Asian Journal of Chemistry; Vol. 37, No. 5 (2025), 1159-1168

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2025.33510

Quantitative Assessment and Chemical Characterization of Secondary Metabolites from Ethnomedicinal Plant *Bergenia ciliata* using ATR-FTIR and NMR Techniques

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Received: 14 February 2025; *Accepted*: 9 April 2025; *Published online*: 30 April 2025; AJC-21983

Bergenia ciliata, an ethnomedicinal plant, is widely recognized for its therapeutic potential. This study aimed to conduct a comprehensive phytochemical screening and chemical characterization of its root and rhizome extracts using ATR-FTIR and NMR techniques. Preliminary qualitative phytochemical analysis was performed on extracts prepared in aqueous, ethyl acetate and methanol solvents to identify bioactive compounds. The presence of tannins, flavonoids, saponins, alkaloids, glycosides, steroids, terpenoids and other secondary metabolites was confirmed. Fourier transform infrared spectroscopy (ATR-FTIR) was employed to determine the functional groups present in the extracts. The spectral analysis revealed key bioactive functional groups including hydroxyl (-OH), carbonyl (C=O), aliphatic nitriles (C≡N) and amides, which contribute to the medicinal properties of plant. Peaks corresponding to stretching and bending vibrations were identified, highlighting the presence of various phytochemical constituents. Furthermore, metabolite profiling using proton nuclear magnetic resonance (^{1}H NMR) spectroscopy provided insights into the chemical structure of bioactive compounds. Bergenin, a bioactive compound known for its pharmacological activities, was detected along with other secondary metabolites. The results of this study confirm the diverse phytochemical composition of *Bergenia ciliata*, supporting its traditional medicinal applications. The integration of ATR-FTIR and NMR spectroscopy provides a reliable approach for characterizing plant-based bioactive compounds, facilitating further study into their pharmacological properties and therapeutic applications.

Keywords: *Bergenia ciliata*, Phytochemical screening, ATR-FTIR spectroscopy, NMR spectroscopy, Bioactive compounds.

INTRODUCTION

Medicinal plants have been integral to traditional healing practices for centuries, offering a rich source of bioactive compounds with therapeutic potential. Among them, *Bergenia ciliata*, commonly known as "Paashanbheda," has been widely used in Ayurvedic and folk medicine for treating various ailments [1]. Native to the Himalayan regions, it is known for its pharmacological properties, including anti-inflammatory, antimicrobial, antioxidant, antidiabetic and anticancer activities [2]. The root and rhizome of *Bergenia ciliata* have traditionally been used for treating kidney stones, wounds, ulcers and respiratory disorders. The medicinal properties of *Bergenia ciliata* are attributed to its diverse secondary metabolites including tannins, flavonoids, alkaloids, saponins, glycosides and phenolic compounds [3]. These compounds exhibit various biological activities such as antioxidant, enzyme inhibition and antimicrobial effects. Despite its extensive medicinal applications, a detailed chemical characterization of its bioactive compounds is essential to validate its therapeutic potential and integrate it into modern medicine.

Bergenia ciliata has been traditionally recognized for its nephroprotective, hepatoprotective and cardioprotective effects, making it a valuable resource for pharmaceutical research [4]. The roots and rhizomes are particularly rich in bergenin, a bioactive compound with anti-inflammatory, hepatoprotective and antiurolithiatic properties. Other important phytochemicals, such as catechins, tannins and flavonoids, further enhance its medicinal potential. Given the increasing global interest in natural remedies, a systematic investigation of the plant's phytochemical profile is necessary to establish its chemical composition and therapeutic applications. Previous studies [5-9] have reported various bioactive compounds in different extracts of *Bergenia ciliata* but detailed spectral characterization using advanced techniques remains limited.

The identification and structural elucidation of bioactive compounds in medicinal plants require sophisticated analytical techniques [10,11]. ATR-FTIR spectroscopy is widely used for the qualitative analysis of functional groups in complex biological samples [12]. It offers significant advantages, including minimal sample preparation, high sensitivity and the ability to analyze various states of matter. ATR-FTIR spectra provide valuable information about the presence of hydroxyl, carbonyl, amide and other characteristic functional groups, which play a crucial role in the biological activity of phytochemicals. On the other hand, NMR spectroscopy is an essential tool for elucidating the structural composition of organic compounds [13,14].

Thus, this study aims to address this gap by systematically investigating the plant's phytochemical profile through ATR-FTIR and NMR spectroscopy. The specific objectives include conducting qualitative phytochemical screening of aqueous, ethyl acetate and methanol extracts to identify major classes of bioactive compounds, characterizing the functional groups present in the plant extracts using ATR-FTIR spectroscopy, determining the structural composition of bioactive metabolites through ^{1}H NMR spectroscopy and correlating the identified phytochemicals with their pharmacological potential. The findings from this study will provide a scientific basis for the traditional use of *Bergenia ciliata*, paving the way for its potential development as a natural therapeutic agent. By employing advanced analytical methods, this research aims to contribute to the growing field of phytopharmacology and support the integration of medicinal plants into evidence-based medicine.

EXPERIMENTAL

Phytochemical screening of *Bergenia ciliata* root and rhizome extracts: Preliminary phytochemical screening was conducted to assess the presence of bioactive compounds in *Bergenia ciliata* root and rhizome extracts using aqueous, ethyl acetate and methanol solvents [15-17]. Standard qualitative procedures were employed for the analysis. The presence of starch was determined using the iodine test, while tannins and phenols were detected using standard protocols [18]. Flavonoids were identified through the alkaline reagent test and Shinoda test. Glycosides were analyzed using the Keller-Kiliani test and Liebermann's test. The presence of saponins was confirmed through foam formation, whereas proteins were detected using the ninhydrin test. Steroids were assessed using standard methods and reducing sugars were identified through Fehling's test and Benedict's test [19]. Terpenoids were evaluated using the Salkowski test [20], while alkaloids were also detected following standard procedures. Fatty acids were examined using established methods [21], whereas carbohydrates were assessed through standard detection assays, while leucoanthocyanins and anthocyanins were tested to determine flavonoid pigment content. Emodins and coumarins were identified based on specific phytochemical protocols [22]. These qualitative tests provided insights into the phytochemical composition of *B. ciliata* extracts.

ATR-FTIR spectral analysis for the functional group characterization of *B. ciliata* extracts: FTIR was used to identify the functional groups present in *B. ciliata* root and rhizome extracts. ATR-FTIR analysis was performed at room temperature using a Perkin-Elmer spectrum-2 with a universal ATR. The scanning range was set between 4000 and 500 cm^{-1}. The Spectrum 10 software (version 10.5.2.636) was used to generate the spectra. For analysis, dried and powdered plant extracts in different solvents (ethyl acetate, aqueous and methanol) were encapsulated in KBr pellets (10 mg extract powder in 100 mg KBr). A mid-infrared deuterated triglycine sulfate (DTGS) detector was used for spectral acquisition. Moreover, a portable programmable temperature controller (4000 series TM High Stability Temperature Controller, Specac, Ltd.) was employed to measure the temperature range (50-120 ºC). The obtained infrared spectra were analyzed to determine peak wavenumbers (cm^{-1}) and vibrational assignments. Based on the absorption spectra, approximate band allocations were developed to identify functional groups such as halogens, carboxylic acids, amides, amines, amino acids and carbonyl compounds.

Nuclear magnetic resonance (NMR) spectral analysis: Metabolite profiling of *B. ciliata* root and rhizome extracts was conducted using ^{1}H NMR spectroscopy. The Bruker AvIII-300 MHz (Switzerland) was used to generate ^{1}H NMR spectra. For analysis, 10 mg of lyophilized plant material was dissolved in 600 µL of deuterated methanol (CD_3OD), extracted using polar aqueous and methanol solvents. The samples were vortexed for 1 min, centrifuged and transferred into 5 mm NMR tubes. The spectral data were processed using Mnova (Mestrelab Products) with phase correction, baseline correction and solvent peak referencing. Peak selection and integration were performed manually, excluding regions corresponding to water signals. The resulting chemical shifts were analyzed and the structures were clarified. To identify the compounds associated with Bergenin, PubChem data were referenced and the top 30 related compounds were converted to .mol files using their isosmiles keys *via* the OpenBabel standalone application. The spectral predictions were generated using nmrdb.org, with chemical shift values reported in ppm.

RESULTS AND DISCUSSION

Bergenia ciliata, a medicinal plant with significant ethnopharmacological relevance, is known to contain numerous bioactive compounds with medicinal potential [23]. This study explores the phytochemical composition of *Bergenia ciliata* through qualitative and quantitative analyses using FTIR and ^{1}H NMR spectroscopy.

Qualitative phytochemical analysis of *B. ciliata* root and rhizome extracts: Phytochemical tests were conducted to assess the presence of various bioactive compounds in different solvent extracts (A1, A2, A3 for root extracts and B1, B2, B3 for rhizome extracts) of *B. ciliata*. The qualitative screening included tests for iodine, tannins, saponins, phenols, flavonoids, steroids, terpenoids, alkaloids, glycosides, proteins, carbohydrates, fatty acids, leucoanthocyanins, anthocyanins, emodins and coumarins. The results revealed the presence of tannins, alkaloids, flavonoids, saponins, amino acids, quinones, coumarins, glycosides, steroids, terpenoids, anthraquinones and phenols, across various extracts. Table-1 presents the detailed phytochemical assessment of root and rhizome extracts, where a '+' indicates the presence and a '–' denotes the absence of a particular phytochemical.

Qualitative analysis of phytochemicals using FTIR spectroscopy: The FTIR spectra of *B. ciliata* extracts including root methanolic (A2) and aqueous (A3) extracts as well as rhizome methanolic (B2) and aqueous (B3) extracts, were analyzed, considering the specific wavenumbers and intensities. The FTIR wavelength ranges and corresponding functional groups are presented in Tables 2-5, while Figs. 1-4 provide a representation of the FTIR spectral profiles for the extracts B2, B3, A2 and A3. Both stretching and bending vibrations were matched with data from the literature [24-27].

The FTIR spectral analysis of the aqueous extract of *B. ciliata* rhizome (B3) identified various functional groups of bioactive compounds, including fluorine, carbodiimide, alkene, carbonyl, aliphatic nitrile, halogen and isonitrile compounds. Multiple absorption peaks were observed across different frequency ranges, with a strong hydroxyl (-OH) stretching vibration at 3328.26 cm^{-1} (49.97% transmittance), dominant in the 3500–3000 cm^{-1} region. A strong metal carbonyl (C=O) stretch was found at 2112.36 cm^{-1} (97.18% T), while an unsaturated hydrocarbon (C=C) stretching vibration appeared at 1635.06 cm^{-1} (73.42% T) with medium bond strength. A fluorine containing compound (C-F) was detected at 1393.73 cm^{-1} (94% T) and another unsaturated hydrocarbon (C=C) bending vibration appeared at 593.66 cm^{-1} (82.75% T).

Similarly, the FTIR analysis of the methanolic rhizome extract (B2) also revealed several functional groups. In the wavelength region below 1000 cm^{-1}, an unsaturated hydrocarbon (C=C) bending vibration was observed at 626.46 cm^{-1} (79.85% T) with medium bond strength. In the 1500–1000 cm^{-1} region, aliphatic ether (C-O) stretching vibrations were recorded at 1020.25 cm^{-1} (30.62% T) and 1111.80 cm^{-1} (91.05% T), both exhibiting strong bonding, along with an aliphatic carbodiimide (N=C=N) asymmetric stretch at 1449.09 cm^{-1}

TABLE-1
PHYTOCHEMICAL ASSESSMENT OF ROOT (A1, A2, A3) AND RHIZOME EXTRACTS (B1, B2, B3) OF *Bergenia ciliata*

Phytochemicals test	Observations	Root extracts			Rhizome extracts		
		A1	A2	A3	B1	B2	B3
Iodine	Appearance of dark blue or purple colour	–	+	+	–	+	+
Phenol	Brownish green or blue-black hues	+	–	+	+	–	+
Tannin	Brownish green or blue-black hues	+	–	+	+	–	+
Flavonoids:							
Alkaline reagent test	Yellow colour becomes colourless	–	+	+	–	–	–
Shinoda test	A pink, orange, or red colour to purple colour	–	+	+	–	–	–
Glycosides:							
Keller-kiiani test	Brown ring in the interphase	+	+	+	+	+	+
Liebermann's test	Violet to blue to green	+	+	+	+	+	+
Saponin	Foaming	–	+	+	–	+	+
Protein:							
Ninhydrin test	Violet colour	–	–	+	–	–	+
Steroid	Greenish hue appears	+	+	+	+	+	+
Terpenoid	Reddish brown colour	+	+	+	+	+	+
Alkaloid	Precipitate's turbidity	+	–	+	+	–	+
Fatty acids	Indicated by the filter paper's appearance of *trans*parency	+	+	+	+	–	+
Carbohydrates:							
Benedict's test	Reddish brown precipitant appears	+	–	+	+	+	+
Fehling's test	Appearance of brick red precipitate						
Leucoanthocyanin	Upper layer is red	–	–	+	+	–	+
Anthocyanins	Pink, red turn blue violet	–	+	+	+	+	+
Emodin	Presence of red colour	+	–	+	+	+	+
Coumarins	Yellow colour appearance	+	+	–	–	–	–

Indication result: + = presence, – = absence

TABLE-2
FTIR SPECTRAL FUNCTIONAL GROUPS OBTAINED FOR THE AQUEOUS EXTRACT OF *Bergenia ciliata* RHIZOME (B3)

Region	Wavelength range (cm^{-1})	Frequency of measured peaks (absorption range, cm^{-1})	Functional group RHA	Vibration bond type
Area 1	1500-1000	1000-1400	CF_3 (C=F)	Strong stretching
		1440-1500	Aliphatic carbodiimide (N=C=N)	Strong stretching, symmetrical
Area 2	2200-1500	1566-1650	Unsaturated hydrocarbon (C=C)	Medium stretching
		1900-2100	Metal carbonyl (C=O)	Strong stretching
Area 3	2500-2200	2222-2260	Aliphatic nitrile, conjugated (C≡N)	Weak stretching
			Aliphatic isonitrile (C≡N)	Weak stretching
Area 4	3500-3000	3328.26	Hydroxy compounds (-OH)	Strong bond stretching

TABLE-3
FTIR SPECTRAL FUNCTIONAL GROUPS OBTAINED FOR THE METHANOLIC EXTRACT OF *Bergenia ciliata* RHIZOME PLANT (B2)

Region	Wavelength range (cm^{-1})	Frequency of measured peaks (absorption range, cm^{-1})	Functional group RHM	Vibration bond type
Area 1	>1000	900-500	Unsaturated hydrocarbon, trisubstituted (C=C)	Medium bending
Area 2	1500-1000	1400-1000	CF_3 (C=F)	Strong stretching
		1440-1500	Aliphatic carbodiimide (N=C=N)	Strong stretching, symmetrical
		1085-1150	Aliphatic ether (C-O)	Strong stretching
Area 3	2000-1500	1770-1800	Substituent group (C=O)	Strong stretching
		1725-1705	Aliphatic acid or carbonyl compound (C=O)	Strong stretching
		1680	Aliphatic *tertiary* amide (C=O)	Strong stretching
		1650-1566	Unsaturated hydrocarbon, cyclic (C=C)	Medium stretching
		1650-1600	Unsaturated hydrocarbon *trans* conjugated (C=C)	Medium stretching
		1760-1780	Cyclic anhydride (CO-O-CO)	Strong stretching
Area 4	2500-2000	1990-2140	Isothiocyanate (N=C=S)	Strong stretching
		2130-2160	Aliphatic carbodiimide (N=C=N)	Strong stretching, asymmetrical
		1900-2100	Metal carbonyl (C=O)	Strong stretching
Area 5	3600-2500	2550-2600	Silanol (Si-O-H)	Weak stretching
		3300-2500	Conjugated carboxylic acid (O-H)	Strong stretching
		3200-3550	Hydroxy compounds (-OH)	Strong stretching

TABLE-4
FTIR SPECTRAL FUNCTIONAL GROUPS OBTAINED FOR THE AQUEOUS EXTRACT OF *Bergenia ciliata* ROOT PLANT (A3)

Region	Wavelength range (cm^{-1})	Frequency of measured peaks (absorption range, cm^{-1})	Functional group RTA	Vibration bond type
Area 1	>1000	515-690	Linear bromo compound (C-Br)	Strong stretching
		550-850	Long chain halogen (C-Cl)	Strong stretching
Area 2	1500-1000	1440-1500	Aliphatic carbodiimide (N=C=N)	Strong stretching, symmetrical
Area 3	2200-1500	1650-1566	Unsaturated hydrocarbon (C=C)	Medium stretching
		1900-2100	Metal carbonyl (C=O)	Strong stretching
Area 4	2500-2200	2260-2222	Aliphatic nitrile, conjugated (C≡N)	Weak stretching
			Aliphatic isonitrile (C≡N)	Weak stretching

TABLE-5
FTIR SPECTRAL FUNCTIONAL GROUPS OBTAINED FOR THE METHANOLIC EXTRACT OF *Bergenia ciliata* ROOT PLANT (A2)

Region	Wavelength range (cm^{-1})	Frequency of measured peaks (absorption range, cm^{-1})	Functional group RTM	Vibration bond type
Area 1	>1000	960-980	Unsaturated hydrocarbon, *trans* conjugated (C=C)	Strong bending
		1085-1150	Aliphatic ether (C-O)	Strong stretching
Area 2	1500-1000	1400-1000	CF_3 (C=F)	Strong stretching
		1440-1500	Aliphatic carbodiimide (N=C=N)	Strong stretching, symmetrical
Area 3	1900-1500	1785-1815	Aliphatic acid halide (C=O)	Strong stretching
		1770-1800	Substituent group (C=O)	Strong stretching
Area 4	2500-1900	1990-2140	Isothiocyanate (N=C=S)	Strong broad stretching
		1900-2100	Metal carbonyl (C=O)	Strong stretching
		2130-2160	Aliphatic carbodiimide (N=C=N)	Strong stretching, asymmetrical
		2222-2260	Aliphatic isonitrile (C≡N)	Weak stretching
Area 5	3000-2500	2550-2600	Silanol (Si-O-H)	Weak stretching
Area 6	3500-3000	3200-3550	Hydroxy compounds (-OH)	Strong broad stretching

(86.53% T). In the 2000-1500 cm^{-1} region, strong stretching vibrations for carbonyl (C=O) and amide (C=O) compounds were detected, while an unsaturated hydrocarbon (C=C) stretch at 1612.26 cm^{-1} (96.66% T) exhibited medium bond strength. In the 2000–2500 cm^{-1} region, the presence of isothiocyanate (N=C=S), aliphatic carbodiimide (N=C=N) and metal carbonyl (C=O) groups was confirmed, all showing strong bonding. Finally, in the 3600-2500 cm^{-1} range, carboxylic acid (COOH) stretching vibrations were observed at 2831.96 cm^{-1} (82.28% T) and 2942.94 cm^{-1} (79.93% T), both with strong bonding, while a hydroxyl (-OH) stretching vibration was detected at 3319.12 cm^{-1} (72.19% T) with strong bond strength. These findings confirmed the presence of diverse bioactive functional groups in both the aqueous and methanolic extracts of *B. ciliata* rhizome, reinforcing its potential medicinal properties.

In the aqueous extract of *B. ciliata* root (A3), the out-of-plane stretching vibrations correspond to C-Br and C-Cl were identified at 586.16 cm^{-1} (87.41% T) with strong bond strength. In the 1500–1000 cm^{-1} region, a peak at 1088.53 cm^{-1} (97.87% T) is due to the symmetrical stretching of aliphatic carbodiimide

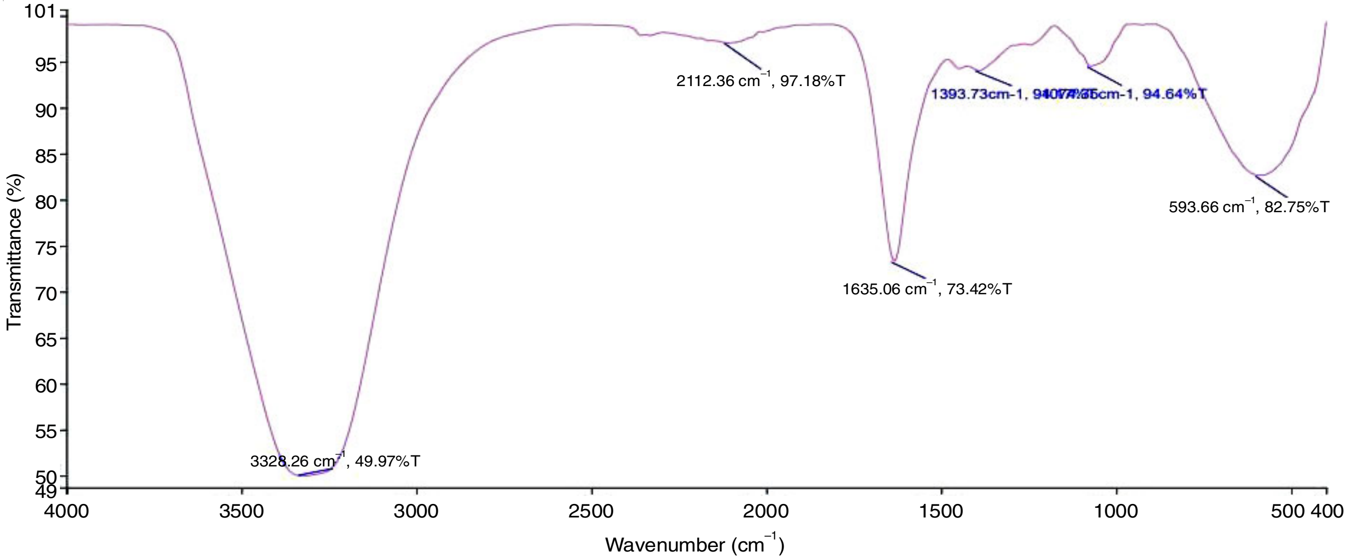

Fig. 1. FTIR analysis of an aqueous extract of *Bergenia ciliata* rhizome plant (B3)

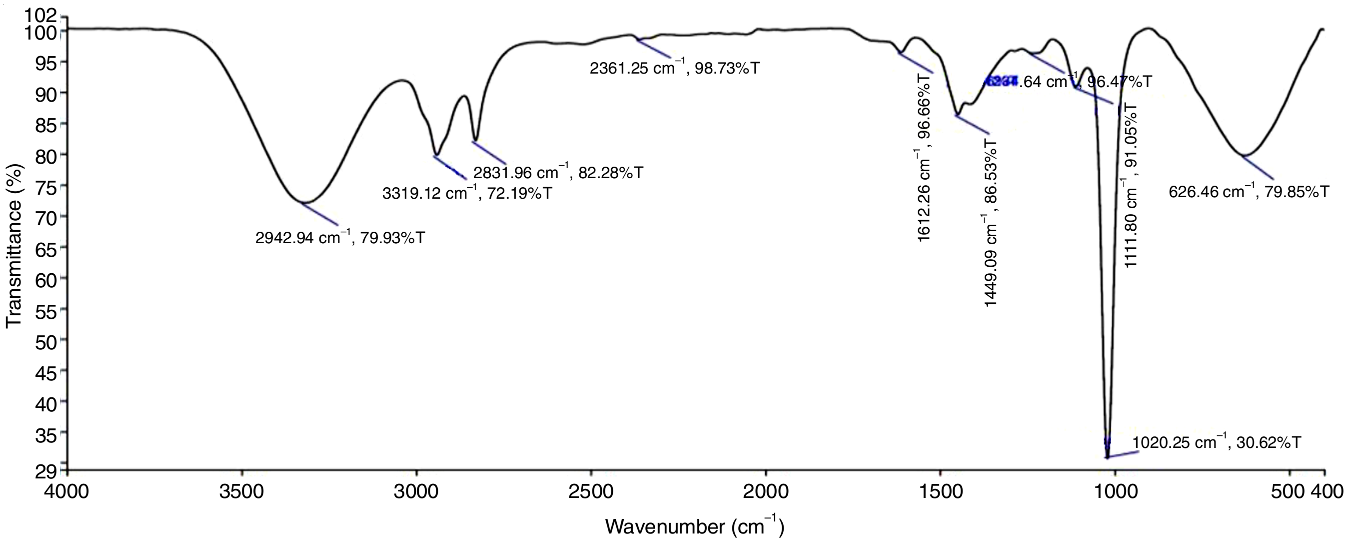

Fig. 2. FTIR analysis of methanolic extract of *Bergenia ciliata* rhizome plant (B2)

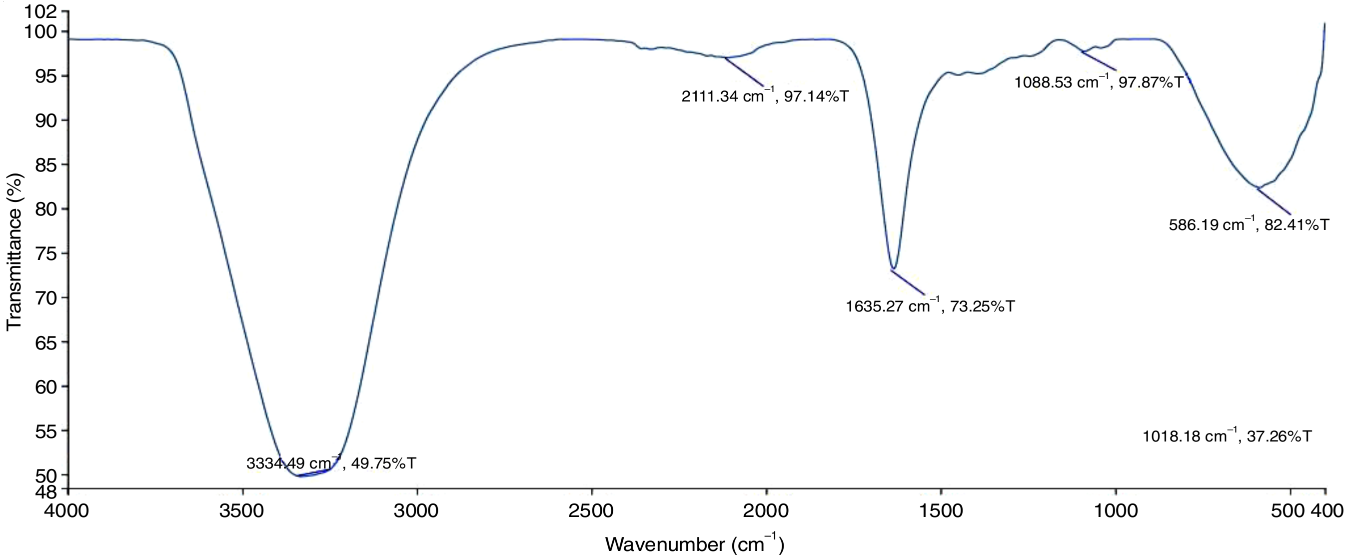

Fig. 3. FTIR analysis of aqueous extract of *Bergenia ciliata* root plant (A3)

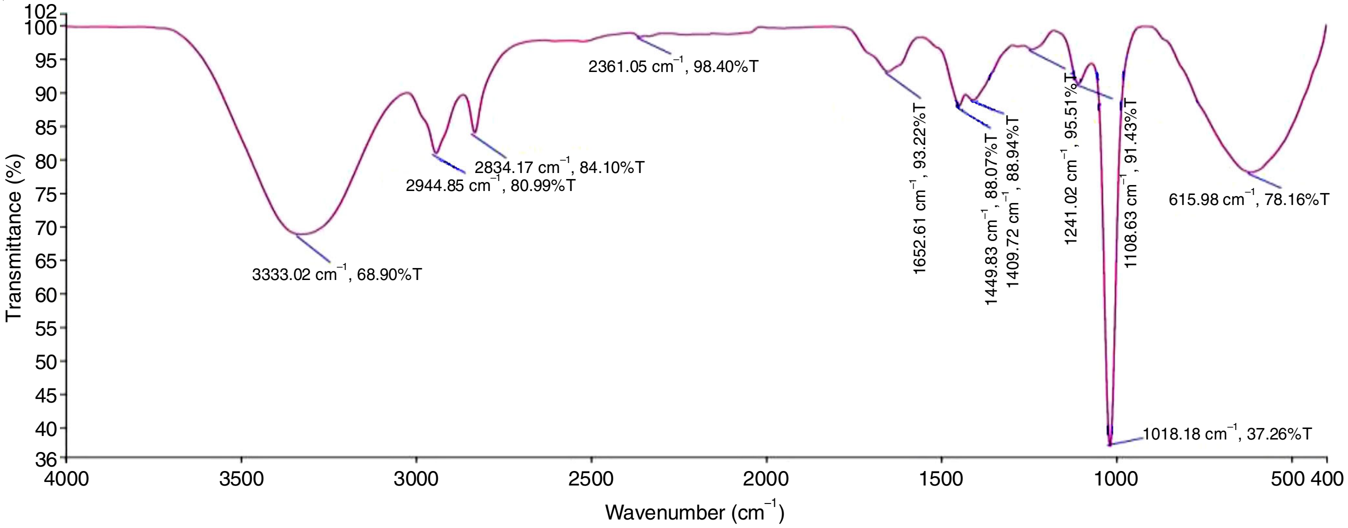

Fig. 4. FTIR analysis of methanolic extract of *Bergenia ciliata* root plant (A2)

(N=C=N), whereas the stretching vibrations for unsaturated hydrocarbons (C=C) and carbonyl (C=O) at 1635.27 cm^{-1} (73.25% T) and 2111.34 cm^{-1} (97.14% T), respectively. Finally, at higher region, 2500–2200 cm^{-1}, weak stretching vibrations corresponding to aliphatic nitrile and isonitrile (C≡N) are observed.

Similarly, the FTIR analysis of the methanolic extract of *Bergenia ciliata* root (A2) identified seven distinct absorption regions. In the lower region (> 1000 cm^{-1}), the bending vibrations of C=C and C-O were recorded at 615.98 cm^{-1} (78.16% T). Whereas in the 1500–1000 cm^{-1} region exhibited strong stretching peaks at 1018.18 cm^{-1} (37.26% T), 1108.3 cm^{-1} (91.43% T), 1241.02 cm^{-1} (95.51% T), 1409.73 cm^{-1} (88.94% T) and 1449.83 cm^{-1} (88.94% T), indicating the presence of C=F, C-O and N=C=N functional groups, respectively. In the 1900–1500 cm^{-1} region, a peak at 1652.1 cm^{-1} (93.22% T) correspond to the C=O stretching in halide-substituted groups, whereas 2500–1900 cm^{-1} region dominated by stretching vibrations of N=C=N, C=O and N=C=S, respectively with a prominent peak at 2361.05 cm^{-1} (98.40% T). The Si-O-H and -OH stretching vibrations, with peaks at 2944.85 cm^{-1} (80.99% T) and 3333.02 cm^{-1} (68.90% T), respectively, was also appeared which indicate a mixture of weak and strong bond strengths.

Comparatively, Kumar *et al.* [28] reported similar FTIR spectra for *B. ciliata*, with prominent peaks for O-H and C=O stretching, while Choudhary *et al.* [29] identified key FTIR bands for bergenin and gallic acid with similar absorption patterns. The FTIR analysis further indicates the presence of flavonoids and phenols due to O-H stretching, terpenes due to C-H groups and alkaloids due to the N-H stretching [30]. The functional groups such as quinones, aldehydes, amides, alkenes, amines, alcohols, carboxylic acids, aromatics, anhydrides, phenols, ethers and organic halogen compounds, all of which belong to secondary plant metabolites, were identified in accordance with previous studies [31,32]. The FTIR spectrum of ellagic acid in *B. ciliata* aligns with findings from other plant species. For example, ellagic acid extracted from *Terminalia chebula* exhibited similar O-H stretching at ~3400 cm^{-1}, C=O stretching at ~1720 cm^{-1} and aromatic C=C stretching at ~1600 cm^{-1} [28].

This study confirmed the presence of diverse functional groups in both the root (A2, A3) and rhizome extracts (B2, B3) of *B. ciliata* including alkynes, alcohols, phenols, alkyl halides, alkanes, aldehydes, aromatics, amines, carboxylic acids, nitro compounds, α-, β-unsaturated esters and alkyl halides. These identified functional groups can be further isolated and tested for their biological activities, paving the way for potential pharmacological applications [32].

Metabolite profiling of *B. ciliata* using ^{1}H NMR spectroscopy: The NMR spectroscopy analysis conducted on *B. ciliata* extracts provided an indepth understanding of the metabolite composition in four different crude extracts labeled as A2, A3, B2 and B3. The study employed ^{1}H NMR spectroscopy using MestreNova software for spectral analysis, allowing the identification and quantification of various secondary metabolites. The spectral data revealed significant variations in chemical shifts, structural fingerprints and metabolite distribution among the different samples. The aliphatic region of the NMR spectra exhibited more pronounced peaks compared to the aromatic region indicating a higher abundance of aliphatic protons (Figs. 5-8), which are often associated with bioactive compounds such as flavonoids, isocoumarins and polyphenols. A metabolite library comprising the top 30 compounds was prepared to streamline data interpretation (Table-6). This library included a diverse range of bioactive molecules such as cuscutin, altenuene, bergenin, monocerin, ellagic acid and several isochromenone derivatives. These compounds have been previously reported for their pharmacological properties including antioxidant, antimicrobial, hepatoprotective, neuroprotective, anti-inflammatory, anticancer and antifungal activities. Among these, bergenin (compound **4**) was successfully identified, as its ^{1}H NMR spectral characteristics matched with previously reported data [33], confirming its presence in the extract.

In this study, the characteristic signals for aromatic protons were observed between δ 6.5-7.5 ppm, indicating the presence of phenolic molecules such as bergenin, ellagic acid and gallic

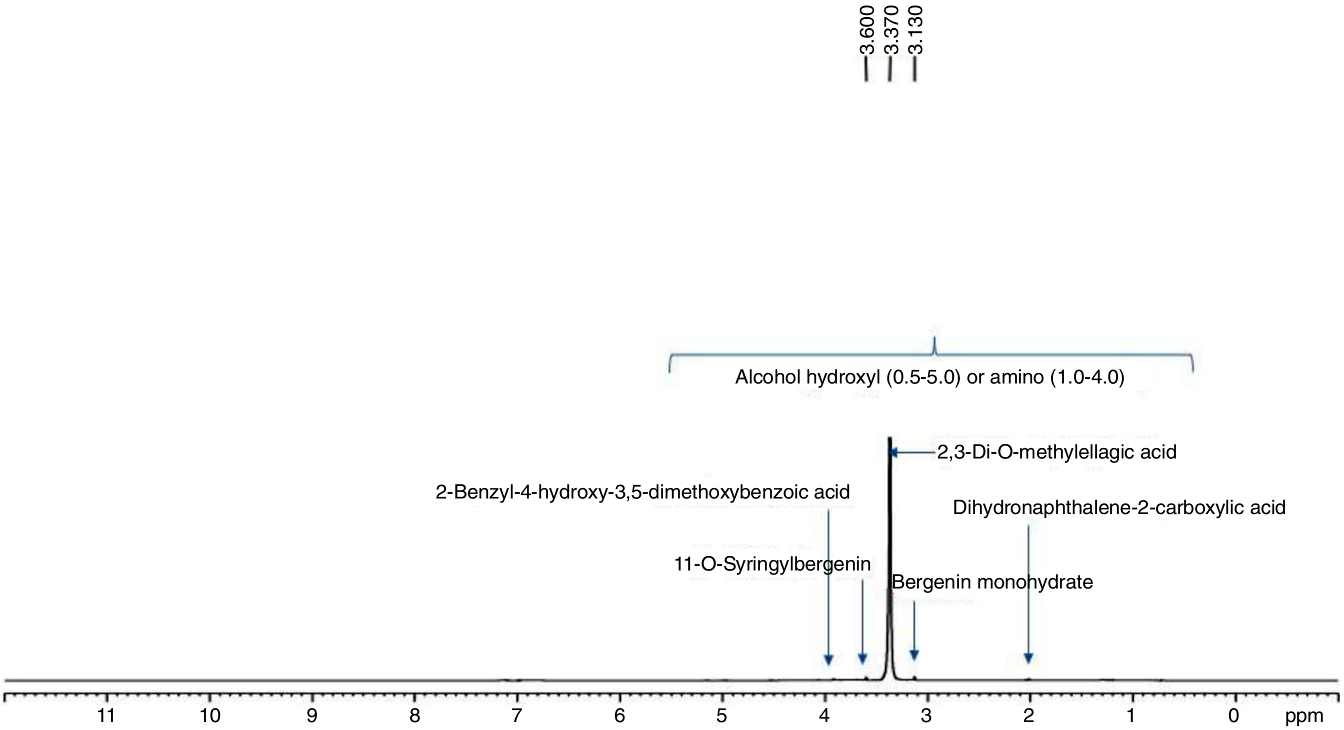

Fig. 5. ^{1}H NMR spectrum of *Bergenia ciliata* root (A2) after methanol treatment (RTM) in the expended area in the range of δ 4–3

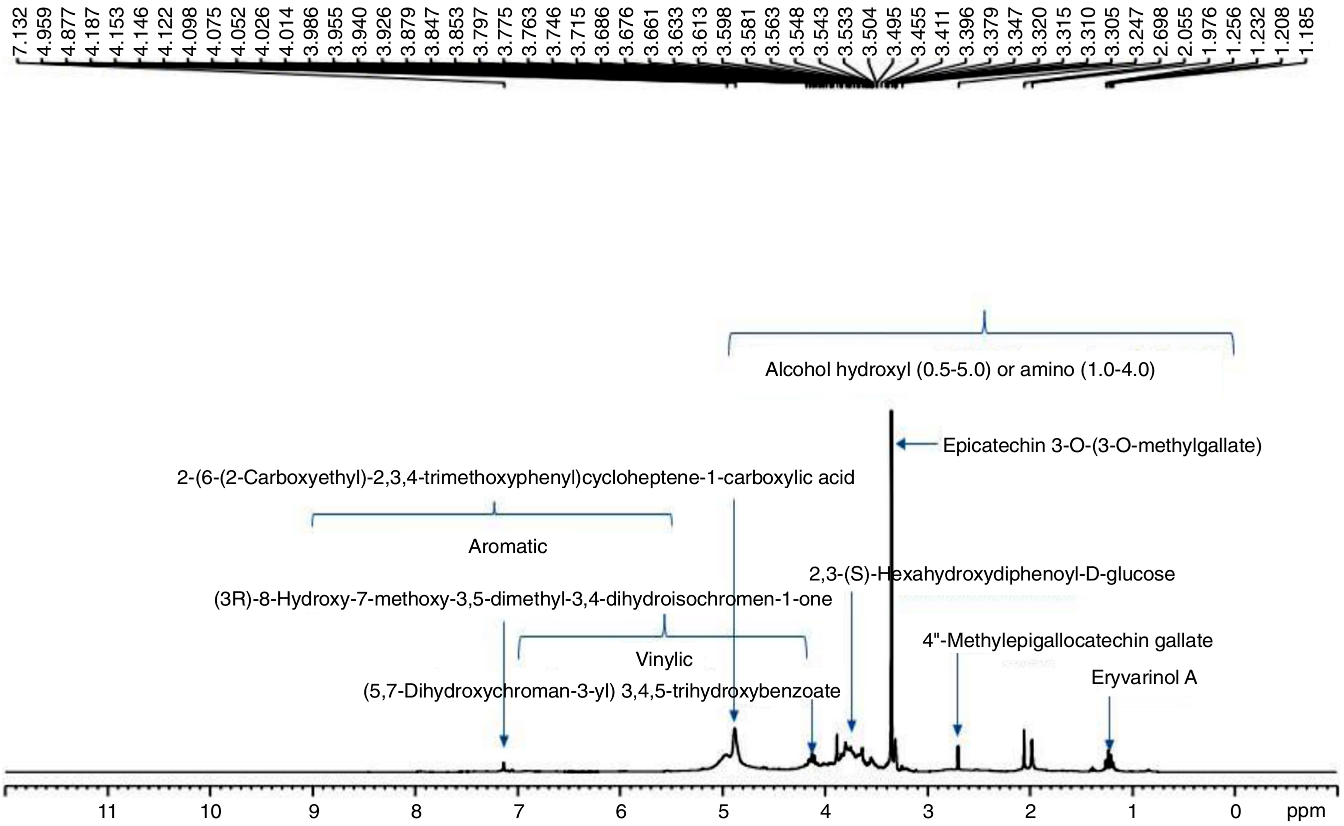

Fig. 6. ^{1}H NMR spectrum of *Bergenia ciliata* root (A3) after aqueous treatment (RTA) in the expended area in the range of δ 7.5–5.5

acid [29]. The presence of methoxyl groups was identified with signals around δ 3.7 ppm, a typical feature of bergenin [27]. Proton signals corresponding to hydroxyl groups attached to aromatic rings were observed around δ 8.5-9.0 ppm, further confirming the presence of phenolic constituents. Comparisons with previous studies reveal similar chemical shifts for these bioactive compounds. For instance, Singh *et al.* [23] reported signals at δ 6.8-7.2 ppm for aromatic hydrogens in bergenin and at δ 3.6-3.8 ppm for methoxy groups, while Kumar *et al.* [27] noted hydrogen-bonded hydroxyl groups at δ 8.8 ppm in

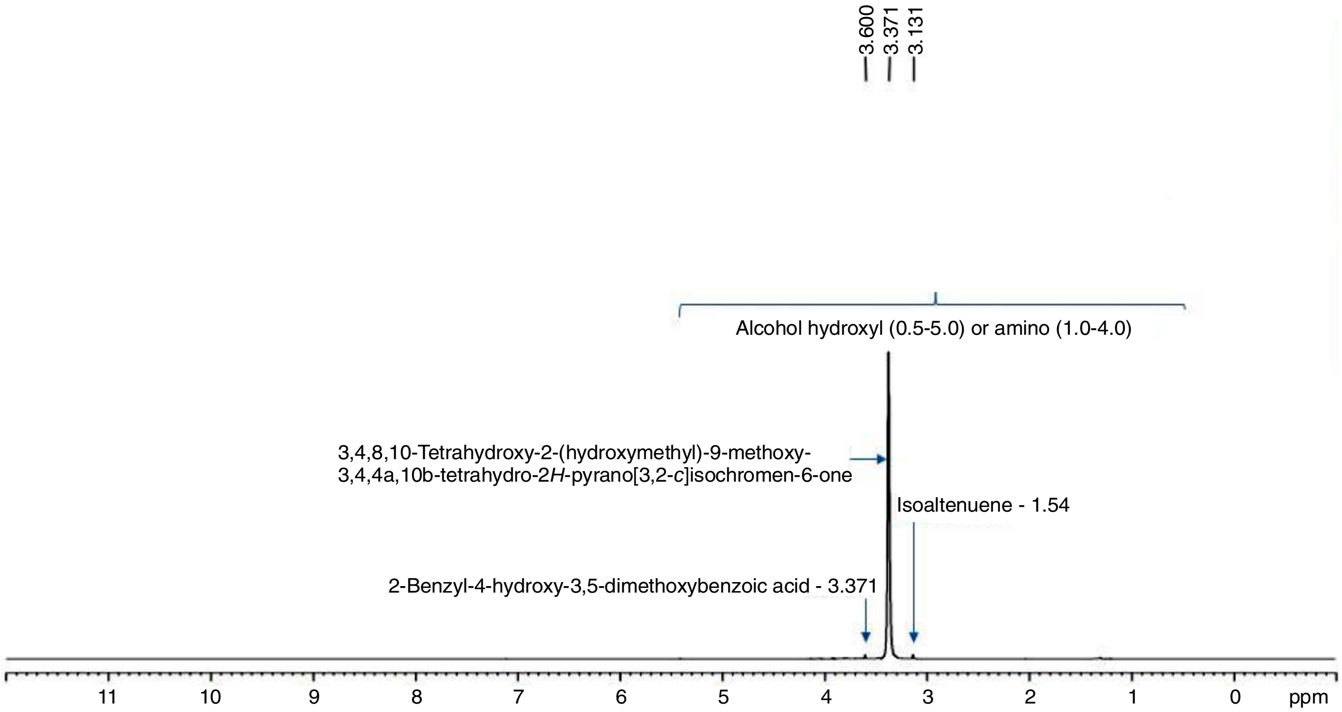

Fig. 7. ^{1}H NMR spectrum of *Bergenia ciliata* rhizome (B2) after methanol treatment (RHM) in the expended area in the range of δ 4–3

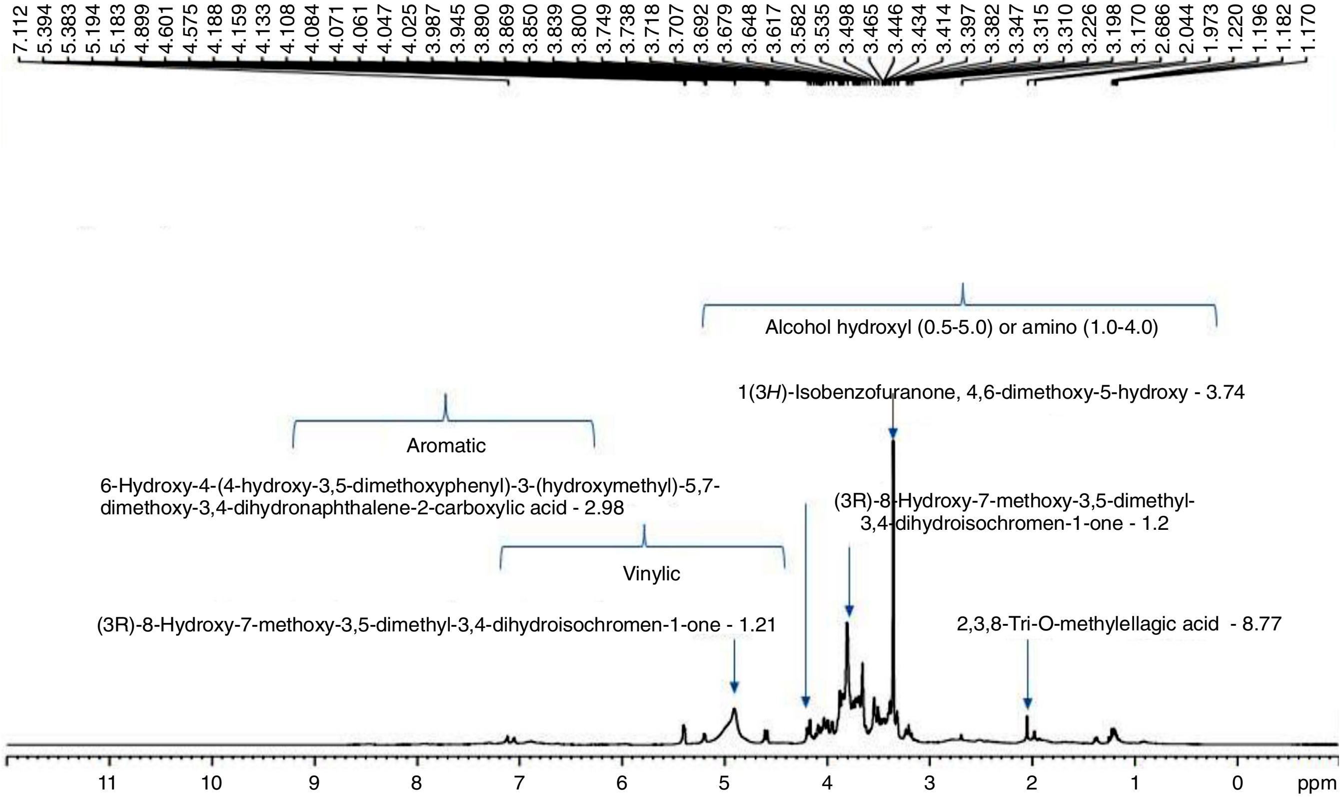

Fig. 8. ^{1}H NMR spectrum of *Bergenia ciliata* rhizome (B3) after aqueous treatment (RHA) in the expended area in the range of δ 7–1

gallic acid, consistent with the present findings. The ^{1}H NMR spectrum also exhibited characteristic signals for ellagic acid, with aromatic protons appearing in the δ 6.5-7.5 ppm range, typical of its aromatic rings. Additionally, hydroxyl protons were observed at δ ~9.5 ppm, indicative of hydrogen-bonded phenolic groups [27]. Similarily, the NMR profiles for ellagic acid have been reported in other plant species, such as pomegranate (*Punica granatum*), where ellagic acid exhibited ^{1}H NMR signals at δ 6.8-7.2 ppm for aromatic hydrogens and δ 9.0-9.5 ppm for phenolic hydroxyls, consistent with the observations in *B. ciliata* [28]. The presence of ellagic acid contributes to the known pharmacological activities of *B. ciliata*, including

TABLE-6
TOP 30 METABOLITES SIMILAR TO STANDARD EXTRACTED FROM THE NMR LIBRARY ALONG WITH THEIR CID, MOLECULAR WEIGHT (m.w.), MOLECULAR FORMULAE (m.f.), H-DONOR, H-ACCEPTOR

S. No.	Compound name	CID	m.f.	m.w.	H-donor	H-acceptor
1	3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydro-2*H*-pyrano[3,2-*c*]isochromen-6-one	2356	$C_{14}H_{16}O_9$	328.27	5	9
2	Altenuene	34687	$C_{15}H_{16}O_6$	292.28	3	6
3	1(3*H*)-Isobenzofuranone, 4,6-dimethoxy-5-hydroxy-	43532	$C_{10}H_{10}O_5$	210.18	1	5
4	Bergenin	66065	$C_{14}H_{16}O_9$	328.27	5	9
5	(2S,3R,4R,4aS,10bR)-3,4,8,9,10-Pentahydroxy-2-(hydroxymethyl)-3,4,4a,10b-tetrahydro-2*H*-pyrano[3,2-*c*]isochromen-6-one	73192	$C_{13}H_{14}O_9$	314.24	6	9
6	Monocerin	92267	$C_{16}H_{20}O_6$	308.33	1	6
7	6*H*-Dibenzo(b,d)pyran-6-one, 4-hydroxy-3methoxy-	148356	$C_{14}H_{10}O_4$	242.23	1	4
8	Isoaltenuene	180444	$C_{15}H_{16}O_6$	292.28	3	6
9	11-O-Syringylbergenin	195481	$C_{23}H_{24}O_{13}$	508.40	5	13
10	Bergenin monohydrate	201412	$C_{14}H_{18}O_{10}$	346.29	6	10
11	6-Hydroxy-4-(4-hydroxy-3,5-dimethoxyphenyl)-3-(hydroxymethyl)-5,7-dimethoxy-3,4-dihydronaphthalene-2-carboxylic acid	266966	$C_{22}H_{24}O_9$	432.40	4	9
12	2-Benzyl-4-hydroxy-3,5-dimethoxybenzoic acid	271363	$C_{16}H_{16}O_5$	288.29	2	5
13	(2R,4S,4aR)-3,4,8,10-Tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydro-2*H*-pyrano[3,2-*c*]isochromen-6-one	378697	$C_{14}H_{16}O_9$	328.27	5	9
14	4"-Methylepigallocatechin gallate	401129	$C_{23}H_{20}O_{11}$	472.40	7	11
15	Epicatechin 3-O-(3-O-methylgallate)	467296	$C_{23}H_{20}O_{10}$	456.40	6	10
16	Epicatechin 3-O-(4-O-methylgallate)	467297	$C_{23}H_{20}O_{10}$	456.40	6	10
17	(2S,3R,4R,4aS,10bR)-3,4,8,10-Tetrahydroxy2-(hydroxymethyl)-9-methoxy-3,4,4a,10b-tetrahydro-2*H*-pyrano[3,2-*c*]isochromen-6-one	471287	$C_{14}H_{16}O_9$	328.27	5	9
18	2,3-(S)-Hexahydroxydiphenoyl-D-glucose	492390	$C_{20}H_{18}O_{14}$	482.30	9	14
19	6*H*-Dibenzo[*b*,*d*]pyran-6-one, 2,3,4,4a-tetrahydro-2,3,7-trihydroxy-9-methoxy-4a-methyl-, (2R,3R,4aR)-rel-	600784	$C_{15}H_{16}O_6$	292.28	3	6
20	6,8-Dihydroxy-7-methoxy-3-methyl-3,4-dihydroisochromen-1-one	614234	$C_{11}H_{12}O_5$	224.21	2	5
21	8-Hydroxy-6,7-dimethoxy-3-methyl-3,4-dihydroisochromen-1-one	617182	$C_{12}H_{14}O_5$	238.24	1	5
22	2-(6-(2-Carboxyethyl)-2,3,4-trimethoxyphenyl)cycloheptene-1-carboxylic acid	631464	$C_{20}H_{26}O_7$	378.40	1	7
23	(3R)-8-Hydroxy-7-methoxy-3,5-dimethyl-3,4-dihydroisochromen-1-one	637303	$C_{12}H_{14}O_4$	222.24	1	4
24	Eryvarinol A	637476	$C_{25}H_{26}O_9$	470.50	4	9
25	2,3,3a,9b-Tetrahydro-6-hydroxy-7,8-dimethoxy-2-*n*-propyl-5*H*-furo[3,2-*c*][2]benzopyran-5-one	3533255	$C_{16}H_{20}O_6$	308.33	1	6
26	(5,7-Dihydroxychroman-3-yl) 3,4,5-trihydroxybenzoate	5270719	$C_{16}H_{14}O_8$	334.28	5	8
27	(5,7-Dimethoxychroman-3-yl) 3,4,5-trihydroxybenzoate	5276404	$C_{18}H_{18}O_8$	362.30	3	8
28	Ellagic acid	5281855	$C_{14}H_6O_8$	302.19	4	8
29	2,3,8-Tri-O-methylellagic acid	5281860	$C_{17}H_{12}O_8$	344.30	1	8
30	2,3-Di-O-methylellagic acid	5316858	$C_{16}H_{10}O_8$	330.24	2	8

its antioxidant and anti-inflammatory effects [32]. These results highlight the structural stability of ellagic acid across different sources and reinforce its identification in *B. ciliata*.

Further analysis of the spectral data revealed that each sample contained at least one metabolite that exhibited a similar chemical shift pattern to a known standard compound. Specifically, sample A2 was found to contain 2,3-di-O-methylellagic acid, a polyphenolic compound known for its potent antioxidant and anticancer activities [34]. In sample A3, two major metabolites were identified *viz*. 3,4,8,10-tetrahydroxy-2-(hydroxymethyl)-9-methoxy-3,4a,10b-tetrahydro-2*H*-pyrano[3,2-*c*] and (2*S*,3*R*,4*R*,4a*S*,10b*R*)-3,4,8,9,10-pentahydroxy-2-(hydroxymethyl)-3,4a,10b-tetrahydro-2*H*-pyrano[3,2-*c*]isochromen-6-one. These compounds, being structurally related polyphenolic derivatives, are known for their strong antioxidant, cardioprotective and anti-inflammatory properties [32].

The B2 extract was characterized by the presence of isoaltenuene, a metabolite with significant antimicrobial and antifungal properties. Meanwhile, B3 was found to contain 1(3*H*)-isobenzofuranone, 4,6-dimethoxy-5-hydroxy-, a bioactive compound known for its antimicrobial, antioxidant and anti-inflammatory effects [35]. The identification of these metabolites further supports the therapeutic potential of *B. ciliata* and highlights its role as a natural source of pharmacologically significant compounds.

Conclusion

The present study provides a comprehensive analysis of the phytochemical composition and the functional groups characterization of *Bergenia ciliata* root and rhizome extracts using qualitative screening, ATR-FTIR and NMR spectroscopy. Phytochemical screening revealed the presence of key bioactive compounds including flavonoids, tannins, alkaloids, saponins, steroids, glycosides, terpenoids and phenols, indicating the plant's medicinal potential. The ATR-FTIR spectral analysis confirmed the presence of various functional groups such as hydroxyl (-OH), carbonyl (C=O), amide, alkene, nitrile and halogen compounds, which contribute to the pharmacological properties of *B. ciliata*. NMR spectroscopy further elucidated the chemical structure of metabolites, particularly Bergenin, reinforcing its role as a bioactive constituent. These findings validate the traditional use of *Bergenia ciliata* in herbal medicine and highlight its potential for pharmaceutical applications. The study also demonstrates the reliability of ATR-FTIR and NMR techniques for profiling secondary metabolites in medicinal plants. Overall, this study enhances the understanding of the phytochemistry of *Bergenia ciliata* and provides a scientific basis for its potential applications in drug development and natural product research.

ACKNOWLEDGEMENTS

The authors are highly grateful to the authorities and the R&D division of Shri Guru Ram Rai University, Dehradun for allowing us to carry out experimentation work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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