

Antioxidant Activity, Total Phenolic and Flavonoid Content of *Berberis aristata* DC. and *Berberis thomsoniana* C.K. Schneid. from Sagarmatha National Park, Nepal

Lok Ranjan Bhatt*, Bina Wagle, Minu Adhikari, Santoshi Bhusal, Anjana Giri, Shandesh Bhattarai

ABSTRACT

Background: Medicinal plants are the major sources of the crude drugs and natural antioxidants. *Berberis* species have been used in the treatment of various ailments around the world including Nepal. However, systematic study on phytochemistry and pharmacology of *Berberis* species from Nepal is lacking. **Objectives:** To evaluate the antioxidant activity, total phenolic and flavonoid contents of *Berberis aristata* and *Berberis thomsoniana* from Sagarmatha National Park. **Materials and Methods:** Antioxidant activity was measured through 2, 2-Diphenyl-1-Picrylhydrazyl assay. Total phenolic and flavonoid content were estimated using Folin-Ciocalteu and aluminum chloride method respectively. **Results:** Samples showed dose-dependent radical scavenging activity. Radical scavenging activity of the methanolic extracts of different parts of *B. aristata* and *B. thomsoniana* ranged from 19.38 to 98.47%, with leaf extracts of *B. thomsoniana* showing the strongest activity. The total phenolic content of the samples varied from 11.04 to 65.30 mg GAE g⁻¹ dry weight whereas total flavonoid content was in between 2.4 to 16.46 mg quercetin/g dry weight. **Conclusion:** Among the tested samples, leaf extracts of *B. thomsoniana* showed the strongest antioxidant activity and contained the highest amount of total phenolic and flavonoid content.

Key words: *Berberis*, Antioxidant activity, Phenolic content, Flavonoid content, Nepal.

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INTRODUCTION

In recent years there have been growing interests among the researchers for the search of new antioxidant molecules with promising activity. Medicinal plants are rich sources of a wide variety of natural antioxidants and exhibit better antioxidant activity than common fruits and vegetables.¹⁻² In recent years, natural antioxidants from medicinal plants have attracted greater interest of researchers and consumers because of their ability to scavenge free radicals³ and being safer than synthetic antioxidants. Free radicals are thought to be responsible in the development of a number of disorders, including cancer, neuro degeneration and inflammation.⁴⁻⁶

Berberis aristata and *Berberis thomsoniana* belong to the family Berberidaceae and are known as Chutro in Nepali. There are 27 species of *Berberis* reported from Nepal.⁷ *Berberis aristata* is distributed between 1300-3400 m and *Berberis thomsoniana* from 3000-3650 m throughout Nepal Himalaya.⁸ *Berberis* species are important medicinal plants of Nepal and have been used to cure various ailments in traditional systems of medicine.⁹⁻¹² *Berberis aristata* is used in skin diseases, diarrhea, dysentery, jaundice, fevers, eye diseases, piles, ulcers, sores and conjunctivitis, stomachache, inflam-

mation, gastric disorders, wound healing, liver and spleen disorders.¹³⁻²² However, little is known about the traditional use, pharmacology and phytochemistry of *Berberis thomsoniana*.

Several compounds such as alkaloids, terpenoids, flavonoids, sterols, anthocyanins, lignans, vitamins, proteins, lipids and carotenoids have been isolated, but alkaloids are the main bioactive chemical constituents of *Berberis* species.²³ Various pharmacological activities such as antipyretic, antimicrobial, hepatoprotective, wounds healing, antihyperglycemic, antitumor, anticancer, antioxidant and several classes of secondary metabolites (alkaloids, flavonoids, phenolic acids etc.) have been reported from *B. aristata*.²⁴

However, only a few pharmacological studies^{22,25} have been conducted and many works have emphasized on the diversity and traditional uses of *Berberis* species from different parts of country.^{13,16,21,26} Little information is available on antioxidant activity, total phenolic and flavonoid contents of *Berberis* species from Nepal. Hence, the present study was conducted to evaluate the antioxidant activity, total phenolic and flavonoid contents of two Nepalese *Berberis*.

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MATERIALS AND METHODS

Collection and identification of plant materials

Plant specimens were collected from Namche, Thame and adjoining areas of the Sagarmatha National Park in May, 2016. Duplicate specimens were collected for the herbarium preparation. The samples were collected by one of us (LR Bhatt), identified by Dr. Bhaskar Adhikari, Botanist at the Royal Botanical Garden Edinburgh-UK and confirmations were done through the available literature.^{7-8,26} Voucher specimens (*B. aristata* BRU 036, *B. thomsoniana* BRU 040) are deposited at Biological Resources unit, Nepal Academy of Science and Technology.

About 500 g of the healthy plant parts (leaf, stem, bark) of each species were collected. The collected samples were packed in the cotton bags and air dried for several days. The air-dried samples were finely powdered using laboratory blender and kept in zip locked bags until further analysis.

Extraction of the samples

One gram dried and powdered sample of each plant was extracted in 20 mL of methanol in shaking incubator (100 RPM) at 37°C for 24 h. The obtained solution was filtered and residue extracted again following the same procedure and filtered. Final volume of organic solution was maintained to 40 mL with the addition of methanol and kept in refrigerator until further analysis.

Antioxidant activity

Antioxidant activity of samples and ascorbic acid (standard) was estimated using DPPH (2, 2-Diphenyl-1-Picrylhydrazyl) as free radical²⁷ with slight modifications.²⁸ DPPH assay is routinely employed for the determination of free radical scavenging activity of plant extracts and is one of the standard and easy colorimetric methods for the evaluation of antioxidant properties of pure compounds.²⁹⁻³⁰ In this test, the antioxidants reduce the DPPH radical to a yellow colored compound and the extent of the reaction depends on the hydrogen donating capacity of the antioxidants.³¹ Different concentrations of sample parts and ascorbic acid were prepared in methanol. Samples and standard solutions were mixed with 0.3 mM DPPH into 96 well plates in 1:1 ratio. The mixture after gentle shaking was incubated at room temperature in dark for about 30 minutes. The absorbance was measured at 517 nm using Epoch 2 ELISA plate reader and the percentage DPPH radical scavenging activity of the samples was determined using following formula.

$$\text{DPPH Radical Scavenging Activity (\%)} = \frac{[(Ac - As) \times 100]}{Ac}$$

Where, Ac and As are absorbances of control and sample respectively.

Total phenolic content

The Folin-Ciocalteu reagent assay was used to determine the Total phenolic content (TPC) with slight modifications.³² An aliquot of the samples (30µL) was introduced into 96 well ELISA plate followed by 150 µL of ten times diluted Folin-Ciocalteu reagent and 120 µL of 7.5 % sodium carbonate. Well plate was incubated for half an hour in dark and absorbance at 765 nm was recorded in an ELISA plate reader. Gallic acid was used as a standard and the results were expressed in mg/g of gallic acid equivalent.

Determination of total flavonoid content

Total flavonoid content (TFC) of the extracts was determined as described previously.³³ About 100 µL of appropriate concentration of sample was added to 96 well ELISA plates. Then 100µL of 2% Aluminum chloride solution was added to it and the reaction mixture was incubated at room temperature for about an hour. After incubation, the absorbance of the solution was measured at 450 nm. Quercetin was used to plot

calibration curve and TFC of the sample was expressed as mg Quercetin equivalents per gram of extract.

Statistical analysis

The data were average of triplicate measurements. Microsoft excel was used to compute means, standard deviation and regression.

RESULTS

In the present study, antioxidant activity of the extracts of various plant parts of *Berberis aristata* and *Berberis thomsoniana* was determined using DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay.

Samples exhibited dose-dependent radical scavenging activity (Table 1). Among the samples, leaf extract of *B. thomsoniana* showed the strongest activity at lowest tested concentration. It exhibited more than 98% radical scavenging activity at the concentration of 0.625 mg/g. However, there was slight decrease in antioxidant activity on increasing the sample concentration. It seems that 0.625 mg/g would be optimum concentration of the sample for this assay and there might not be enough free radicals to neutralize the higher concentration of sample. It was followed by the stem and bark extracts of *B. aristata* (Table 1). However, at higher concentration all the samples exhibited similar radical scavenging activity. Ascorbic acid, a natural antioxidant and positive control showed 96% radical scavenging activity at 100 µg/mL concentration.

All the tested samples contained different amount of phenolics. The total phenolic content varied from 11.04 to 65.30 mg GAE g⁻¹ dry weight of the sample (Table 2). Leaf extract of *B. thomsoniana* contained the highest phenolic content followed by leaf extract of *B. aristata*. Stem extract of *B. thomsoniana* contained the least amount of total phenolics.

There is large variation in the total flavonoid content of the plant samples, which ranges from 2.4 to 16.46 mg quercetin/g dry weight (Table 2). Among the tested samples, leaf extract of *B. thomsoniana* had the highest amounts of flavonoids. It was followed by leaf extract of *B. aristata*.

DISCUSSION

Antioxidant activity, total phenolic and flavonoid content of leaf, stem and bark extracts of *B. aristata* and *B. thomsoniana* was determined. Considerable amount of variation was observed among the samples regarding antioxidant ability, total phenolic and flavonoid contents. Among the tested samples, leaf extract of *B. thomsoniana* exhibited highest antioxidant activity. However, with increasing concentration, there was decrease in radical scavenging activity (RSA). It is likely that 0.625 mg/g concentration of the sample is quite enough to quench the available DPPH radicals. Unavailability of DPPH radicals would have contributed in decreasing the RSA of *B. thomsoniana* leaf extract on increasing the sample concentration.

On analyzing the total phenolic and flavonoid content of the samples, leaf extracts of both the plants possessed the higher content of total phenolic and flavonoid whereas the bark extracts of *B. aristata* contained the least amount of phenol. Likewise stem and bark extract of *B. thomsoniana* were the poorest in total flavonoid content. Based on previous research there is variation in amount of phenolic and flavonoid content in different parts of the plant.³⁴⁻³⁵

Strong DPPH radical scavenging activity of *Berberis* species was reported from the North-West Himalayan Region of India.³⁶⁻³⁷ While comparing different plant parts of *Berberis* species, higher antioxidant activity and greater amount of phenols and flavonoids in leaves of *B. vulgaris* and *B. croatica* was reported.³⁸ The antioxidant activity varied mostly in relation to the plant parts while statistically no significant differences were found between *B. vulgaris* and *B. croatica*.³⁸ Similarly, the amount of total phenolics of root extract of *Berberis aristata* from western Indian

Table 1: Antioxidant activity of *B. aristata* and *B. thomsoniana* by DPPH assay

Plant name	Parts used	% DPPH radical scavenging activity of sample at different concentrations (†)				
		0.625 mg/g	1.25 mg/g	2.5 mg/g	5 mg/g	10 mg/gm
<i>B. aristata</i>	Leaf	19.38±0.51	38.72±2.51	70.24±2.66	95.42±0.70	94.42±0.20
<i>B. aristata</i>	Stem	80.53±1.52	93.74±0.94	97.37±0.64	97.50±0.98	96.04±0.79
<i>B. aristata</i>	Bark	59.10±0.85	71.02±3.33	75.66±1.47	93.96±0.17	96.92±0.39
<i>B. thomsoniana</i>	Leaf	98.47±0.35	97.83±0.26	97.31±0.25	95.33±0.50	93.18±1.07
<i>B. thomsoniana</i>	Stem	53.36±2.11	67.80±1.30	91.59±0.06	96.43±2.73	96.66±0.28
<i>B. thomsoniana</i>	Bark	54.56±2.49	70.07±0.79	87.18 ±0.56	95.75± 1.30	97.468±0.17
% DPPH radical scavenging activity of ascorbic acid						
Concentration (µg/mL)	10	20	40	60	80	100
% RSA	59.05±0.90	64.48±0.23	73.23±0.89	79.45±0.60	89.14±3.99	95.66±2.88

-Values are means of triplicate measurements ± standard deviation; †concentration is expressed in mg/g dry weight of sample.

Table 2: Total phenolic and flavonoid content in *B. aristata* and *B. thomsoniana*.

Plant name	Part used	Total phenolic content	Total flavonoid content
<i>B. aristata</i>	Leaf	50.44±8.10	7.82±0.78
<i>B. aristata</i>	Stem	17.70±2.97	3.03±0.32
<i>B. aristata</i>	Bark	11.04±2.20	6.08±0.50
<i>B. thomsoniana</i>	Leaf	65.30±10.72	16.46±0.30
<i>B. thomsoniana</i>	Stem	13.04±1.86	2.38±0.03
<i>B. thomsoniana</i>	Bark	13.73±1.65	2.40±0.10

-Values are expressed as the average of triplicate measurements ± standard deviation; TPC and TFC is expressed in mg/g dry wt. of sample

Himalayas was found quite low and exhibited poor antiradical efficiency.³⁹ Stem extract of *Berberis lycium* Royle showed medium radical scavenging activity and contained moderate amount of total phenols and flavonoids.⁴⁰ Other species of *Berberis* also possess good amount of phenolics.^{38,41}

Among the tested samples leaf extract of *B. thomsoniana* exhibited the highest antioxidant ability and contained higher amount of phenols and flavonoid. It should be further evaluated using multi model antioxidant assays to know its actual antioxidant potential. Similarly, future studies needs to be focused on isolation and purification of active constituents from it.

CONCLUSION

In the present study extracts from different parts of *B. aristata* and *B. Thomsoniana* showed concentration dependent antioxidant activity. Among the samples, leaf extract of *B. thomsoniana* showed strong antioxidant activity and possess higher amount of total phenols and flavonoids. There is no previous report on the traditional use, phytochemistry and pharmacology of *B. thomsoniana*; further research on this plant should be carried out. The results revealed that extracts of both the plants possess substantial amount of phenols and flavonoids and exhibited strong DPPH radical scavenging activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

DPPH: 2, 2-Diphenyl-1-Picrylhydrazyl; **ELISA:** Enzyme-linked immunosorbent assay; **TPC:** Total phenolic content; **TFC:**Total flavonoid content.

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GRAPHICAL ABSTRACT



SUMMARY

- Present study showed that leaf extract of *B. thomsoniana* showed promising antioxidant ability and contained higher amount of phenolics and flavonoids.

ABOUT AUTHORS

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