

# Phytochemicals, Antioxidant and Antimicrobial Potential of Ethnomedicinal Plants of Sikkim Himalaya

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## Singh *et al.*: Biological Activities of Medicinal Plants of Himalaya

In the present study, four important ethnomedicinal plants of Sikkim Himalaya viz., *Astilbe rivularis* Buch-Ham ex. D. Don, *Eupatorium adenophorum* Spreng., *Bergenia ciliata* (Haw.) Sternb and *Artemisia vulgaris* L. were investigated to ensure its ethno pharmacological potency. The methanolic root extracts of the selected plants were investigated for total phenolic content, total flavonoid content, antioxidant and antimicrobial activities. Among the tested extracts, the root methanolic extract of *Bergenia ciliata* contained the highest total phenolic content (911.4±0.124 mg gallic acid equivalents/g extract) and showed exceptional activity in 2,2-diphenyl-1-picrylhydrazyl and 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid assays with half-maximal inhibitory concentration value of 10 µg/ml and 1.0 µg/ml, respectively. The minimum inhibitory concentration of *Bergenia ciliata* root methanolic extract was also found to be the lowest for gram-negative bacteria *Escherichia coli* (600 µg/ml) and actinomycetes *Streptomyces* sp. (800 µg/ml), suggesting high and effective potency against microbes. *Bergenia ciliata* and *Astilbe rivularis* root extracts were further investigated for phenolic compounds using high performance liquid chromatography. The extracts of *Bergenia ciliata* and *Astilbe rivularis* were found to contain substantial amount of bergenin, gallic acid, chlorogenic acid, catechin, p-coumeric and m-coumeric acid. In conclusion, this study gives scientific evidence on the biological activities of the selected plant species and recommends *Bergenia ciliata* and *Astilbe rivularis* as candidate plants for pharmaceutical drug development.

**Key words:** Medicinal plants, antioxidant, antimicrobial, phenolic acid, bergenin, Sikkim Himalaya

Herbal medicine is one of the most important traditional legacies, a pioneer component of the traditional trend being followed all over the world for centuries. In recent years, the use of herbal remedies to treat and cure various ailments has been exponentially growing. Traditional knowledge regarding the use of various medicinal plants in primary healthcare practices is abundant in the Indian Himalayan region. Likewise, Sikkim endows many medicinal plants that are used by the local inhabitants for the cure of diseases such as wound healing, gout, diabetes, arthritis, fever, inflammatory disease, immune modulation, respiratory disease, vaginal problems, antimicrobial, etc.,<sup>[1-3]</sup>. The healing and curing properties of medicinal plants are mainly due to their secondary metabolites such as phenol, terpenoids, alkaloids, etc. Secondary metabolites

accumulate in different parts of the plant such as root, stem, leaves, fruit, seeds and the amount of these metabolites vary from species to species depending on its age or various ecological and climatic factors. Of all the plant secondary metabolites, phenolic and polyphenolic groups of compounds are gaining much more importance with their ability to act as redox elements allowing them to act as reducing agents, hydrogen donors and even oxygen quenchers. They inhibit many oxidizing enzymes and counteract free radicals<sup>[4]</sup>. Recent studies corroborate that

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antioxidant obtained from medicinal plants are effective against free radicals which are the main cause of cancer, diabetes, inflammatory disease, asthma, cardiovascular diseases, neurodegenerative diseases and premature aging<sup>[5,6]</sup>.

In recent years, aboriginal knowledge regarding locally available medicinal plants of Sikkim is being documented enormously, but a huge gap still exists. So far, very limited efforts have been made towards validation of pharmacological properties of medicinal plants. Moreover, medicinal plants quality with respect to bioactive constituents is also not known. Therefore, the present study was aimed to investigate four medicinal plants of Sikkim viz. *Astilbe rivularis* (*A. rivularis*), *Artemisia vulgaris* (*A. vulgaris*), *Bergenia ciliata* (*B. ciliata*) and *Eupatorium adenophorum* (*E. adenophorum*) (Table 1) for their phytochemicals, antioxidant and antimicrobial activities.

## MATERIALS AND METHODS

### Sample collection:

The root samples of four medicinal plants namely, *A. vulgaris*, *E. adenophorum*, *A. rivularis* and *B. ciliata* were collected during the flowering season (March 2014) from the plants growing in the arboretum of G. B. Pant National Institute of Himalayan Environment (GBPNIHE), Sikkim Regional Centre, Gangtok, India (latitude 27° 21' 35.7" N; longitude 88° 37' 24.4" E), located at the elevation of 2047 m above sea level. The plant materials were washed thoroughly under running tap water, followed by distilled water and dried on blotting paper at room temperature. Thereafter, washed materials were dried in an oven at 40°±2° until a constant weight was achieved. The dried plant samples were crushed using a grinder into a fine powder, packaged in a polythene bag and kept

in a cool dark place for further experimentations.

### Chemicals and reagents:

Seven standards namely (+)-catechin, p-coumaric acid, m-coumaric acid, caffeic acid, ferulic acid, gallic acid and bergenin were procured from Sigma Aldrich, India. 2,2-Azinobis-3-Ethylbenzothiazoline-6-Sulphonic Acid (ABTS), Butylatedhydroxytoluene (BHT), chlorogenic acid, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), High Performance Liquid Chromatography (HPLC) grade methanol, quercetin and Folin and Ciocalteu's phenol (FC) reagent were purchased from HiMedia, Pvt. Ltd. (Mumbai, India).

### Extract preparation:

To prepare the plant extract, 2 g of powdered sample was taken in a conical flask and soaked in 10 ml methanol solution for 24 h. The supernatant was transferred into a new tube and the residue was re-extracted twice with 10 ml solvent. Extracts were filtered using Buckner funnel and Whatman filter paper no.1. The accumulated filtrate was concentrated through drying in an oven maintained at 40°. The concentrated extract was suspended in methanol to yield a 50 mg/ml stock solution.

### Determination of Total Phenolic Content (TPC):

TPC was evaluated according to the method of Meda *et al.*<sup>[7]</sup>. Briefly, 100 µl of 5 mg/ml extract was transferred in 2.5 ml of 10-fold diluted FC reagent and after 3 min, 2.0 ml of 7.5 % sodium carbonate solution was added. Reaction mixtures were incubated for 30 min at room temperature and then optical density was measured using Ultra Violet (UV)-spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan) at 760 nm. Gallic acid was used as a standard and TPC of plant extracts was expressed in mg Gallic Acid Equivalents (mg (GAE)/g

**TABLE 1: BOTANICAL NAME/AUTHORITY, FAMILY, TRADITIONAL USES AND REFERENCES OF SELECTED SIKKIM HIMALAYAN MEDICINAL PLANTS USED IN TRADITIONAL MEDICINE**

S. no	Botanical name	Common name	Family	Traditional uses and references
1	<i>Astilbe rivularis</i> Buch-Ham ex. D. Don	Burokhati, Pango	Saxifragaceae	Rhizome is used to treat toothache, body pain, and menstrual disorder. Leaves are used as blood purifier
2	<i>Artemisia vulgaris</i> L.	Titaypati	Asteraceae	Leaf extract used on cuts and bruises to stop bleeding mostly in nose bleeding. It possesses detergent effect and used as cleansing agent.
3	<i>Bergenia ciliata</i> (Haw.) Sternb	Pakhanbed	Saxifragaceae	Rhizome is used to treat fractured bones, fresh cuts, wounds, diarrhoea, pulmonary infections, vomiting, fever, cough and boils by locales
4	<i>Eupatorium adenophorum</i> Spreng.	Banmara, Namnong	Asteraceae	Leaves extract is used in cuts and wounds.

extract).

### Determination of Total Flavonoid Content (TFC):

TFC was estimated by aluminum chloride colorimetric method, with minor modification<sup>[8]</sup>. In brief, the individual test samples were dissolved in methanol and 1 ml sample solution was properly mixed with 1 ml solution of 2 % aluminium chloride methanolic solution. After 10 min of incubation at ambient temperature, the optical density of the solution was measured at 410 nm using a spectrophotometer. TFC was expressed as milligram Quercetin Equivalent (mg QE/g extract).

### Antioxidant Potential:

**DPPH free radical scavenging ability:** DPPH assay was performed according to the method of Sarikurku *et al.*<sup>[9]</sup>. Extract dilutions (1 ml) were added to 0.004 % (4 ml) methanolic solution of DPPH. The optical density of the mixture was measured at 517 nm using a spectrophotometer after 30 min of incubation at room temperature. BHT was used as the standard. The ability of a sample to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \left[ \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \right] \times 100$$

Where, Abs<sub>control</sub> is the absorbance of DPPH radical+methanol; Abs<sub>sample</sub> is the absorbance of DPPH radical+extract/standard. The radical scavenging activity of the extracts was determined by the half maximal Inhibitory Concentration (IC<sub>50</sub>) value. The IC<sub>50</sub> value is the concentration of extracts at which DPPH radicals are scavenged by 50 %. The lowest IC<sub>50</sub> value indicates higher radical scavenging capacity and vice versa.

### Determination of ABTS radical scavenging ability:

The ABTS radical scavenging assay was done by preparing a stock solution in which an equal amount of ABTS (7 mM) and ammonium per sulfate were mixed<sup>[10]</sup>. The working solution was prepared by diluting the above stock solution with methanol until the absorbance at 734 nm was 0.706±0.02. Fresh ABTS solution was prepared for each assay. Medicinal plant extracts (1.5 ml) were allowed to react with the equal amount of ABTS working solution and after 7 min of incubation, the optical density was taken at 734 using the spectrophotometer. The ABTS scavenging capacity was compared with that of BHT.

$$\text{ABTS radical scavenging activity (\%)} = \left[ \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{(\text{Abs}_{\text{control}})} \right] \times 100$$

Where, Abs<sub>control</sub> is the absorbance of ABTS radical+methanol; Abs<sub>sample</sub> is the absorbance of ABTS radical+extract/standard

The radical scavenging activity of the extracts was determined by IC<sub>50</sub> value as mentioned in above DPPH assay.

### Antimicrobial potential:

8 mg (2 g-positive, 2 g-negative bacteria, two actinomycetes and two fungal phytopathogens) were taken from the microbial culture collection established in the microbiology laboratory of GBPNIHE, Almora, India. Methanolic root extracts were used to determine the antimicrobial activity by the disc diffusion method. The test microorganism i.e. bacteria and actinomycetes were grown in tryptone yeast extract broth whereas fungi in potato dextrose broth. 24 h grown culture of bacteria and 5 d grown culture of actinomycetes and fungi were used for antimicrobial assays. For determination of antimicrobial activity against test microorganisms, 100 µl of broth culture was evenly spread on the respective agar medium in petri plates and sterilized 5 mm disc filters were placed over it. 15 µl of root methanolic extract was then poured over each disc. The plates were incubated at 25° for 2 d in case of bacteria and 5 d for actinomycetes/fungi. A solvent control for each test microorganism was also maintained to nullify any inhibition by it. Following incubation, the zone around the disc was calculated for each test microorganism. All the experiments were performed at least in triplicates and the mean value was calculated.

Minimum Inhibitory Concentration (MIC) of extracts against test microorganisms was determined by following standard protocol of clinical and laboratory standard institute<sup>[11,12]</sup>.

### HPLC analysis for bioactive compounds:

Eight bioactive compounds *viz.* chlorogenic acid, (+)-catechin, p-coumaric acid, m-coumaric acid, caffeic acid, ferulic acid, gallic acid and bergenin detection and quantification were done using reverse-phase HPLC (Shimadzu, LC-2030 Plus) equipped with Prominence Diode Array Detector. The separation was carried out using a C<sub>18</sub> column (Shimadzu-pack solar, 5 µm, 4.6×250 mm). The mobile phase used for the determination of chlorogenic acid, (+)-catechin, p-coumaric acid, m-coumaric acid, caffeic acid, ferulic acid and gallic acid was methanol (A) and 0.1 % ortho-phosphoric acid in water (B) in 40:60 with a flow rate of 1 ml/min for the total run time of 20 min. A stock

solution of 1 mg/ml for each phenolic acid was prepared in methanol. The stock (1 mg/ml) of each known compound was diluted to different concentrations for the preparation of calibration curves. The curve linearity for each standard was determined based on the correlation coefficient. Identification and quantification of the compound were done based on retention time and absorption wavelength spectra profile of each standard.

For the detection and quantification of bergenin, methanol in isocratic mode was used with a flow rate of 0.5 ml/min and injection volume was 20 µl. The detection wavelength for bergenin was 272 nm.

### Statistical analysis:

The investigated parameters, except phenolic acid quantification and MIC, were analyzed for Analysis of Variance (ANOVA) and significance was determined at  $p < 0.05$  using Statistical Package for the Social Sciences (SPSS) (version 16) software. The significant differences among the mean values were assessed based on Duncan's multiple range tests. The mean and standard deviation were calculated using MS-Excel. Each experiment was repeated thrice.

## RESULTS AND DISCUSSION

The content of the extractable phenolic compounds was deduced through a linear gallic acid standard curve ( $y = 2.468x + 0.088$ ;  $R^2 = 0.996$ ). In the investigated root extracts, TPC was detected in the following decreasing order: *B. ciliata* > *A. rivularis* > *A. vulgaris* > *E. adenophorum*. The highest TPC ( $911.4 \pm 0.124$  mg GAE/g extract) was detected in *B. ciliata* extract as shown in fig. 1.

TFC was determined through a linear quercetin standard curve ( $y = 28.47x + 0.103$ ;  $R^2 = 0.998$ ). TFC of the studied extracts was found in the following order: *A. vulgaris* > *E. adenophorum* > *B. ciliata* > *A. rivularis*. The highest TFC of  $151.6 \pm 1.1$  mg QE/g extract was observed in *A. vulgaris* extract whereas the lowest TFC was detected in *A. rivularis* extract as shown in fig. 1.

TPC results indicated that among all the studied four plants, the roots of Saxifragaceae family plants contained comparatively higher amount of phenolic compounds than the roots of Asteraceae family plants. Results of flavonoid analysis revealed that root is a reservoir of flavanoids in Asteraceae plants whereas in Saxifragaceae plants root contain comparatively less amount of flavonoids. Earlier, researchers have reported that secondary metabolite accumulation in the medicinal plant is cell and organ-specific and it varies

from plant to plant<sup>[13]</sup>.

Previous investigations in *Bergenia* species showed that *Bergenia* contained simple phenols, flavonoids, coumarins and tannins making it one of the most potent therapeutic sources which could be used for drug development by pharmaceutical industries in future<sup>[14-16]</sup>. Likewise, in our study, the root methanolic extract of *B. ciliata* estimated a high amount of phenolic compounds. Another potent medicinal plant *A. rivularis* has also exhibited high phenolic content in its root. Other previous studies that have analyzed the total phenolic compounds present in *A. rivularis* came to the same conclusion depicting high phenolic compounds in the plant which has led to its increased therapeutic potential<sup>[17]</sup>. All the studied plant species contained a fairly good amount of phenol and flavonoid content, therefore, providing an insight into its pharmacological activity and traditional use.

The antioxidant capacities of methanolic extracts of selected plant species were determined using two assays viz. DPPH and ABTS which are based on different reaction mechanisms. The results of the antioxidant assays revealed that the selected plant extracts possessed a significant variability in their inhibitory activity against DPPH and ABTS radicals. In both the assays, *B. ciliata* and *A. rivularis* root methanolic extracts showed high radical scavenging activity with DPPH  $IC_{50}$  values of 10.8 µg/ml and 20.0 µg/ml respectively and ABTS  $IC_{50}$  values of 1.0 and 2.0 µg/ml, respectively (fig. 2). The plant extracts which contained higher TPC such as *B. ciliata* and *A. rivularis* exhibited better antioxidant activity. *A. vulgaris* root methanolic extract also exhibited good antioxidant activity which could be due to higher flavonoid content ( $151.6 \pm 2.88$  mg QE/g extract). Recently, Subba *et al.*<sup>[18]</sup> reported antioxidant activity (DPPH  $IC_{50}$  value  $32.05 \pm 2.59$  µg/ml) of the methanolic root extract of *A. vulgaris* from the Nepal Himalaya.

Correlation analysis of TPC and TFC with DPPH and ABTS assays revealed that there is a significant correlation between the TPC and antioxidant assays (Table 2). The negative significant correlation was found between TPC and ABTS ( $R^2 = -0.97$ ,  $p < 0.05$ ) which suggests that TPC contribution in antioxidant property of the plant is higher than TFC. These results are in well agreement with Kainama *et al.*<sup>[19]</sup> work. Moreover, it is well established that antioxidant compounds such as phenolics and flavanoids exert their effect through different mechanism which in turn depend on their structure and functional groups.



Due to the detrimental effects of synthetic antioxidants such as Butylate hydroxyanisole and BHT, traditional medicinal plants are extensively being investigated worldwide in search of novel plant-derived antioxidant compounds. In this study, the root methanolic extracts of *B. ciliata* and *A. rivularis* have shown better antioxidant activity than the synthetic antioxidant BHT. These results suggest that natural antioxidants provided by these plants could be used to develop safe drugs with no side effects. Earlier studies on the phytochemical screening and antioxidant activity of *B. ciliata* and *A. rivularis* have always shown them to comprise high phenolic compounds and subsequently high antioxidant activity making them highly ethnopharmacologically important<sup>[16,17,20]</sup>.

The antimicrobial activity of studied plants (i.e. *B. ciliata*, *A. vulgaris*, *E. adenophorum* and *A. rivularis*) varied with respect to the test organisms. Generally, bacteria and actinomycetes were found more sensitive to antimicrobial compounds present in methanolic extracts, whereas fungus did not show any zone of inhibition (Table 3). Out of the four plant extracts, *E. adenophorum* extract showed maximum zone of inhibition against *Bacillus megaterium* (*B. megaterium*) (4.17 mm) followed by *B. ciliata* (4.00 mm) and *A. rivularis* (3.83). *A. vulgaris* extract showed no activity against *B. megaterium*. Similarly, maximum inhibition against *Bacillus subtilis* (*B. subtilis*) was observed by root extract of *E. adenophorum* (6.83 mm) which was followed by *B. ciliata*, *A. rivularis* and *A. vulgaris* extracts. In the case of Gram-negative bacteria, extracts of *B. ciliata* and *E. adenophorum* showed maximum inhibition against *Serratia marcescens* and *Escherichia coli* (*E. coli*), respectively. Root extracts of all selected

species showed no activity against *Nocardia tenerifensis*, whereas *Streptomyces* sp. was inhibited maximum by root extract of *A. vulgaris*. The antimicrobial activity of medicinal plant extracts are shown in fig. 3. MIC was determined for studied plant root extracts. *B. ciliata* and *A. rivularis* showed MIC of 1000 µg/ml and 1800 µg/ml against *B. megaterium* and *Serratia marcescens*, respectively. In case of *B. subtilis*, *A. rivularis* showed the lowest MIC 600 µg/ml. *B. ciliata* showed 600 µg/ml MIC for *E. coli*. *B. ciliata* exhibited a MIC of 800 µg/ml against *Streptomyces* sp. Results of MIC are given in Table 4. Recently, Khan *et al.*<sup>[21]</sup> noted *B. ciliata* root extract antibacterial activity against both gram-positive (*B. subtilis* and *Bacillus atrophoeus*) and gram-negative (*Klebsiella pneumonia* and *Pseudomonas aeruginosa*) bacteria with no antifungal activity. The methanolic leaf extract of *A. rivularis* had also been reported of having good antibacterial activity against gram-positive *B. subtilis*, *B. megaterium*, *Streptococcus pyogenes* and *Staphylococcus aureus* as well as gram-negative bacteria like *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysenteriae* with MIC value ranged from 8-64 µg/ml against the tested bacterial pathogen<sup>[22]</sup>. Rai *et al.*<sup>[23]</sup> reported antibacterial activity of the rhizome extract of *A. rivularis* against both gram-positive and gram-negative bacterial pathogens such as *B. subtilis*, *Bacillus amyloliquefaciens*, *Aeromonas liquefaciens*, *Flexibactor* sp., and *Pseudomonas* sp. Adhikary *et al.*<sup>[24]</sup> reported antimicrobial activity of rhizome methanolic extract of *A. rivularis* against *E. coli* with MIC of 0.011 mg/ml. As literature indicates, this is the first report on antimicrobial activity of *A. rivularis* from Sikkim Himalaya. Moreover, so far, very scant information on antimicrobial property of root extracts of selected plants is available.

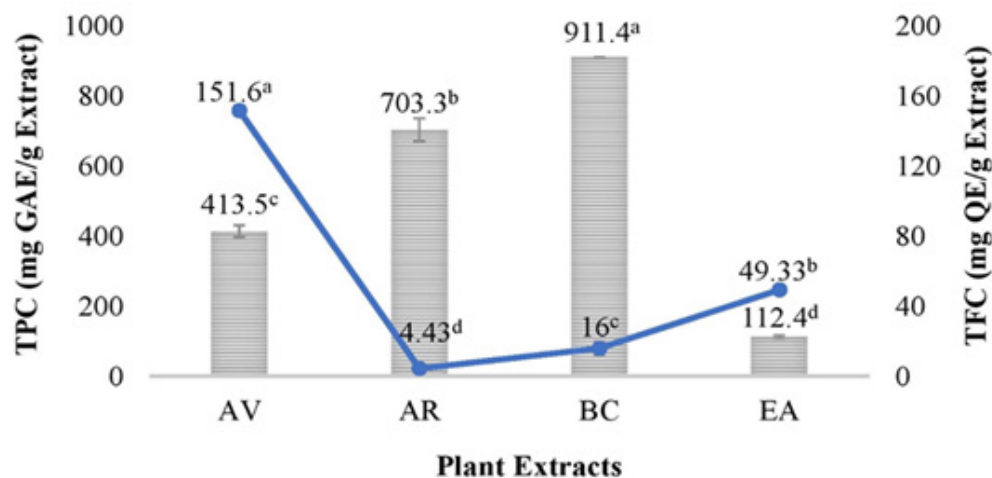


Fig. 1: Total phenolic and flavonoid contents of root methanolic extracts of selected medicinal plants

Note: Values are the mean±standard deviation of triplicate experiments. Different letter in a column shows significant differences at  $p < 0.05$ . AV: *Artemisia vulgaris*; AR: *Astilbe rivularis*; BC: *Bergenia ciliata*; EA: *Eupatorium adenophorum*; (■): TPC and (●): TFC

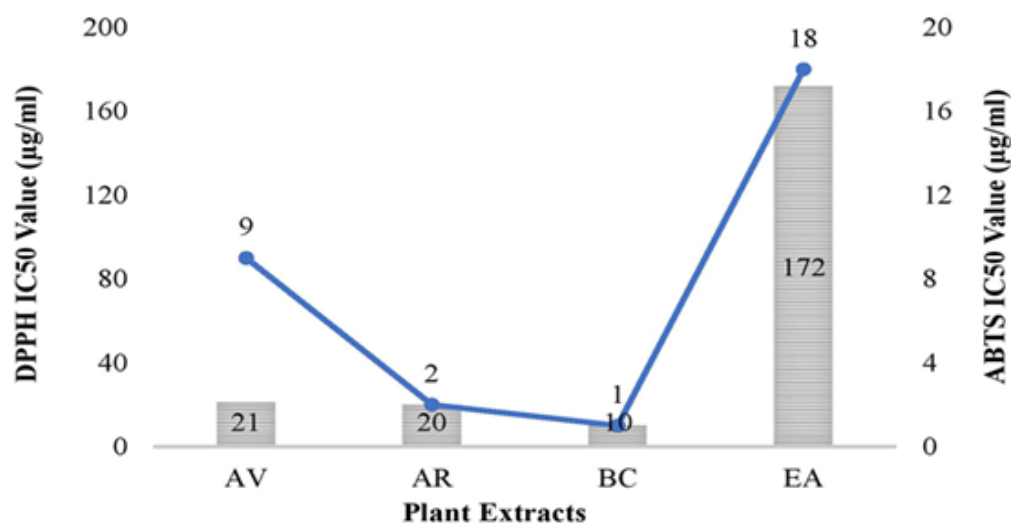


Fig. 2: Radical scavenging activity of root methanolic extracts of selected medicinal plants. Note: Values are the mean±standard deviation of triplicate experiments. AV: *Artemisia vulgaris*; AR: *Astilbe rivularis*; BC: *Bergenia ciliata*; EA: *Eupatorium adenophorum*; (■): DPPH and (●): ABTS

**TABLE 2: CORRELATIONS COEFFICIENT (R2) BETWEEN THE IC50 VALUES OF ANTIOXIDANT ASSAYS AND TOTAL PHENOLIC AND FLAVONOIDS CONTENT**

Antioxidant assays	TPC	TFC
ABTS	-0.97475*	0.391268
DPPH	-0.84042	-0.02718

Note: \*Correlation significance at  $p < 0.05$

**TABLE 3: ANTIMICROBIAL ACTIVITY OF ROOT EXTRACTS AGAINST TEST MICROORGANISMS BY DISC DIFFUSION METHOD**

Microorganisms	Zone around disc (mm)			
	BC	AV	EA	AR
Gram-positive bacteria	0.59±0.07ab	0.59±0.07ab	0.59±0.07ab	0.59±0.07ab
<i>Bacillus megaterium</i>	4.00±0.00	NA	4.17±0.16	3.83±0.16
<i>Bacillus subtilis</i>	6.17±0.16	2.67±0.16	6.83±0.43	5.17±0.16
Gram-negative bacteria	0.59±0.07ab	0.59±0.07ab	0.59±0.07ab	0.59±0.07ab
<i>Escherichia coli</i>	2.17±0.43	1.00±0.28	4.00±0.00	0.67±0.32
<i>Serratia marcescens</i>	3.5±0.00	1.00±0.30	3.00±0.30	3.20±0.30
Actinomycetes	0.59±0.07ab	0.59±0.07ab	0.59±0.07ab	0.59±0.07ab
<i>Nocardia tenerifensis</i>	NA	NA	NA	NA
<i>Streptomyces</i> sp.	3.33±0.16	4.17±0.16	2.33±0.16	3.67±0.16
Fungi	0.59±0.07ab	0.59±0.07ab	0.59±0.07ab	0.59±0.07ab
<i>Fusarium oxysporium</i>	NA	NA	NA	NA
<i>Fusarium solani</i>	NA	NA	NA	NA



Fig. 3: Antimicrobial activity of different medicinal plants roots methanolic extract in (a): *Bacillus megaterium*; (b): *Bacillus subtilis*; (c): *Escherichia coli*; (d): *Serratia marcescens* and (e): *Streptomyces sp.*

Note: (BCR: *Bergenia ciliata* root; AR: *Artemisia vulgaris* root; EAR: *Eupatorium adenophorum* root; AAR: *Astilbe rivularis* root)

TABLE 4: MIC OF PLANT ROOTS EXTRACTS AGAINST TEST MICROORGANISMS

Microorganisms	MIC ( $\mu\text{g/ml}$ )			
	BC	AV	EA	AR
Gram positive bacteria	100	100	100	100
<i>Bacillus megaterium</i>	1000	-	1800	1000
<i>B. subtilis</i>	800	1200	800	600
Gram negative bacteria	100	100	100	100
<i>Escherichia coli</i>	600	800	1000	1000
<i>Serratia marcescens</i>	1800	2000	2000	1800
Actinomycetes	100	100	100	100
<i>Streptomyces sp.</i>	800	2000	1600	1400

As *A. rivularis* and *B. ciliata* root extracts had shown excellent antioxidant and antimicrobial activities, these extracts were further selected for quantification of phenolic compounds namely chlorogenic acid, (+)-catechin, p-coumaric acid, m-coumaric acid, caffeic acid, ferulic acid, gallic acid and bergenin (fig. 4). Phenolic compounds were detected and identified according to their maximum absorption spectra, retention time and the coefficients of determination ( $R^2$ ) of standard compounds (Table 5). The phenolic compounds (chlorogenic acid, (+)-catechin, p-coumaric acid, m-coumaric acid, caffeic acid, ferulic acid and gallic acid) in the root extract of *A. rivularis* in decreasing order were catechin ( $46.8 \pm 8.1$  mg/100 g EW), gallic acid ( $36 \pm 0.3$  mg/100 g EW), p-coumaric acid ( $35 \pm 0.0$  mg/100 g EW), chlorogenic acid ( $21 \pm 0.1$  mg/100 g EW) and m-coumaric acid ( $20 \pm 0.0$  mg/100 g EW). Ferulic acid and caffeic acid could not be detected in *A. rivularis* extract. Earlier, the preliminary investigation of *A. rivularis* rhizome extract had revealed the presence of alkaloids, flavonoids,

coumarins and glycosides<sup>[25]</sup>. The present study, for the first time, reported presence of bioactive compounds such as catechin, gallic acid, p-coumaric, m-coumaric acid and chlorogenic acid in *A. rivularis* root extract. Hori *et al.*<sup>[26]</sup> isolated seven potent antioxidant constituents i.e. bergenin, 11-ogalloylbergenin, (+)-catechin, (-)-catechin, (-)-afzelechin, (-)-epiafzelechin and 2-( $\beta$ -D-glucopyranosyloxy)-4-hydroxybenzenacetonitrile from the rhizome of *A. rivularis*. Earlier, GC-MS analysis revealed the presence of terpenoids and fatty acids in the methanolic rhizome extract of *A. rivularis*<sup>[22]</sup>.

The decreasing order of phenolic compounds in the methanolic root extract of *B. ciliata* was recorded as gallic acid ( $162 \pm 7.9$  mg/100 g EW), chlorogenic acid ( $149 \pm 0.1$  mg/100 g EW), catechin ( $137 \pm 2.2$  mg/100 g EW), p-coumaric acid ( $37 \pm 0.0$  mg/100 g EW), ferulic acid ( $24 \pm 0.0$  mg/100 g EW) and m-coumaric acid ( $22 \pm 0.1$  mg/100 g EW).

In spite of high medicinal value and frequent use in

traditional medicinal practices, so far, there are very limited reports on the quantification of phenolic acids in *B. ciliata*. Phenolic compounds like bergenin, arbutin, gallic acid and catechin had been reported since decades in *B. ciliata*<sup>[16,27-30]</sup> but efforts to investigate other phenolic compounds such as chlorogenic acid, p-coumaric acid, ferulic acid and m-coumaric acid have never been made.

Bergenin, a C-glucoside of 4-O-methyl gallic acid, is reported in many species such as *B. crassifolia*, *B. cordifolia*<sup>[29]</sup>, *B. ciliata*, *Bergenia ligulata* (*B. ligulata*)<sup>[31]</sup>, *Corylopsis spicata*, *Caesalpinia digyna*, *Mallotus japonicas*, *Sacaoglottis gabonensis*<sup>[32]</sup> and *A. rivularis*<sup>[25]</sup>. In the present study, bergenin quantification was done in the methanolic root extract of *B. ciliata* and *A. rivularis* (fig. 4). HPLC analysis results showed that the roots of both the species contain substantial amount of bergenin (fig. 5). *B. ciliata* root extract accumulated comparatively higher amount of bergenin i.e. 459±1.4 mg/100 g EW than *A. rivularis* (98±2.8 mg/100 g EW). Boros *et al.*<sup>[29]</sup> quantified bergenin in two *Bergenia* species in three different sampling series and content ranged from 0.32±0.01-5.34±0.03 mg/g in *Bergenia crassifolia* and 0.20±0.01-4.96±0.02 mg/g in *B. ligulata*. Srivastava *et al.*<sup>[33]</sup> reported bergenin in three different species of *Bergenia* namely *B. ligulata*, *B. ciliata*, and *Bergenia stracheyi* as 2.42 %, 3.28 %, and 3.28 %. Similarly, these species were also evaluated by Rajbhandari *et al.*<sup>[34]</sup> for bergenin content and quantified as *B. ligulate* (5.73 %), *B. ciliata* (5.63 %) and *Bergenia stracheyi* (5.99 %). Bergenin and

bergenin-like derivatives have been isolated from the rhizome and aerial parts of *A. rivularis*<sup>[35]</sup>. Other species of Astilbe were also reported for bergenin including *A. chinensis*<sup>[36-38]</sup>, *A. myriantha*<sup>[39]</sup> and *A. thunbergii*<sup>[40]</sup>.

As the literature indicates that bergenin have potential pharmaceutical application including antihepatotoxic, antiulcerogenic, anti-human immunodeficiency virus, neuroprotective, anti-inflammatory, immunomodulatory<sup>[41]</sup>, hepatoprotective<sup>[42]</sup> and also various biological activities such as antiulcer, antifungal and wound healing effect<sup>[43,44]</sup>. Therefore, presence of significant amount of bergenin and other phenolic compounds in *B. ciliata* and *A. rivularis* root extract might be responsible for the antimicrobial activity against both gram-positive and gram-negative bacterial pathogen and also for their antioxidant properties.

The present study investigated the four ethnomedicinal plants of Sikkim Himalaya viz. *A. rivularis*, *A. vulgaris*, *B. ciliata* and *E. adenophorum* for phytochemicals, antioxidant and antimicrobial activities. Among the selected species, *B. ciliata* and *A. rivularis* exhibited potent antimicrobial activity and antioxidant property. Further, HPLC analysis confirmed that *B. ciliata* and *A. rivularis* roots are reservoir of phenolic compounds. The high contents of phenolic compounds including bergenin in root extracts of both the studied species might be responsible for their biological activities. In the light of these results, roots of *A. rivularis* and *B. ciliata* are anticipated as prospective source of novel drugs.

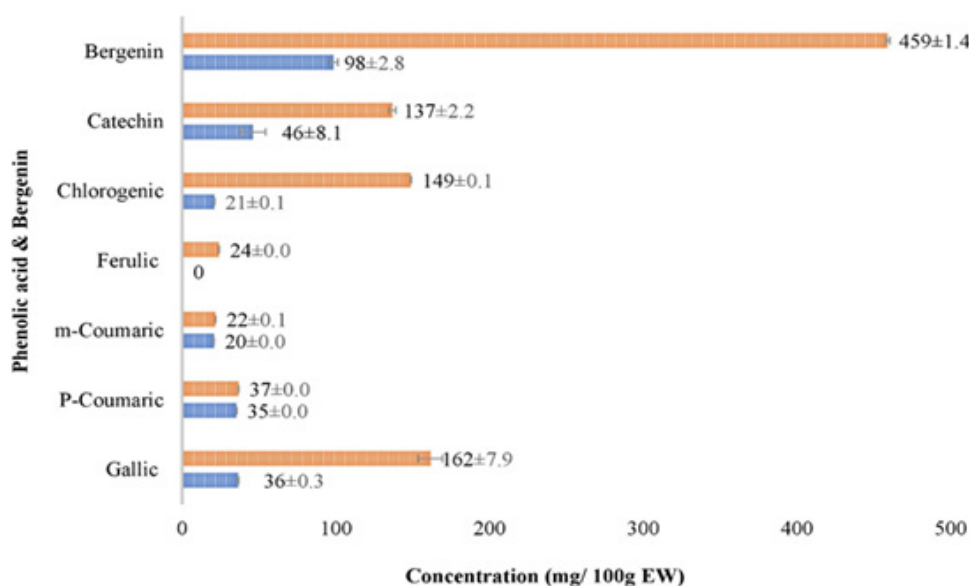


Fig. 4: Quantification of different phenolic compounds in methanolic root extracts of selected medicinal plants

Note: ARR: *Astilbe rivularis* root; BCR: *Bergenia ciliata* root; (■): BCR and (■): ARR



TABLE 5: STANDARD CURVE ANALYSIS FOR QUANTIFICATION OF BIOACTIVE COMPOUNDS

Compounds	Retention time (min)	Wavelength (nm)	Standard equation	R2
Gallic acid	3.79	278	$y=31527x+-2030$	0.999
p-Coumeric	12.38	309	$y=112389x-279726$	0.995
m-Coumeric	15.47	278	$y=22095x-7454$	0.998
Ferulic acid	13.53	325	$y=64147x-8782$	0.994
Chlorogenic acid	5.51	325	$y=46335x-12687$	0.998
Caffeic acid	7.73	325	$y=66668x-2270$	1
Catechin	3.79	216	$y=86202x-4672$	0.999
Bergenin	5.67	272	$y=21313x+25411$	0.988

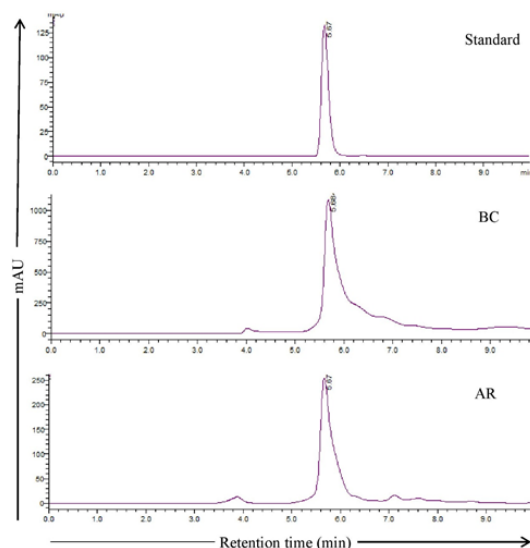


Fig. 5: Chromatogram of bergenin content in methanolic root extracts of selected medicinal plants

Note: AR: *Astilbe rivularis* and BC: *Bergenia ciliata*

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The authors declared no conflict of interests.

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