ($C_7H_6O_5$), and bergenin ($C_{14}H_{16}O_9$), (HPLC-DAD) was used. Standards were prepared using catechin hydrate (98% purity; Sigma-Aldrich, 036k1604), gallic acid (99.5% purity; Loba Chemie, L169241509), and bergenin (98% purity; LGC, 17-GHZ-190-1). These high-purity Certified Reference Materials (CRMs) were essential for ensuring accuracy, with calibration curves generated using working standards at concentrations of 25 ppm, 50 ppm, and 100 ppm. The analysis was conducted on an Agilent 1260 Infinity HPLC system (Serial No. DEAAX08560), equipped with a reverse-phase C18 column and a Diode Array Detector (DAD). Detection wavelengths were set to 280 nm for catechin and gallic acid, and 270 nm for bergenin.

The mobile phase contained 20 mM (Millipore) ammonium acetate (75%, Channel A) and acetonitrile (25%, Channel B), filtered through a Millipore system, operating under isocratic conditions at ambient temperature. Formic acid in the mobile phase optimized compound stability and peak sharpness by adjusting pH. A flow rate was maintained to prevent column overload, and an injection volume of 15 µL sample was consistently used. Compounds were separated based on their polarity and hydrophobic interactions with the stationary phase, with retention times and spectral profiles compared against the standards for identification. Quantification was performed by measuring the area under each peak, correlating with concentration using calibration curves. This method confirmed precise and reproducible results in assessing the phytochemical composition of plant extracts.

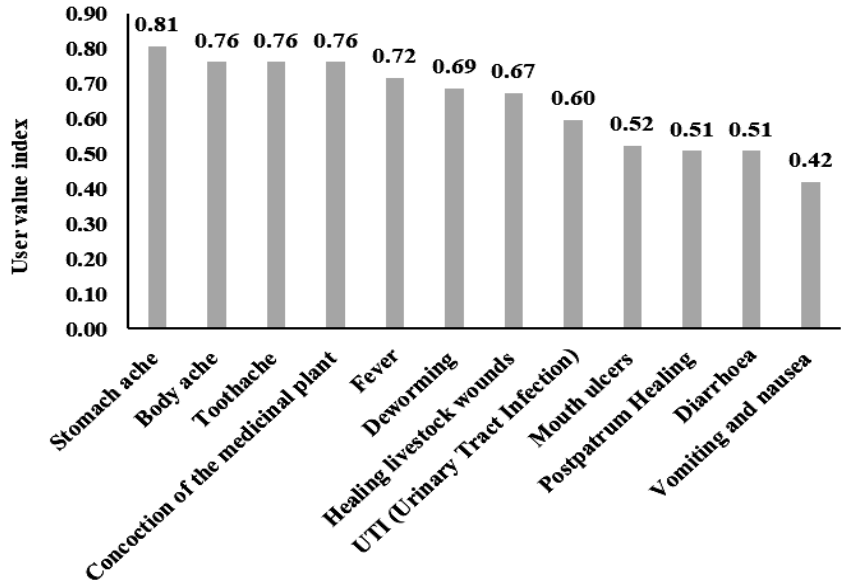

Figure 3: Ethno-medicinal uses of *Bergenia ciliata*

Data analysis

Microsoft Excel 2013 and SPSS version 23 were used for data processing and analysis. The User Value (UV) was calculated to determine the relative importance of *Bergenia ciliata* using the formula:

$$UV = \frac{Uc}{n}$$

Where 'Uc' is the number of use reports cited by each informant for a given plant species (*Bergenia ciliata*) and 'n' is the total number of informants interviewed. For bergenin, catechin, and gallic acid mean and standard deviations were calculated. Since the data were not normal Kruskal-Wallis test was done to compare the concentrations of phytochemicals; total tannin content, total phenolic, and total flavonoid content across the three study sites. Post hoc analysis using Dunn's test was

done for pairwise comparisons. Correlation was performed to evaluate the effect of elevation on the concentrations of phytochemicals. For bioactive compounds, the Shapiro-Wilk test confirmed a normal distribution of the data sets; therefore, an analysis of variance (ANOVA) was performed. Additionally, correlation analysis was performed to assess the effect of elevation on phytochemical concentrations

Results and Discussion

Ethnomedicinal uses of Bergenia ciliata

The plant is used in traditional medicine to treat a range of ailments such as pain-related conditions (stomach ache, body ache, and toothache), digestive and gastrointestinal issues (diarrhea, vomiting, nausea, deworming, and mouth ulcers), infectious and inflammatory conditions (fever and urinary tract infections (UTIs)), and livestock health and veterinary uses (healing livestock wounds). According to Ahmad et al. (2018), 104 different types of illnesses are cured by using rhizomes of *Bergenia ciliata*. According to respondents, both leaves and rhizomes are utilized, with rhizomes being the most frequently used, because they can accumulate secondary metabolites in larger amounts than leaves or stems. The metabolites are easily stored and may be dried without losing much of their efficacy making them useful in traditional medicine. The primary method of consumption is oral, often involving mixing the dried powdered

Table 2: Ethno-medicinal uses of *Bergenia ciliata*

Sl. No	Uses	Methods of consumption	Plant parts used
1	Stomach ache	Powdered extract mixed with water	Rhizomes
2	Body ache	Powdered extract mixed with water	Rhizomes
3	Toothache	Powdered extract mixed with milk for adults and mixed with honey for children	Rhizomes
4	Concoction with dried rhizome of *Astilbe rivularis*	Powdered extract of the concoction mixed with milk	Rhizomes
5	Fever	Powdered extract mixed with water	Rhizomes
6	Deworming	Powdered extract mixed with water	Rhizomes
7	Healing livestock wounds	Leaves are mixed with fodder and fed to livestock	Leaves
8	UTI (Urinary Tract Infection)	Powdered extract mixed with milk	Rhizomes
9	Mouth ulcers	Powdered extract mixed with honey	Rhizomes
10	Postpartum healing	Powdered extract mixed with milk	Rhizomes
11	Diarrhea	Powdered extract mixed with milk	Rhizomes
12	Vomiting and nausea	Powdered extract mixed with water	Rhizomes

form with milk or water. To cover the bitter taste, particularly for children, the powder is also mixed with honey. Honey is preferred over milk for ulcer treatment to avoid worsening gastritis because, honey has anti-inflammatory and antibacterial qualities that help calm the stomach lining and fight *Helicobacter pylori*, a primary cause of gastritis. Additionally, sliced sun-dried rhizomes are chewed for toothaches and mouth ulcers. Leaves are also used to treat livestock wounds, highlighting the plant's broad applications for both human and animal health.

Different consumption methods are employed based on the intended use, with leaves used for nausea, vomiting, and postpartum issues, and rhizomes chewed fresh, consumed orally, sliced sun-dried, or powdered (Phull et al.,2016).

Globally, *Bergenia ciliata* has diverse traditional uses: the Jaunsari tribe in Uttarakhand, India, uses it for boils, piles, diabetes, kidney stones, and appendicitis (Mehta et al.,2022); in China, bergenin is used in cough medicine (Xu et al.,2017); in North Waziristan, Pakistan, it is used for dental issues like toothache (Almasoud et al.,2023); in Nepal, the rhizome juice is taken for hemorrhoids and asthma (Banerjee et al.,2014); in Sikkim, India, it is used for fractures and heart diseases (Singh et al.,2017); in Peshawar, Pakistan, it is used for minor cuts and constipation (Khan, 2017); and Mongols use it for typhoid, gastrointestinal issues, diarrhea, and lung inflammation (Koul et al.,2020). This indicates that while *B. ciliata* is used in various ways across different regions, some applications, such as treating boils, piles, typhoid, appendicitis, hemorrhoids, asthma, fractures, heart disease, and lung inflammation, are not mentioned in the study areas in Bhutan, suggesting potential for further exploration of these uses locally.

User value

In ethnobotanical studies, the User Value (UV) is a measure of value that is used to evaluate the relative importance of plant species in a given socio-cultural environment. In this study, as shown in Figure 4, the UV of *Bergenia ciliata* ranged from 0.42 to 0.81, indicating the plant's importance in traditional healthcare. The highest UV (0.81) was related to the treatment of stomach aches, which was followed by fever (0.72), body aches, and toothaches (0.76). High UV indicates a high degree of understanding and common knowledge across respondents, suggesting that these uses are widely recognized and valued within the community (Sapkota et al.,2022). Species with higher UV levels are thought to be more important based on the quantity of use reports for those species (Yousaf et al.,2018). Higher UV values also indicate that plants are more likely to be biologically active and have medicinal properties (Biswas et al.,2017). The study of Herrera-Feijoo et al. (2023) conducted in Zamora Chinchipe, Ecuador, found that B. ciliata had the highest UVs, ranging from 0.82 to 0.92. This indicates that it is widely used and is popular.

Phytochemical analysis of Bergenia ciliata

Kruskal-Wallis test result for phytochemical contents in *Bergenia ciliata* is shown in Table 3. There was a significant difference in total tannin content (H [2] = 58.471, p = 0.001), with a median of 0.79 in Chapcha, 0.28 in Chendebji, and 0.50 in Patshaling. Post-hoc comparisons using Dunn's test for multiple comparisons indicated that the total tannin content of Chapcha was significantly higher than that of Chendebji (p = 0.000), but there was no significant difference to Patshaling (p = 0.087). There was a significant difference between Patshaling and Chendebji (p = 0.020). One of the reasons for the significant differences in total tannin content between Chendebji to the other two locations could be because of young sample plants found and collected from Chendebji.

The total tannin content in *Bergenia ciliata* reported was much higher in other countries as compared to that of this study in which the maximum tannin content was found in sam-

ples from Chapcha with a mean of 0.77± 0.08 µg GAE/ml, which is very low as compared to tannin contents reported from other countries. It ranged from 37.0 ± 50.1 mg/ml in Hungary (Rafi et al.,2018), 28.6 ± 1.0 mg/ml to 36.9 ± 1.2 mg/ml in Nepal (Srivastava et al.,2014), and 24.7 ± 0.9 mg/ml to 32.4 ± 1.2 mg/ml in Himalchal Pradesh, India (Jayamohan et al.,2013).

Results for total phenolic content showed a significant difference (H [2] = 73.819, p = 0.001) with a median of 1.76 in Chapcha, 2.18 in Patshaling, and 2.81 in Chendebji. Dunn's post-hoc comparison showed no significant difference between the phenolic content of Patshaling and Chendebji (p = 0.084) and between Chendebji and Chapcha (p = 0.24). However, there was a significant difference in the phenolic content between Patshaling and Chapcha (p = 0.00). Differences in microclimatic conditions such as temperature, humidity, and light exposure could have affected plant metabolism and secondary metabolite production. Chendebji is colder as compared to the other sites as shown in Figure 2. However, the rainfall in Patshaling is high compared to the other two places. The secondary metabolite production takes place as a defense mechanism therefore, it is difficult to ascertain the reasons from this study.

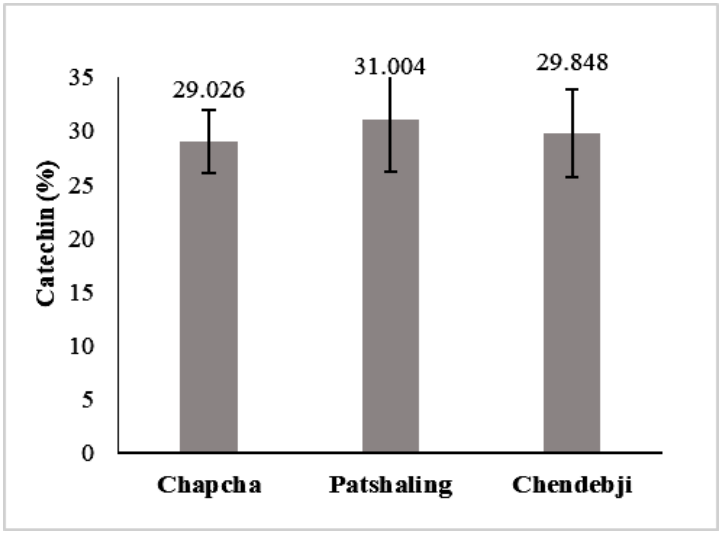

Figure 4: Total percentage of catechin

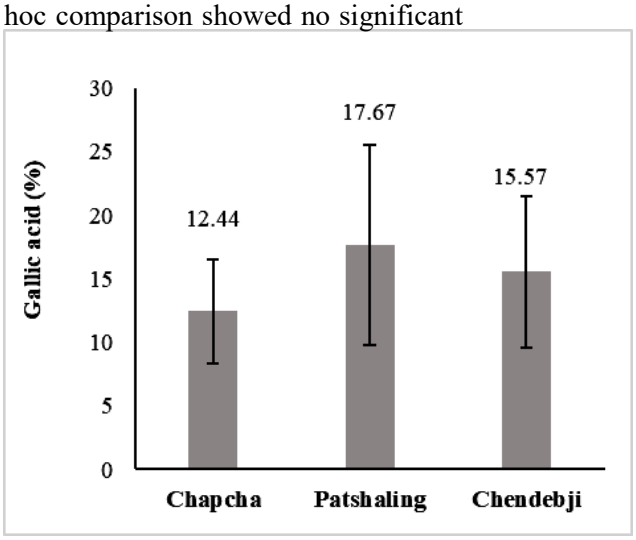

Figure 5: Total percentage of gallic acid

Similar to tannin, phenolic content was found to be lower in this study as compared to other studies with a maximum of 2.85±0.19 µg GAE/ml in Chendebji. In Shimla, India, it ranged from 8.94 ± 0.09 mg GAE/g (Dulta et al.,2021), 24.55 to 82.56 mg GAE/g in Nepal (Pant et al.,2021), 21.44 ± 0.60 mg GAE/g in Ralwalkot, Pakistan (Mehmood et al.,2022), and 80.96 ± 1.74 mg/ml in Amritsar, India (Kanth et al.,2019).

Likewise, the total flavonoid content result showed a significant difference (H [2] = 42.341, p = 0.001) with a median of 187.80 in Chapcha, 327.44 in Patshaling, and 286.04 in Chendebji.

Post-hoc comparisons using Dunn's method

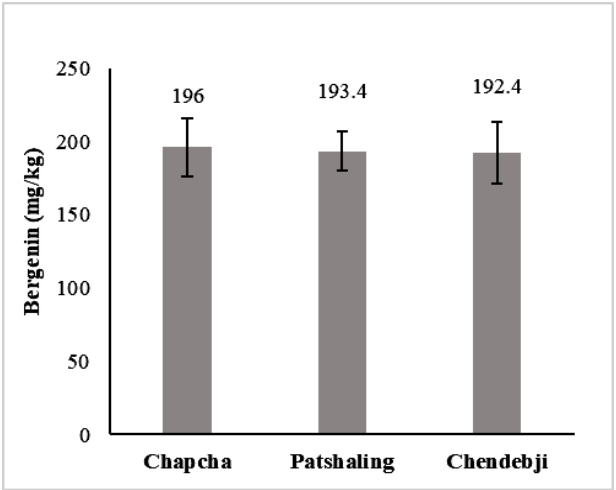

Figure 6: Concentration of bergenin from three different locations (mg/kg)

for multiple comparisons indicated that there is a significant difference between the flavonoid content of Chapcha and Chendebji (p = 0.05), and Chapcha and Patshaling (p = 0.001). However, no significant difference between the flavonoid content of Chendebji and Patshaling (p = 1.00) was observed.

Therefore, the results showed a higher total flavonoid content than tannin and phenols in *Bergenia ciliata*. However, it is still lower as compared to the reports from other studies such as 16.68 ± 0.58 mg QE/g in Amritsar, India (Mehmood et al.,2022), 208.4 ± 0.6 mg QE/g in Lucknow, India (Deeba et al.,2024), 249.7 mg QE/g in Udaipur, India (Bishnoi et al.,2018), 18.94 to 45.76 mg QE/g in Nepal (Pant et al.,2021), and 70.65 ± 0.86 mg/ml in Udaipur, India (Hegazi et al.,2019). Patshaling with a total flavonoid content of 340.86 ± 94.82 µg QE/ml is the highest from this study. *Bergenia ciliata* samples from the study areas have lower concentrations of total tannins, phenolics, and flavonoids than samples from other countries. This could be due to a combination of environmental, genetic, and methodological factors. According to Chaves et al. (2020) and Ramírez et al. (2021), fluctuating rainfall and lower average temperatures could decrease plant stress, which then reduces the production of secondary metabolites like tannins and flavonoids. However, greater UV stress at higher elevations generally encourages the formation of phenolic and flavonoid compounds (Sharma et al.,2020). Genetic variation is a contributing factor (Sharma et al.,2020), as the *B. ciliata* populations in Bhutan may exhibit phenotypic differences compared to those from other regions (Sharma et al.,2020). Additionally, post-harvest practices may influence results. Advanced techniques like freeze-drying, commonly used in other studies, may better preserve phytochemical content, whereas traditional methods like sun-drying could harm heat-sensitive compounds such as flavonoids (Wang et al.,2019). These factors collectively emphasize the need for further research into the genetic and environmental determinants of phytochemical composition in *B. ciliata*.

Table 3: Kruskal-Wallis test on phytochemical concentration

Variables	Location	Mean	Std. Dev	Median	df	Sig.
Total tannin content	Chapcha	0.77	0.08	0.79	2	<.001
	Patshaling	0.5	0.23	0.5		
	Chendebji	0.28	0.02	0.28		
Total phenolic content	Chapcha	1.79	0.23	1.76	2	<.001
	Patshaling	2.23	0.19	2.18		
	Chendebji	2.85	0.19	2.81		
Total flavonoid content	Chapcha	189.76	33.39	187.8	2	<.001
	Patshaling	340.86	94.82	327.44		
	Chendebji	298.02	86.1	286.04		

Key phytochemical analysis

The results for catechin, gallic acid, and bergenin are shown in Table 4. Patshaling had the maximum catechin at 31.00 ± 4.78%, followed by Chendepji at 29.85 ± 4.09%, and Chapcha showed a mean of 29.03 ± 2.88%. The catechin levels in *Bergenia ciliata* from the study area were lower compared to those from other regions, such as 92.37 ± 4.58% to 138.59 ± 2.17% in Gujarat, India (Dharmender et al.,2010) and 100.06 ± 4.10% in Lucknow, India (Srivastava et al.,2014). However, it

Table 4: Comparison of Phytochemical Concentrations in *Bergenia ciliata* Across Locations and with Other Studies

Phytochemical	Location	Values (This Study)	Units	Values (Other Studies)	Units	Reference
Catechin	Chapcha	29.03 ± 2.88	%	92.37 ± 4.58 – 138.59 ± 2.17	%	Dharmender et al.,2010
	Patshaling	31.00 ± 4.78	%	100.06 ± 4.10	%	Srivastava et al.,2014
	Chendebji	29.85 ± 4.09	%	0.08 ± 0.03	%	Singh et al.,2017
Gallic Acid	Chapcha	17.67 ± 7.88	%	6.57 ± 0.99	%	Boros et al.,2014
	Patshaling	12.44 ± 4.07	%	99.9 ± 2.09	%	Dharmender et al.,2010
	Chendebji	15.57 ± 5.94	%	99.96 ± 2.13	%	Srivastava et al.,2014
Bergenin	Chapcha	196 ± 19.63	mg/kg	194.01 ± 4.30 – 92.00 ± 0.28	mg/kg	Ali et al.,2021
	Patshaling	193.4 ± 13.22	mg/kg	160.25 ± 3.50	mg/kg	Dharmender et al.,2010
	Chendebji	192.4 ± 21.14	mg/kg	38.3 ± 1.90	mg/g	Pandey et al.,2017

was higher than the value reported from Sikkim, India, at 0.08 ± 0.03% (Singh et al.,2017).

The mean gallic acid content was highest in Chapcha at 17.67 ± 7.88% followed by 15.57 ± 5.94% in Chendebji and 12.44 ± 4.07% in Patshaling. A study conducted in Hungary reported 6.57% gallic acid (Boros et al.,2014), while studies from Gujarat, and Lucknow, India, had significantly higher concentrations, with 99.9% (Dharmender et al.,2010) and 99.96 ± 2.13% (Srivastava et al.,2014), respectively.

However, the bergenin content in Bhutan was comparable with reports from other regions with Chapcha showing a mean of 196 ± 19.63 mg/kg, Patshaling with 193.4 ± 13.22 mg/kg and Chendepji with 192.4 ± 21.14 mg/kg compared to 194.01 ± 4.30 mg/kg to 92.00 ± 0.28 mg/kg in Lahore, Pakistan (Ali et al.,2021), 160.25 ± 3.50 mg/kg in Gujarat, India (Dharmender et al.,2010), and 38.30 mg/g in Sikkim, India (Pandey et al.,2017).

Differences in phytochemical content within samples from Bhutan and between Bhutan and other countries could be because of various factors such as plant sample maturity (Jayamohan et al.,2013) soil profile (Pant et al.,2021), harvesting time (Magray et al.,2022), and soil moisture (Yang et al.,2018) among other factors. Moreover, drought conditions often increase secondary metabolite production as a stress response (Qaderi et al.,2023), while excessive moisture can reduce metabolite concentrations and disrupt their production (Laftouhi et al.,2023). The metabolite concentration is also affected by extraction methods (Zhang et al.,2011). The concentration and composition of phytochemicals in *Bergania ciliata* can vary with the growth cycle, including flowering season (Hasdiana, 2018). Some phytochemicals may reach peak concentration levels during flowering, while others may be more abundant during different stages of growth (Banerjee et al.,2014). During the flowering stage, phenolics and flavonoids accumulate in flowers and reproductive structures to attract pollinators or protect seeds from predators.

Elevated phenolic concentration may suggest stronger antioxidant qualities (Rashid et al.,2022). The results of this study indicated that, in comparison to Chapcha and Patshaling, Chendebji had a different chemical pro-

file, especially in terms of its higher phenolic content and lower tannin levels causing less bitterness while tasting and its medical uses may be affected.

Moreover, the difference in the phytochemical concentration could also be due to the solvent used in extraction. In some studies, methanol has been used as the solvent because of its high extraction efficiency for polar and moderately polar compounds and methanol is found to yield higher concentrations of flavonoids, phenolics, and alkaloids (Singh et al.,2017). Yousaf et al. (2018) reported that the methanol extract of the rhizome had the highest flavonoid content (14.81 ± 0.021 mg/ml), followed by ethanol and aqueous extracts, while the n-hexane fraction exhibited the lowest flavonoid content (7.91 ± 0.07 mg/ml). In this study, ethanol was chosen as the solvent for extraction, which could be one of the reasons for the lower phytochemical content observed.

Conclusion and Recommendation

This study documented 12 different ethnomedicinal uses of *Bergenia ciliata* in three different areas of Bhutan. It is traditionally used to treat various ailments, including stomach ache, body ache, toothache, fever, urinary tract infections, diarrhea, vomiting, nausea, mouth ulcers, postpartum recovery, deworming, and healing wounds in both humans and livestock, often in concoction with dried rhizome and liquids (honey, water, and milk). The most used plant part is rhizome and it is used mostly in powdered form. The highest user value was for stomach aches. It is a valuable medicinal plant with beneficial therapeutic properties and should be conserved. Since *B. ciliata* is found in other parts of Bhutan and is also used by other communities, documentation of ethnomedicinal practices is recommended.

The total tannic, total phenolics, and total flavonoids varied across the study areas which could be due to differences in the sample plant maturity and environmental factors. However, catechin, gallic acid, and bergenin showed no significant difference across the three areas. Moreover, these phytochemicals were found to be in lower concentration compared to the concentration of phytochemicals from other countries except bergenin, which could be because of differences in sample maturity and the solvent used for extraction. Since there is no previous study done on the phytochemical analysis of *Bergenia* in Bhutan, this first attempt could be supported with further studies. In the future, studies should focus on detecting a broader range of bioactive compounds, utilizing improved methodologies for more comprehensive analysis.

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