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Research Article

Assessment of *In vitro* Antidiabetic Activity in Selected Species of *Berberis* L.

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ABSTRACT

Species of *Berberis*, especially their roots, have long been used in traditional medicine to manage hyperglycemia. However, only a few species are commonly used for this purpose. Although more than 20 species of *Berberis are* found in Nepal, only two species, namely *B. aristata* and *B. asiatica,* are widely utilized in traditional medicinal practices. The present study aims to evaluate the therapeutic potential of five *Berberis* species (*B. concinna, B. everestiana, B. hamiltoniana, B. insignis,* and *B. jaeschkeana*) by assessing their total phenolic content, total flavonoid content, and *in vitro* α-glucosidase inhibitory activity. On the basis of parameters evaluated, *B. hamiltoniana* emerged as the most promising species exhibiting the highest total phenolic content (66.97 ± 2.56 mg GAE/g) and the lowest half maximal inhibitory concentration (IC_{50} 10.64 µg/ml) against α-glucosidase. Its inhibitory activity surpassed that of acarbose, the standard drug commonly used in the management of hyperglycemia.

Keywords: α-glucosidase; *Berberis* species; Flavonoid; Phenols

Introduction

Diabetes mellitus has emerged as one of the most prevalent metabolic disorders, representing an intimidating challenge to global public health (Karalliedde & Gnudi, 2016). It is classified into four main categories: Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes, and other specific types of diabetes (American Diabetes Association, 2019; Perišić et al., 2022; Salguero et al., 2023; Ortega et al., 2025). More than 90% of diabetes cases are attributed to T2DM, which is characterized by persistent hyperglycemia resulting from insulin resistance and an inadequate insulin response, accompanied by disturbances in carbohydrate, lipid, and protein metabolism (Weyer et al., 1999; Farzaei et al., 2017). According to the International Diabetes Federation (2025), approximately 11.11% of adults aged 20-79 years, which is nearly 589 million individuals, were living with T2DM globally in 2024.

The federation identifies unhealthy dietary patterns, physical inactivity, obesity, adverse lifestyle behaviors, and rapid urbanization as major contributors to the global rise in T2DM. In the case of Nepalese adults, diabetes is also one of the major health problems with prevalence rates ranging from 6.3% to 8.5% (Sharma et al., 2011; Shakya-Vaidya et al., 2013).

In recent years, there has been increasing interest in alternative therapeutic strategies for diabetes management, particularly using medicinal plants. Plant-derived bioactive compounds exhibit diverse mechanisms of glycemic regulation, including inhibition of carbohydrate-digesting enzymes, enhancement of insulin sensitivity, and reduction of oxidative stress, often with fewer or no side effects (Wei et al., 2023). Furthermore, several herbal remedies have long been used to treat diabetes across various traditional medical systems; however, their active constituents and precise mechanisms of action remain incompletely characterized and have not been systematically integrated into modern medical practice (Prasathkumar et al., 2021; Liu et al., 2024).

Species of *Berberis* L. (Family Berberidaceae) have been widely used as herbal medicines in various traditional medicine systems, including Ayurveda, Sowa-Rigpa (Tibetan-based), Unani, Siddha, homeopathy, naturopathy, and Yogic culture systems. In addition to their medicinal value, these species are utilized as natural dyes, consumed as wild edible fruits, and used in the preparation of wines, pickles, sauces, and coloring agents (Manandhar, 2002; Tutak & Korkmaz, 2012).

Several species of the genus *Berberis* have traditionally been consumed as herbal medicines to treat numerous ailments, including diabetes (Manandhar, 2002; Gaire & Subedi, 2011; Subba & Gaire, 2022). Notably, species like *B. asiatica*, *B. orthobotrys*, and *B. chitria* have been specifically reported for their antidiabetic properties in regions including India and Pakistan (Uniyal et al., 2006; Singh et al., 2017; Dwivedi et al., 2019; Majid et al., 2019)

Experimental and clinical studies have confirmed the antidiabetic efficacy of several *Berberis* species, supporting their traditional use in the management of T2DM or hyperglycemia (Belwal et al., 2020). Phytochemical studies on some of these species have revealed the presence of isoquinoline alkaloids, especially berberine (Hussaini & Shoeb, 1985; Lee et al., 2006; Gomes et al., 2012; Liu et al., 2015), palmatine, columbamine, oxyacanthine, and berbamine (Papiya et al., 2010; Bajpai et al., 2015; Alamzeb et al., 2018). These alkaloids have been reported to exhibit various pharmacological activities, including antidiabetic (Upwar et al., 2011; Mittal et al., 2012; Tiwari et al., 2024; Abid et al., 2025), antioxidant (Alamgeer et al., 2017; Bhatt et al., 2018), and hepatoprotective activities (Tiwari and Khosa, 2010).

In Nepal, the genus *Berberis* comprises 21 species (Adhikari et al., 2012). However, limited information is available regarding the ethnomedicinal uses and therapeutic potential of several of these species. Therefore, the present study aims to evaluate the hypoglycemic potential of extracts from five *Berberis* species: *B. concinna*, *B. everestiana*, *B. hamiltoniana*, *B. insignis* and *B. jaeschkeana*. Among these, *B. concinna* is an endemic species of Nepal. *B. insignis* is confined to eastern Nepal, whereas *B. jaeschkeana* is confined to western Nepal. Similarly, *B. hamiltoniana* occurs in central and western Nepal, while *B. everestiana* is distributed across eastern, central, and western regions of the country (Adhikari et al., 2012). All of these species are being screened for the first time for their *in vitro* antidiabetic potential.

Materials and Methods

Plant materials and sample preparation

Plant samples of different *Berberis* species were collected from various geographical locations across Nepal and identified using the available taxonomic literature. The details of collection sites, including their GPS coordinates, are provided in Table 1, and representative photographs are presented in Figure 1.

Table 1: *Berberis* species collected and their parts used for the study.

S.N.	Species	Parts used	Altitude (m.)	*Locality
1	*B. concinna* Hook.f.	Aerial parts	3692	A
2	*B. everestiana* Ahrendt	Aerial parts	4335	B
3	*B. hamiltoniana* Ahrendt	Aerial parts	2975	C
4	*B. insignis* Hook.f. & Thomson	Bark	2944	D
5	*B. jaeschkeana* C.K. Schneid.	Aerial parts	2975	C

*A: Kharpu Bhanjyang, Rasuwa (26.1689 N; 85.2187 E); B: Timbung Pokhari, Taplejung (27.4268 N; 88.0431 E); C: Patarasi Jumla (29.1906 N; 82.2148 E); D: Sankhuwasbha, Jaljale (27.1962 N; 87.2961 E).

The collected samples were packed in cotton bags at the collection site, thoroughly cleaned to remove adhering soil and debris. Then, shade-dry under proper aeration at room temperature for one week to eliminate excess moisture. Herbaria were prepared from voucher specimens using the method described by Bridson &

Forman (1992). The dried samples were homogenized using an electric grinder and sieved through a 60-mesh screen. The pulverized samples were portioned, vacuum-packed, and stored until further use.

Figure 1: Photographs of different species of *Berberis* L. (A: *B. concinna*; B: *B. everestiana*; C: *B. hamiltoniana*; D: *B. insignis*; E: *B. jaeschkeana*).

Solvent extraction

The extraction of *Berberis* samples was carried out using 80% (v/v) aqueous methanol. The ratio of powdered sample to extraction solvent was kept at 1:10. Initially, the mixture was vortexed and allowed to stand for 24 hours with intermittent vortexing for better infusion. On the subsequent day, the tubes were shaken well and filtered using a Whatman No. 1 filter paper. The residue was re-suspended in the solvent and subjected to extraction for another 24 hours. After 24 hours, the mixture was filtered again. The filtrates were mixed and allowed to evaporate under reduced pressure in a rotary evaporator (RE 100-Pro, Dragon Lab China) at 65 °C. The condensed filtrate was then transferred to pre-weighed petri-plates and allowed to evaporate in a laminar air flow hood until a constant weight was achieved. Thereafter, the dried extracts were scraped off the surface, collected in 2 ml polypropylene tubes, and stored at -20 °C.

Estimation of total phenolic content

The total phenolic content (TPC) was estimated following the method of Ainsworth & Gillespi (2007) with minor procedural modifications. The method involves the color reaction of Folin-Ciocalteu (FC) reagent. The FC reagent (Fisher Scientific, India) was diluted to 10 times the original concentration. The total volume of the reaction mixture was 300 µl. Each well was loaded with 20 µl of plant extract (2.5 mg/ml) and 200 µl of diluted FC reagent and incubated for 5 minutes at 25 °C. Then, 80 µl of 1 M Na_2CO_3 solution was followed by incubation for 25 minutes at 25 °C. Methanol (80%) was used as a blank. Absorbance was measured at 765 nm using an EPOCH 2 microplate reader (Agilent Technologies). The experiment was also carried out using gallic acid solution of different concentrations (10-60 µg/ml) instead of plant extract, and absorbance values obtained at different concentrations of gallic acid were used to obtain a calibration curve. The concentration of phenolic content in the plant extract was determined by using the equation of the standard curve (Figure 2) of gallic acid ($y = 0.006x$, $R^2 = 0.999$). TPC of the plant extract was calculated according to the formula:

$$TPC = (c \times v)/m$$

Where *c* represents the phenolic concentration obtained from the gallic acid calibration curve, *v* is the volume of the extract used, and *m* is the dry weight of the sample.

TPC was expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g).

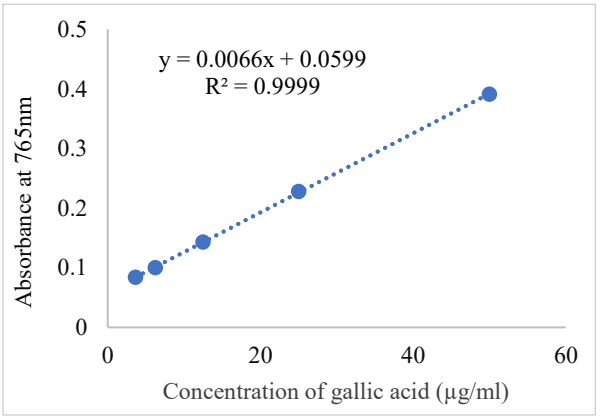

Figure 2: Standard curve of gallic acid.

Estimation of total flavonoid content

Total flavonoid content (TFC) estimation was done by following the protocol of Roy et al. (2011). The plant extracts dissolved in respective solvents at a concentration of 10 mg/ml. Then, 25 µl of each sample solution was separately mixed with 75 µl of 10% aqueous solution of $AlCl_3$, 5 µl of 1 M aqueous solution of CH_3COOK and 140 µl of distilled water. Methanol, instead of plant extract, was used as a blank. After vigorous shaking and subsequent incubation at room temperature for half an hour, absorbance was measured at 415 nm using a spectrophotometer. In a separate

experiment, quercetin solution in methanol in the concentration range 10 to 100 µg/ml was used instead of the plant extract to prepare a standard curve of quercetin. TFC in the extract was determined based on the equation of standard curve (Figure 3) of quercetin ($y = 0.0068x + 0.025$; R^2=0.9962). From this concentration value TFC of the plant extract was calculated by using the formula:

$$TFC = (c \times v)/m$$

Where *c* represents the flavonoid concentration obtained from the quercetin calibration curve, *v* is the volume of the extract used, and *m* is the dry weight of the sample.

The value of TFC was expressed as milligrams of quercetin acid equivalent per gram of dry weight (mg QE/g).

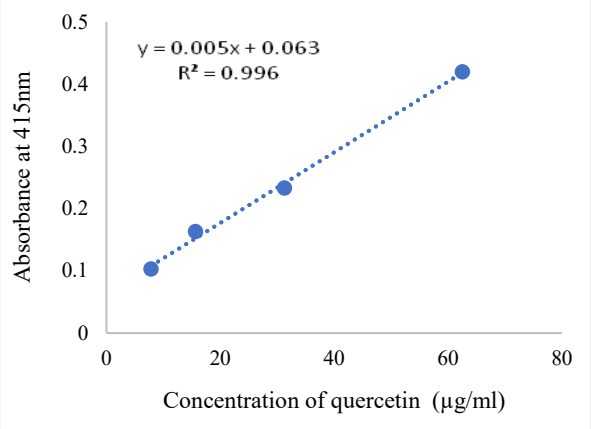

Figure 3: Standard curve of quercetin.

Evaluation of α-glucosidase inhibition activity

The modified protocol of Si et al. (2010) was employed for the α-glucosidase enzyme inhibition assay. One hundred fifty microlitres of p-nitrophenyl α-D-glucopyranoside (PNPG) solution (Sigma Aldrich, Germany) prepared in 0.1 M potassium phosphate buffer (pH 6.4) was pipetted into each well of 96 well Plate. In wells marked as blank and negative control, 10 µL of 80% methanol was added, while in the wells marked as sample, the same amount of either plant extract or acarbose solution (1 mg/ml) prepared in 80% methanol was added. Then, 40 µl of 1 M Na_2CO_3 (British Drug Houses) was added to the wells, representing the blank. The reaction mixture was pre-incubated at room temperature (28°C) for 10 minutes. After pre-incubation, 10 µl of α-glucosidase (Sigma Aldrich, Germany) enzyme solution (0.2 U/ml) prepared in 0.1 M phosphate buffer (pH 6.4) was added, and the plate was incubated at 28 °C for a further 20 minutes. The reaction was terminated by adding 40 µl of 1 M Na_2CO_3 to all the wells except the blank. The absorbance was measured at 405 nm using a microplate reader (EPOCH-2, Agilent Technologies). Inhibition of α-glucosidase activity was calculated by using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Abs. control} - \text{Abs. sample}}{\text{Abs. control}} \times 100$$

In order to calculate concentrations of plant extracts and acarbose that inhibit the enzyme activity by 50% (i.e., IC_{50} value), an enzyme inhibition assay was carried out using plant extracts and acarbose solution of different concentrations. From the value of enzyme inhibition at different concentrations, IC_{50} was calculated using the following formula.

$$IC_{50} = \text{Exp (Ln (conc pi>50) – ((pi>50 - 50) / (pi>50 - pi<50)) * Ln (conc pi>50 / conc pi<50))}$$

Where, Exp – exponential function; Ln – Natural log; Conc pi>50- extract concentration at which enzyme inhibition is just above 50%; Conc pi<50 - extract concentration at which enzyme inhibition is just below 50%; Pi>50 – observed percentage inhibition value that is immediately above 50%; Pi<50– observed percentage inhibition value that is just below 50%

Data analysis

All experimental data obtained for each sample were the average value of three independent measurements. Values were reported as mean ± standard deviation. Microsoft Office Excel (2007) was used to compute means, standard deviation, and one-way analysis of variance (ANOVA), followed by post hoc analysis by Tukey's test. Percentage values were subjected to log transformation before ANOVA.

Results and Discussion

Total phenolic content

The total phenolic content (TPC) of different species of *Berberis* extract is presented in Figure 4. The highest TPC (66.97 ± 2.56 mg GAE/g) was observed in *B. hamiltoniana* extract, whereas the lowest TPC (5.8±0.04 mg GAE/g) was observed in *B. concinna* extract. The TPC of *B. concinna* was significantly lower, while that of *B. hamiltoniana* was significantly higher compared to other species ($p<0.05$).

Phenolic compounds are widely acknowledged secondary natural metabolites produced biogenetically (Cheynier et al., 2013). Phenol and polyphenols are abundant in fruits, berries, cereals and vegetables, and they play a crucial role in mitigating oxidative stress due

to their redox properties, hydrogen-donating ability and metal-chelating potential (Pandey & Rizvi, 2009; Wang et al., 2011; Wootton-Beard & Ryan, 2011; Zhang & Tsao, 2016; Minatel et al., 2017). Studies have shown that increased intake of polyphenol-rich diet reduces the risk of degenerative diseases like diabetes, cardiovascular diseases, cancer, etc. (Nisar, 2022). As a result measurement of total phenolic and flavonoid content has been a standard practice in studies on medicinal plants and those on functional foods and dietary substances.

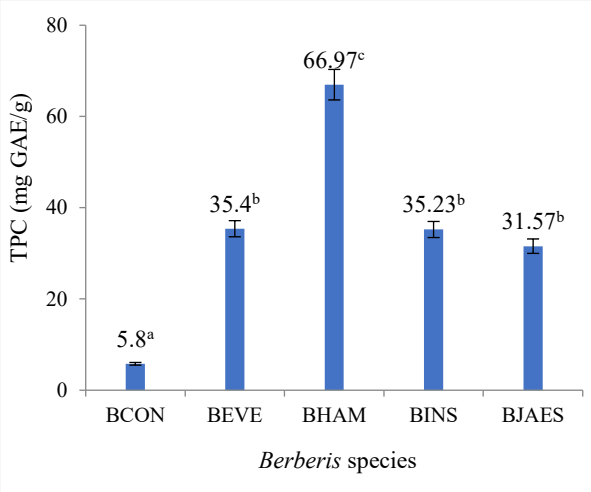

Figure 4: Total phenolic content (TPC) in extracts of different species of *Berberis* (BCON: *B. concinna*; BEVE: *B. everestiana*; BHAM: *B. hamiltoniana*; BINS: *B. insignis*; BJAES: *B. jaeschkeana*). Values are expressed as mean ± SD (n = 3). Means followed by different superscript letters are significantly different at $p < 0.05$ (one-way ANOVA followed by Post-hoc Tukey's HSD test).

Earlier studies have reported the presence of phenols in various parts of *Berberis*, including fruit, leaves, stem bark, and roots (Dhungel et al., 2016; Bhatt et. al, 2018; Awal et al., 2025).The abundance of phenolic content of methanolic extract of *B. hamiltoniana* from the aerial-part is quite higher among the studied taxa of *Berberis*. The elevated phenolic concentration of *B. hamiltoniana* indicates significant variation in accumulation of phenolic among the evaluated species.

Total flavonoid content

The total flavonoid content (TFC) in extracts of different species of *Berberis* is presented in Figure 5. Among the studied species, *B. everestiana* and *B. hamiltoniana* exhibited the highest TFC (7.61 ± 0.15 and 7.46 ± 1.15 mg QE/g resectively), while *B. insignis* showed the lowest (1.89 ± 0.25 mg QE/g). The TFC values in extract of *B. everestiana* and *B. hamiltoniana* were significantly higher ($p \leq 0.05$), while the extract of *B. insignis* was significantly lower ($p \leq 0.05$) than that of rest of the species.

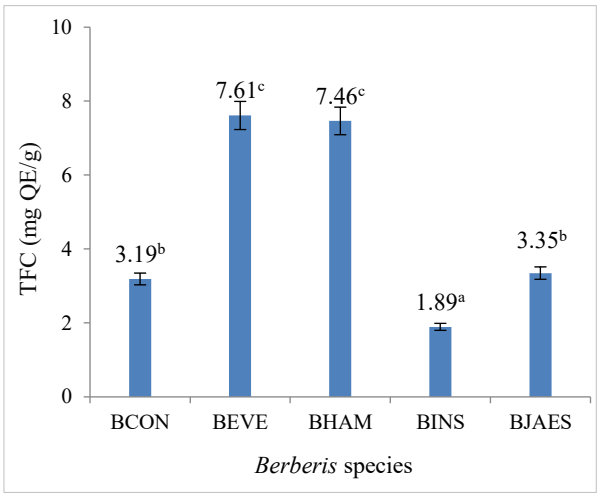

Figure 5: Total flovonoid contentment (TFC) of different *Berberis* species (BCON: *B. concinna*; BEVE: *B. everestiana*; BHAM: *B. hamiltoniana*; BINS: *B. insignis*; BJAES: *B. jaeschkeana*). Values are expressed as mean ± SD (n = 3). Means followed by different superscript letters are significantly different at $p \leq 0.05$ (one-way ANOVA followed by Post-hoc Tukey's HSD test).

Flavonoids are one of the most crucial secondary metabolites belonging to the phenol family (Panche et al., 2016). They are well known for their antioxidant, anti-inflammatory, and antidiabetic properties. The most commonly occurring flavonoids in certain beverages, vegetables, fruits, spices, and soup include quercetin and anthocyanins (Hertog et al., 1993; Pandey & Rizvi, 2009; Panche et al., 2016). Epicatechin, a natural flavonoid present in *Camellia sinensis* (tea), has been reported to mediate the activation of the insulin receptor in T2DM (Ganugapati et al., 2011). Additionally, earlier studies have shown that flavonoids in *Berberis* species contribute significantly to free radical scavenging and enzyme inhibition activities (Imenshahidi & Hosseinzadeh, 2016).

While studying the total flavonoid content in different parts of *Berberis aristata* and *B. thomsoniana*, Bhatt et al. (2018) reported that there is variation in total flavonoid content in species- specific as well as organ-specific manner. The species-specific variation in total flavonoid content in the present study supports the findings of Bhatt et al. (2018). Awal et al. (2025) further reported that flavonoid content decreases while total phenol increases with rising soil moisture and elevation. Since the samples in this study were collected from different elevations, these factors may also have contributed to the observed variations. Additionally, such variations could also be influenced by soil physicochemical properties, biotic and abiotic stresses, thermal exposure, ecological interactions, genetic variation, or differences in metabolite pathways (Andola et al., 2010, 2019; Thompson et al., 2010; War et al., 2012; Cheynier et al., 2013; Gan et al., 2017).

α-glucosidase enzyme inhibition

The ability of crude extracts obtained from different *Berberis* species to inhibit the activity of enzyme α-glucosidase is presented in Figure 6. Among the tested species, the extract of *B. hamiltoniana* exhibited the highest enzyme inhibition (88.01% ± 0.34), whereas *B. everestiana* showed the lowest inhibition (0.76% ± 0.034) under experimental conditions. Two species, *B. concinna* and *B. hamiltoniana,* were further evaluated to determine their maximal half inhibitory concentration (IC_{50}) values.

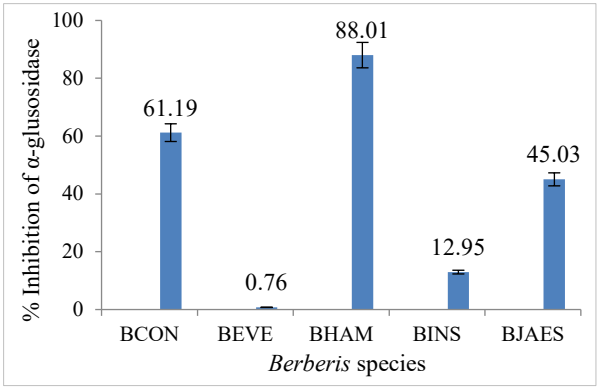

Figure 6: Percentage inhibition of α-glucosidase enzyme by *Berberis* species extract (BCON: *B. concinna*; BEVE: *B. everestiana*; BHAM: *B. hamiltoniana*; BINS: *B. insignis*; BJAES: *B. jaeschkeana*).

The comparison of IC_{50} values of extracts from *B. concinna* and *B. hamiltoniana* with the standard drug acarbose against α-glucosidase is shown in Figure 7. The extract of *B. hamiltoniana* showed the most promising inhibitory potential, exhibiting an IC_{50} value significantly lower than that of acarbose. In contrast, the extract of *B. concinna* showed an IC_{50} value significantly higher than that of acarbose. Based on these screening results, *B. hamiltoniana* (with lowest IC_{50} value against α-glucosidase, i.e., highest inhibition) is identified as the most promising species for further investigation.

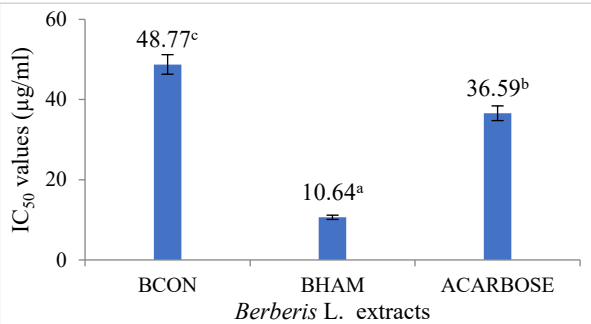

Figure 7: α-glucosidase IC_{50} value of *Berberis* extracts (BCON: *B. concinna* & BHAM: *B. hamiltoniana*). Values are expressed as mean (n = 3). Means followed by different superscript letters are significantly different ($p \leq 0.05$).

Although several *Berberis* species, such as *B. aristata, B. asiatica, and B. orthobotrys,* have been traditionally used to manage diabetes, none of the species examined in the present study has reported medicinal uses. Notably, *B. hamiltoniana* exhibited significant α-glucosidase inhibitory activity, surpassing even that of acarbose. Since the antidiabetic effects of various *Berberis* species have been linked to phytoconstituents such as berberine, palmatine, columbamine, and berbamine (Tiwari et al., 2024; Abid et al., 2025), the hypoglycemic potential observed in *B. hamiltoniana* may be due to the presence of similar or other novel bioactive compounds.

Conclusion

Comparative evaluation of TPC, TFC, and *in vitro* antidiabetic potential of extracts of selected species of *Berberis* from Nepal revealed species specific variations in these parameters. Though none of the species selected for study are used in traditional medicine, their therapeutic potential as revealed by high TPC values and high inhibitory potential against α-glucosidase *in vitro* indicate towards their potential use as substitutes to species of *Berberis* like *B. aristata, B. asiatica* and others used for various therapeutic applications. However, a detailed study on their phytochemical constituents and potential toxicity should be carried out before their use as substitutes to those highly demanded species.

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