Indigenous knowledge and bioactive compounds of *Berberis aristata* confirm its therapeutic potential: An ethnopharmacological appraisal in Nepal

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Research

Abstract

Background: The prevalence of ethnomedicinal plant usage can be attributed to a combination of factors, primarily driven by local preferences towards traditional herbal remedies. This tendency is exacerbated by a dearth of practicable alternatives and the pervasiveness of economic distress. Additionally, this phenomenon is greatly influenced by a persistent belief in the effectiveness of folkloric herbal cures and the alleged medicinal qualities of local flora. Nepal stands as a region abundant in biological resources, showcasing a noteworthy reservoir of therapeutic plant species employed extensively in Ayurvedic medicine.

Methods: Berberis aristata DC (also called Daruharidra / Chutro), known for its therapeutic leads, was the focus of this ethnobotanical survey, examining its chemo-diversity and medicinal properties based on its traditional use by the indigenous communities throughout Nepal. We chose to perform the qualitative phytochemical screening along with other biological analyses of *B. aristata* to complement and validate our findings obtained from the ethnobotanical studies.

Results: Our findings revealed that 14 distinct ethnic communities residing in 24 districts utilize *B. aristata* for diverse therapeutic purposes, with the Tamang indigenous group being the primary users. Most ethnic communities employing *B. aristata* for medicinal purposes reside in the Bagmati and Koshi Provinces. Analytical chemistry revealed higher phenol and flavonoid content in *Berberis* leaves. The free radical scavenging assay revealed the highest values for bark methanolic extract and demonstrated strong antimicrobial properties against *Staphylococcus aureus* and *Klebsiella pneumoniae*. The inhibitory effects on α-amylase found varying levels of inhibition in different plant parts.

Conclusion: Overall, the present study highlights the importance of investigating and utilizing the vast natural resources available in Nepal. Finally, we conclude traditional medicinal plants, such as *B. aristata*, possess enormous potential for developing novel therapeutic medications that are safe, affordable, and effective for human consumption.

Keywords: Conservation, Distribution, Indigenous knowledge, Medicinal plant, Therapeutic uses

Background

Collection of medicinal plants for herbal remedies follows two main strategies, i.e., random approach, where no regard is taken for the taxonomic affinities, ecological clues, organoleptic properties, therapeutic potentials, cultural contexts, or other intrinsic qualities, and focused selection approach is based on ecological, cultural, geographical, biological, taxonomic, therapeutic, and ethnopharmacological appraisals (Cox 1990, Farnsworth & Bingel 1977). It is assumed that the harvesting of medicinal plants in Nepalese traditional pharmacopeias is non-random and influenced in part by the therapeutic efficacy of the plant (Kutal *et al.* 2021), and in part by geographical and socio-cultural factors (Bhattarai *et al.* 2022, Kunwar *et al.* 2018, Kutal *et al*. 2021). Some collection was influenced by culture (Kunwar *et al*. 2020b) as *Paris polyphylla* has been collected in the belief that its seven leaves are useful for curing seven ailments (Kunwar *et al.* 2020a). *Berberis aristata* DC is also culturally preoccupied as a medicine for jaundice and it has been reported to be collected for jaundice and fever for decades in Nepal (Bhandari *et al*. 2021, Bhattarai 1992, Kunwar *et al.* 2006).

Berberis aristata, with the vernacular name Chutro, is a rare Himalayan medicinal plant that belongs to the Berberidaceae family (Bhatt *et al.* 2018). This spinous, hard, and yellowish plant displays an erratic pattern of distribution both globally and regionally in addition to a large geographic spread throughout Nepal (Fig. 1). Globally, *B. aristata* is endemic to the Himalayan region, including Nepal, India, Sri Lanka, Bhutan, and Tibet (Choudhary *et al.* 2021). Regionally, this species is distributed between the easternmost region of Taplejung and the westernmost region of Darchula, at elevations between 1500 m and 4000 m above sea level. This species is frequently distributed and collected in Solukhumbu, Dolakha, Ramechhap, Mustang, Dolpa, etc (Thusa & Mulmi 2017). However, climatic, and environmental conditions, such as temperature, rainfall, and soil type, have a significant impact on the distribution and localization of *B. aristata* (Bhatt *et al.* 2018). Moreover, this plant's distribution is linked to a variety of ecological niches, such as forested regions, riverbanks, and rocky slopes, underscoring its adaptability to many ecological circumstances.

Figure 1. Morphological features of *Berberis aristata*. a) Plant twig, b) Roots, c) Bark of *Berberis aristata*, d) Trimmed branches

B. aristata holds a long history of usage in Ayurvedic medicine. *B. aristata* has been demonstrated to have several pharmacological properties, such as antipyretic, antibacterial, antimalarial, and anticancer characteristics (Komal *et al.* 2011). Alkaloids, flavonoids, and berberine are some prominent compounds isolated from *Berberis* with sufficient antioxidant and antibacterial properties (Gaurav *et al.* 2020, Jahan *et al.* 2022). Jatrorrhizine, an isolated alkaloid from the plant, is used to treat gastritis and other digestive problems (Jahan *et al.* 2022). Other secondary metabolites, such as oxyberberine, palmatine, berberamine, karachine, palmatine chloride, tetrahydropalmatine, pseudopalmatine chloride, etc. found in the plant can treat several ailments including diarrhea, hemorrhoids, gynecological disorders, and malarial fever (Azumder *et al*. 2011, Chander *et al*. 2017, Jahan *et al*. 2022).

From an ethnobotanical perspective, various parts of *Berberis* are used in treating several ailments, as leaves are used for treating skin diseases, cholera, jaundice, diarrhea, skin and eye infection, and menorrhagia, while root decoction is mainly utilized for cleansing wounds to prevent pathogenic infection and healing (Bhaila *et al.* 2022). Various extracts of *B. aristata* have also been used for curing hepatotoxicity (Gilani & Janbaz 1995). Available literature suggests extraction done with 50% hydrochloric acid shows promising antimicrobial activity against different pathogens (Singh *et al.* 2007). Moreover, alcoholic and aqueous extracts of root possess remarkable antibacterial and antifungal activity against several tested microorganisms in clinical and standard lab strains and were found excellent for treating cholera (Bhatt *et al.* 2018, Mazumder *et al*. 2011, Potdar *et al*. 2012, Shahid *et al*. 2009). Considerable attention has been given to knowing and utilizing therapeutic leads

present in plants with promising activity towards hepatoprotection, anti-diarrhea, cardiotonic, anti-diabetic, anti-cancer, anti-inflammatory, anti-microbial, ophthalmic, and activities related to CNS (Bhattarai *et al.* 2022). Our goal was to validate the traditional uses of the plant by Indigenous communities of Nepal through increased utilization, combining their knowledge with experimental findings to identify various therapeutic biomolecules within this species.

Material and Methods

Ethnobotanical survey

Our research focused on discovering plants with unique therapeutic biomolecules that are being extensively used by various communities throughout Nepal. As part of our initial efforts, we conducted an in-depth survey utilizing a carefully constructed questionnaire to collect vital data from various communities. But, the COVID-19 pandemic's unprecedented difficulties, such as travel restrictions and prohibitions on direct physical contact, created obstacles for us to gather primary data and samples from various regions. To complement our research findings, we turned to a thorough literature review. We documented and assessed the geographical sites where these plants were located, performed an in-depth analysis of previous research works, and then chose a practical location to harvest the plant for future analysis (extraction of bioactive molecules and tests for antimicrobials). Remarkably, the chosen medicinal plant, *B. aristata*, offered great promise for the generation of novel therapeutic products in addition to being of cultural and economic value to the Nepalese population.

Plant extraction and phytochemical screening

All chemicals and reagents used in this study were of analytical grade and were procured from Sigma-Aldrich (Germany) unless otherwise stated**.** The leaves, barks, and roots of *B. aristata* were harvested from Arghakhachi at an altitude of 2,270 m above sea level, based on available ethnobotanical knowledge of Indigenous people and various available scientific literature. The plant was identified based on herbarium collected and compared with other stored herbarium samples housed in the National Herbarium and Botanical Laboratory (KATH) Godawari, Lalitpur, Nepal. Specimens of the herbarium samples are housed in the Institute of Biological Resources (IBr), Kathmandu, Nepal with an accession number of UKIB_127.

Extraction of *Berberis* secondary metabolites was performed according to the protocol as devised by Bhattarai *et al*. (2022) with minor modifications. Briefly, the harvested samples (leaf, bark, and root) were air-dried in the shade for a week and ground to a fine powder, followed by extraction using different organic solvents. The collected samples were percolated using methanol as an extracting solvent. The filtrate was collected after frequent extraction for 72 h, and the extracted solvent along with extracts was separated using a Rota-evaporator (Rotavapor® R-100, Germany). The extract was made solvent-free by drying it in a water bath at 40°C. The dry extracts were used for various test performances. The methanol extract of bark was subjected to further fractionation using different organic solvents. Thin-layer chromatography (TLC) performed on the extract revealed the presence of multiple spots on paper, indicating the potential presence of several secondary metabolites. Thus, fractionation of the methanol extract of bark was carried out by adding 200 mL of distilled water to a separating funnel containing the crude bark extract, and an equal volume of ethyl acetate (EtOAc), DCM, and hexane solvents. The solvents were added over a water-bath to make the sample dry after the fractionation process was completed.

Qualitative phytochemical screening and assessment of biological properties

The qualitative phytochemical screening of plant extract was performed according to the protocol devised by Sasidharan *et al.* (2010) and Siddiqui (2021) with modifications. The Folin-Ciocalteu (TFC) test which was used to determine the total phenolic content of the sample extract was performed as per the protocols suggested by Singleton *et al.* (1965) with minor modifications. Various amounts of gallic acid and plant extract in three different technical replicates were used. To prepare various concentrations, a stock solution containing 0.5 mg/mL gallic acid was diluted with distilled water. Additionally, a plant extract from a 5 mg/mL solution was added to the plate in triplicate with 20 µL of extract. Following the addition of an F-C reagent to each well-containing sample and standards, a microplate reader was used to detect the absorbance at 765 nm. Final absorbance was measured following the addition of 80 µL after the plate had been incubated in the dark for 15-30 min. Following the addition of 80 µL of sodium carbonate solution into each well-containing standard and samples, the plate was incubated in the dark for 15-30 min, followed by measurement of absorbance. The gallic acid standard curve was plotted using various concentrations. The TPC was calculated as mg GAE/g of dry weight or milligrams of gallic acid equivalent per gram weight of the dry sample.

The TFC of the sample extract was calculated using the colorimetric method as devised by Chang *et al*. (2002), with minor modifications. Altogether, 130 µL of different concentrations of standard quercetin solutions were loaded into the 96-well plate. 20 µL of plant extract with a concentration of 5 mg/mL was loaded. The initial reading was measured after adding 110

µL distilled water into the well-containing plant sample. After that, 60 µL ethanol was loaded in each well-containing standard and sample. 5 µL of 10% aluminum chloride and 5 µL of 1.0 M potassium acetate were added separately in each well, reaching a total volume of 200 µL. The plate was incubated for 30 min in the dark. The final absorbance was measured at 415 nm in a microplate reader. All samples and standard solutions were loaded in triplicate.

Antioxidant activity

Free radical scavenging activity of the sampled plant extracts was carried out by using 1, 1-diphenyl-2-picrylhydrazyl (DPPH) following a protocol described by Huang *et al*. (2005) with slight modifications. For this, quercetin and 50 % DMSO (Dimethyl sulfoxide) were used as positive and negative controls, respectively. Firstly, 100 µL of the control (either positive or negative) was loaded into the plate and 100 µL different concentrations of plant samples were loaded in triplicates. Initial absorbance was recorded at 517 nm. After that 100 µL DPPH solution was added to each well-containing sample and control, followed by incubating it in the dark for 30 min, and measurement of the absorbance (517 nm) in a microplate reader. Percentage (%) inhibition was calculated using the following equation:

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

Where, 'Abs. of control' is the absorbance of negative control that is 30% DMSO solution and 'Abs. of sample' is the absorbance of the plant extract analyzed.

α-amylase activity

The enzyme activity assay for *in-vitro* α-amylase was performed in a 96-well plate method, following the standard protocol by Senger *et al*. (2012) with modifications. The assay was performed in triplicates. For this, 100 µL of substrate solution from 0.5 mM was loaded onto the plate, followed by the addition of 20 µL extracts and 80 µL enzyme solutions.

The initial absorbance was taken at 405 nm in the ELISA (enzyme-linked immunosorbent assay) plate reader. 30 % DMSO was used as a negative control. The plate was incubated for 15 min at RT. The final absorbance was taken after 15 min at the same wavelength. The percentage inhibition was calculated and whose inhibition was calculated above 50 %, the IC_{50} was calculated for the same by diluting the stock into various final concentrations. The equation for calculating the inhibiting percentage was identical to that for antioxidant activity.

Antimicrobial activity

For the determination of the antimicrobial activity of root, leaf, and bark extract, the standard protocol of antimicrobial susceptibility testing (AST) was followed as described in the manual of AST (Morgan 1995). Screening and evaluation of the antibacterial activity of different extracts were performed in agar well-diffusion method against four bacterial species (*S. aureus* ATCC 25293, *E. coli* ATCC 25922, *Salmonella* Typhi ATCC 14028, and *Klebsiella pneumoniae* ATCC 13883) and inhibitory zone of bacterial growth (ZOI) was scored. Each well comprised 20 µL 50% DMSO as a negative control, neomycin as a positive control, and plant extracts of 50 mg/mL. Plates were incubated at 37°C overnight and ZOI was measured.

Minimal inhibitory concentration (MIC)

MIC represents the lowest concentration of sample sufficient to suppress the microbial growth, which is usually expressed in terms of microgram or milligram per milliliter of bacterial broth solution required to inhibit the growth. This expresses the better effectiveness of the fractions/compound against the corresponding microorganisms. The methanolic sample extract which shows significant antibacterial activity is selected for the determination of MIC. The MIC test was performed on a 96-well culture plate (Morgan 1995), which is a rapid and sensitive method for MIC determination. The 50 µL neomycin antibiotic was used as a positive control and the DMSO solution was taken as a negative control. 5 µL of bacterial inoculum (1×10^6 CFU/mL) was used for testing MIC. Experiments for all bacterial strains, plant extracts, and controls were performed in triplicate. The inoculated plate was incubated at 37°C for 18-20 h, followed by the addition of 30 µL resazurin in each well. Observation of the plate was made after 3-4 h of incubation. The appearance of pink color indicated the live bacteria, while the blue color indicated dead or inactive bacteria. Data for the MIC measurement was recorded from the concentration of inactive wells (Baral *et al*. 2011, Baral & Maharjan 2011).

Minimal bactericidal concentration (MBC)

MBC represents the lowest concentration of a compound sufficient to kill the bacteria and is determined by the micro-dilution method using a serial dilution of plant extract. This simple, rapid, and sensitive method was used for measuring the

viability of bacteria. For this, 50 mg/mL of methanol extract solution was diluted to the final concentration of 0.78 mg/mL by a two-fold dilution method. The MBC was performed in a nutrient agar (NA) plate. The MBC was done based on MIC, suggesting that the concentration for MBC has experimented above the MIC concentration. Bacterial cells from MIC plates were cultured in an NA media, followed by incubation at 37°C for 12 h. MBC was calculated based on the growth of bacterial colonies, which are expressed in terms of mg/mL (Baral *et al*. 2011, Baral & Maharjan 2011).

Isolation of bio-active compounds

The column chromatography of the hexane fraction of bark sample extract was carried out using silica gel. The 10 mm column was used for the 8 g hexane fraction sample. The isolation of active constituents from the sample was performed with column chromatography and Thin-layer chromatography (TLC). 10 % EtOAc (subfraction 8-10) in hexane and 60% EtOAc in hexane (subfraction 23-24) solvent systems were tested as single spots. After TLC, the FT-IR measurements of the active fractional samples were performed at room temperature (*ca.* 28°C) using a Perkin Elmer, USA spectrophotometer to observe the presence of the functional group. The FT - IR of 10 % (subfraction 8-10) and 60% (subfraction 23-24) EtOAc sample eluent was reported.

Statistical analysis

All experiments were performed in triplicates and data are presented as mean ± standard error of the mean. The TPC, TFC, antioxidant activity, and enzyme assay results were processed by using Gen5 microplate data collection, Agilent, USA, and analyzed by MS Excel 2021. The IC_{50} values were calculated using GraphPad Prism version 10.0.0.

Results

Traditional uses

We reported the extensive use of *B. aristata* in 24 districts of Nepal, particularly in the rural areas of mid-hills and mountains of the country (Fig. 2a). A total of 14 different ethnic groups, namely Tamang, Sherpa, Limbu, Newar, Gurung, Rai, Banskharka, Dolpo people, Lama, Majhi, Newah, Vaidhya and Dhami-Jharkri, Bhotia, and Thakuri use *B. aristata* as a source of therapeutic agent (Fig. 2b). For instance, indigenous people inhabiting Kaski district, mainly Gurung use *Berberis* for different ailments, such as skin infections, ulcers, diarrhea, bronchitis, asthma, fever, jaundice, stomach disorders, respiratory infections, and fungal infections. However, no information on the usage of *Berberis* as therapeutic implications was found by the dominant ethnic group Magar in the Kaski district. The use of species for jaundice is found highly consented to as it was found to be used in 11 districts out of 24 districts reported. The species was found to be commonly used in seven districts of Bagmati and Koshi provinces each and four districts of Gandaki province. The species was not found and used at all by the ethnic groups of Madhesh province. The species is being used mostly for curing diseases for digestion troubles followed by musculoskeletal, liver, and endocrine systems (Fig. 2c).

Our investigation has determined that the main ethnic group in the Baglung region is the Magar ethnic group, which makes up 28% of the population. The Kami ethnic group comes in second place with 26% of the population, closely followed by the Sarki ethnic group with 6% of the total. These ethnic groups mostly use the root bark of the *Berberis* plant to cure a variety of illnesses like jaundice, malarial fever, diarrhea, and peptic ulcers (Table 1). Similarly, the main ethnic groups living in the Darchula region, the Chhetri ethnic group (65% of the population), the Kami ethnic group (5%), and the Thakuri ethnic group (5%), utilize the medicinal benefits of *Berberis* bark to treat ailments like diarrhea, piles, and malaria. It is captivating that a significant Chhetri ethnic group can be found in various districts, including Dhankuta, Sankhuwasabha, Ramechhap, Dolakha, Dolpa, Surkhet, and Darchula (Fig. 3). These populations use *Berberis* to treat a variety of illnesses, such as jaundice, diarrhea, fever, infections of the eyes, skin, and digestive issues. Furthermore, the Limbu ethnic group, which predominately inhabits the Panchthar, Taplejung, and Tehrathum districts, is renowned for using *Berberis* for a variety of health purposes, including the treatment of ringworms and fungal infections, as well as for curing jaundice, malarial fever, digestive complaints, as a tonic, laxative, and remedy for dental pain and bone fracture healing. Additionally, the Tamang ethnic group uses *Berberis* to treat illnesses including diarrhea, jaundice, liver problems, fever, and skin issues. The Tamang ethnic community makes up a significant proportion of the population in Nuwakot, Kavrepalanchowk, Rasuwa, and Makwanpur (Fig. 2).

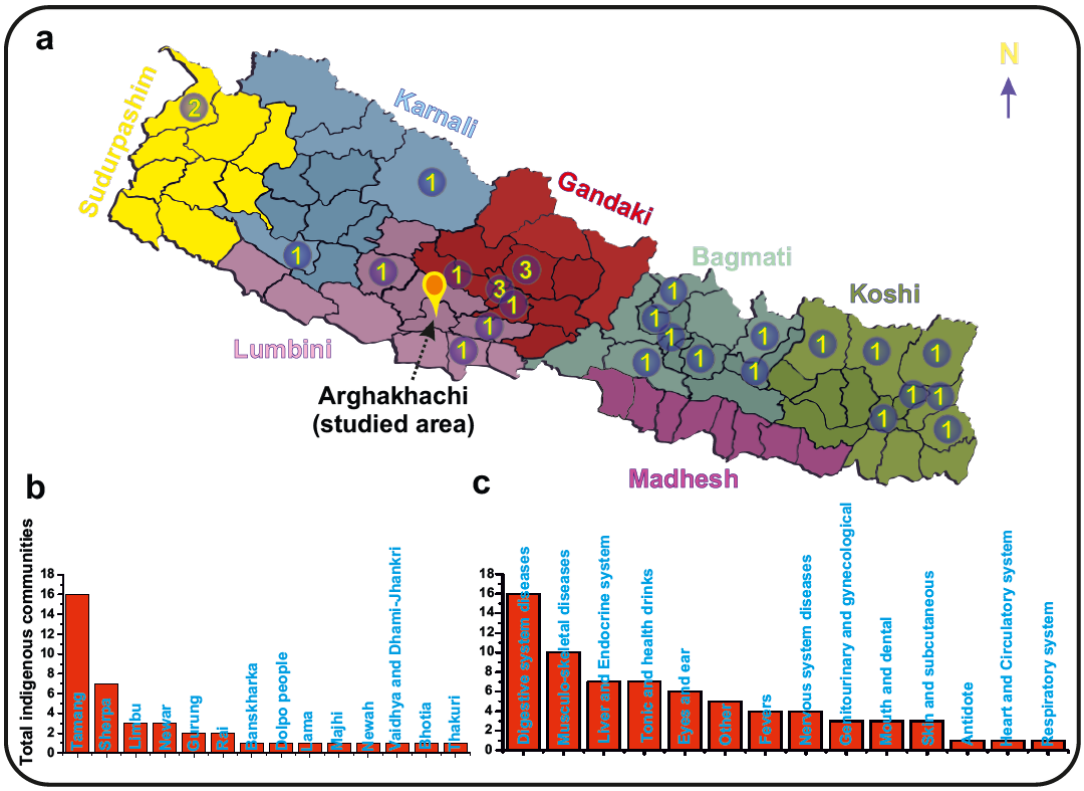

Figure 2. Geographical map of Nepal. a) Nepal map depicting the distributional study with a focus on the therapeutic importance of *B. aristata* in previously published literature. The currently studied area, where no previous studies were done, namely Arghakachi district has been indicated with a yellow pointer. Different color codes refer to different provinces of Nepal, altogether 7. Circles within the map indicate the geographical distribution of *B. aristata*, and the numbers within the circles (1, 2, and 3) indicate their previous report as therapeutic potentials in scientific publications. b) Total indigenous people in Nepal utilizing *B. aristata* for therapeutic purposes. c) Use of *Berberis* for different therapeutic purposes based on available literature.

The Brahman-hill ethnic groups which occupy a higher proportion in most districts use *Berberis* in their traditional medical practices to treat a variety of illnesses, such as gastrointestinal problems, liver diseases, respiratory infections, malaria, skin infections, diabetes, ulcers, diarrhea, bronchitis, asthma, fever, jaundice, stomach problems, and fungus infections. These ethnic groups are primarily found in Kathmandu, Kaski, Parbat, Syangja, Rupandehi, and Arghakhanchi districts. Notably, the Newar and Banskharka populations of Kathmandu and Kavrepalanchowk districts utilize *Berberis* for a variety of medical applications However, it might be difficult to determine with accuracy what proportion of Banskharka population in Kavrepalanchowk district utilize this plant for medicinal purposes (Table 1).

Phytochemicals and active biological compounds

Methanolic crude extract of leaf, bark, and root of *B. aristata* had a slightly different physical appearance. Leaf extract had an oily dark green color, while the bark and root extract had a yellowish brown and yellow color, respectively. The percentage yield of methanolic extracts of various parts was found to be similar, i.e., 12%, 10%, and 10% (W/W) for leaf, root, and bark, respectively. Preliminary phytochemical analysis revealed the most similar class of compounds in different parts of the plant analyzed. Surprisingly, none of the plant parts assessed possess tannins, while steroids were reported only in roots and barks (Table 2).

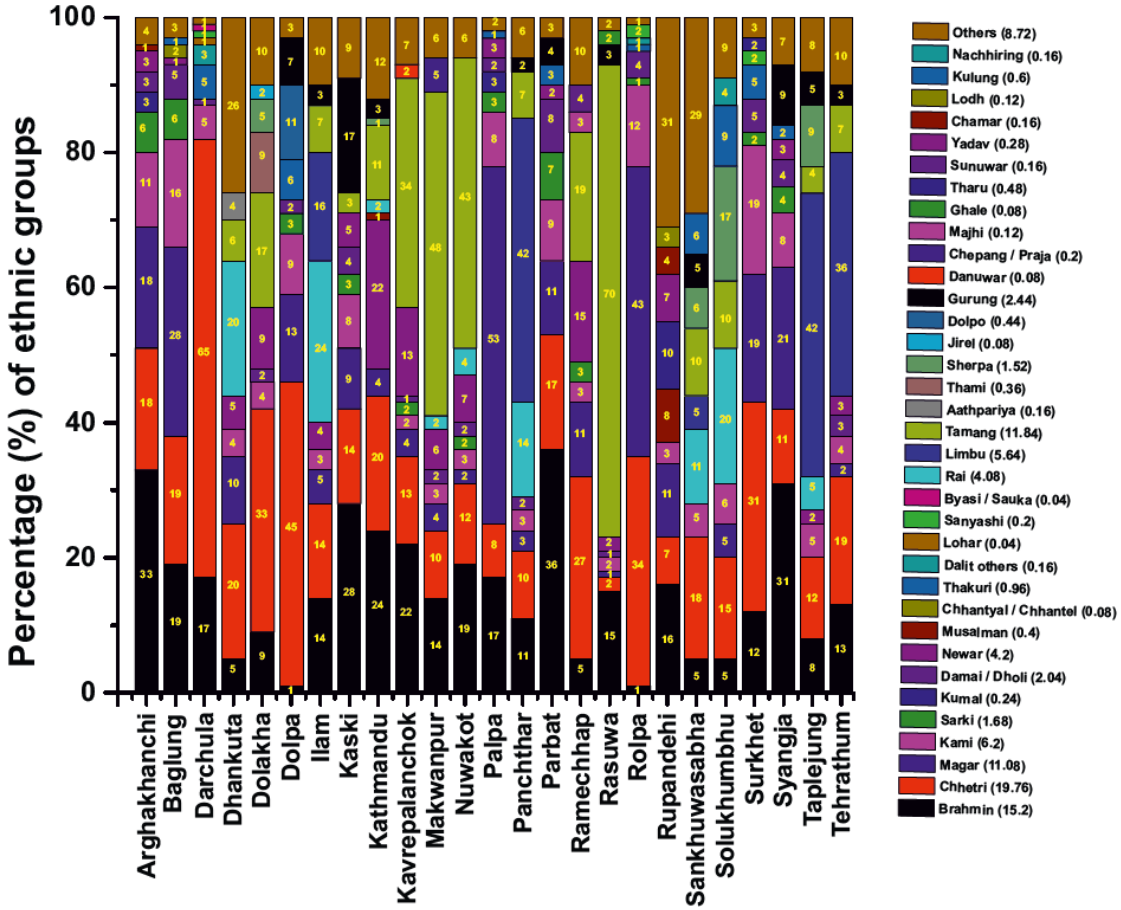

Figure 3. Total populations of the studied districts. Numbers within column bars represent ethnic groups' population percentages in currently studied districts. The numbers in brackets (on the right legend) indicate the combined total population percentage of the ethnic groups in the whole districts mentioned in the figure.

Table 1. Literature search for the distribution and therapeutic uses of *Berberis aristata* by various indigenous communities in Nepal

Districts where *B. aristata* is studied in Nepal	**Compounds behind the consented uses**	**Experimental organism**	**Quantity Amount of compound used/suggested**	**Extraction process**	**Name of ailments**	**Ethnopharmacological findings (Type of disease/ cure)**	**Indigenous communities utilizing the plant for traditional treatment**	**References**
Baglung	Berberine, palmatine, jatrorrhizine, oxyacanthine, and magnoflorine	Antioxidant, antimicrobial against bacterial strains	Root bark	Unknown	To treat jaundice, malarial fever, diarrhea, and peptic ulcers	Juice or decoction is used for jaundice, diarrhea, fever, and eye infection.	Magar	(Sapkota 1970)
Darchula	Berberine, oxyacanthine, aristataquinine, palmatine, berbamine, jatrorrhizine, and magnoflorine	Unknown	Bark	Unknown	To treat diarrhea, Piles, and malaria	Digestive aid, fever, cough, diabetes, skin infections	People of Darchula district, Bhotia and Thakuri	(Aryal *et al.* 2018)
Dhankuta	Berberine, palmatine, and jatrorrhizine	Antimicrobial, antifungal	Bark	Ethanol extraction	Unknown	Jaundice, diarrhea, fever	Tamang and Sherpa	(Adhikari *et al.* 2012)
Dolakha	Berberine, palmatine, jatrorrhizine, and oxyacanthine	In vitro experiments, antimicrobial, antifungal	Bark	Ethanol extraction	Inhibited growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*	Jaundice, diarrhea, fever	Tamang and Sherpa	(Adhikari *et al.* 2012)
Dolpa	Berberine	Hypoglycemic	Unknown	Ethanol extraction	Oral administration	Diabetes, jaundice, skin infections	Dolpo people	(Kunwar & Adhikari 2005)
Illam	Berberine, palmatine, jatrorrhizine, and magnoflorine	In vitro experiments showed potential antimicrobial activity against various pathogens, including *S. aureus, E. coli*,	Bark	Methanol extraction	Antimicrobial activity	Fever, jaundice, skin diseases, gastrointestinal disorders, Type 2 diabetes, diarrhea, hepatitis	Tamang and Sherpa	(Adhikari *et al.* 2012)

		and *C. albicans*, anti-inflammatory activity, anti-hyperglycemic effects						
Kaski	Berberine, palmatine, jatrorrhizine, magnoflorine, and oxyacanthine	In vitro and in vivo experiments done on mice, rabbits, and rats	Doses ranging from 10 mg/kg to 1000 mg/kg	Methanolic and aqueous extracts	Anti-inflammatory, antioxidant, and antimicrobial effects	Skin infections, ulcers, diarrhea, bronchitis, asthma, fever, jaundice, stomach disorders, respiratory infections, and fungal infections	Gurung	(Bastakoti 2019, Khadka *et al.* 2020)
Kathmandu	Berberine, berbamine, oxyacanthine, berberrubine, and columbamine	Antimicrobial, antifungal, and antiprotozoal	Doses ranging from 10 mg/kg to 500 mg/kg	Cold extraction method	Acts as an antimicrobial and antioxidant	Digestive disorders, liver diseases, respiratory infections, Malaria, skin infections, liver diseases, and diabetes	Newar	(Balami 2004)
Kavrepalanchowk	Unknown	Unknown	Unknown	Unknown	Unknown	Use most commonly with other plants for different purposes (medicines and fruits). Fruits, bark, root	Banskharka community	(Acharya 2022)
Makwanpur	Berberine, palmatine, jatrorrhizine, and columbamine	Antimicrobial, anti-inflammatory, and antiulcer	Unknown	Unknown	Unknown	Jaundice and liver disorders	Newah Community	(Joshi *et al.* 2020)
Nuwakot	Berberine, berbamine, and palmatine	In vitro: Antibacterial, antifungal, antiviral, antioxidant, anticancer	Stem	Soxhlet extraction	Jaundice, fever, skin disease	Various bacterial, fungal, viral infections, inflammation, cancer	Lama and Tamang	(Tamang 1970)
Panchthar	Berberine, palmatine, jatrorrhizine, oxyacanthine, and magnoflorine	Antimicrobial	Root bark	Unknown	Unknown	Jaundice, malarial fever	Limbu and Rai	(Gautam 2013)

Palpa	Berberine, palmatine, jatrorrhizine, and magnoflorine	In vitro: Antibacterial, antifungal, antiviral, antioxidant, anticancer	Stem and leaves	Soxhlet extraction	Unknown	Various bacterial, fungal, and viral infections, inflammation	Magar and Newar	(Thusa & Mulmi 2017)
Parbat	Berberine, palmatine, jatrorrhizine, and magnoflorine	In vitro: Antibacterial, antifungal, antiviral, antioxidant, anticancer	Root and Bark	Soxhlet extraction	Piles, sore throat and skin	Various bacterial, fungal, viral infections, inflammation, cancer	Gurung, Magar and Majhi	(Acharya 2022, Malla *et al.* 2014)
Ramechhap	Berberine	In vitro: Antibacterial, antifungal, antiparasitic, antioxidant, anticancer	150 mg/kg	Soxhlet extraction	Reduced serum glucose levels	Various bacterial, fungal, parasitic infections, inflammation, cancer, Type 2 diabetes	Tamang and Newar	(Pradhan *et al.* 2020)
Rasuwa	Berberine, palmatine, jatrorrhizine, oxyacanthine, and magnoflorine	Antioxidant, antimicrobial against bacterial strains	Root bark	Unknown	Unknown	Diarrhea and jaundice. Also used as a blood purifier.	Tamang and Sherpa	(Shrestha *et al*. 2014, Uprety *et al*. 2010)
Rolpa	Berberine, palmatine, jatrorrhizine, magnoflorine, oxyacanthine, berbamine, and columbamine	Mouse model, antimicrobial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans*	Unknown	Hydro-alcoholic extraction method	Anti-inflammatory, antipyretic, analgesic, antidiarrheal, antimicrobial, and antifungal properties	Various diseases including diarrhea, dysentery, fever, and infections	Magar	(Budha-Magar *et al.* 2020)
Rupandehi	Berberine, palmatine, jatrorrhizine, magnoflorine, oxyacanthine, berbamine, and columbamine	Unknown	Unknown	Unknown	Used in traditional medicine	Antibacterial, antioxidant, and anti-inflammatory	Unknown	(Acharya & Acharya 2009)

Sankhuwasabha	Berberine, palmatine, jatrorrhizine, magnoflorine, oxyacanthine, berbamine, and columbamine	Mouse model, antimicrobial activity against *E. coli*, *P. aeruginosa*	Bark	Unknown	Used in traditional medicine	Antibacterial, antioxidant, and anti-inflammatory	Limbu and Rai	(Adhikari *et al.* 2012)
Solukhumbu	Berberine, palmatine	In vitro experiments	100 mg/kg	Hot water extraction	Inhibited growth of *Leishmania donovani*	Leishmaniasis, Type 2 diabetes	Sherpa	(Bhatt *et al.* 2018, Rawal *et al*. 1970)
Surkhet	Berberine, palmatine, jatrorrhizine, magnoflorine, oxyacanthine palmatine	In vitro experiments	Unknown	Unknown	Unknown	Diarrhea, antihelminthic	Raji community	(Maria Almeida *et al*. 2020, Poudel & Singh 2016)
Syangja	Berberine, jatrorrhizine, palmatine, oxyacanthine	Antibacterial activity against gram-positive and gram-negative bacteria	Root, bark	Unknown	Unknown	Bacterial infections	Most people in the area possess traditional knowledge and customs.	(Aryal & Thapa 2019)
Taplejung	Berberine, palmatine, jatrorrhizine, magnoflorine, oxyacanthine	In vitro studies on bacterial and fungal strains	Plant paste	Soxhlet extraction method	Aid in digestion, such as tonics, laxatives, teeth pain, joining of bones, ringworms, and fungal infections.	Bacterial and fungal infections	Limbu, Sherpa, and Tamang	(Thapa 2021)
Terhathum	Berberine, palmatine, jatrorrhizine, oxyacanthine, and magnoflorine	Antioxidant activity, antimicrobial activity against bacterial strains	Fruit	Unknown	Jaundice, malaria, piles, sores, eye diseases	Antioxidant, bacterial infections	Vaidhya and Dhami-Jhankri	(Adhikari *et al*. 2012, Rai 1970)

Table 2. Physical appearance of the plant extracts and yield percentage

Plant parts	Phytochemicals	Color of the extract	Dry weight of the sample (g)	Dry weight of extract (g)	% yield of methanolic extracts (W/W)	TPC (mg GAE/g)	TFC (mg QE/g)
Leaf	Alkaloids, Flavonoids, Saponin, Terpenoids, Glycosides, oils, fats, reduced sugar	Oily dark green	200	24	12	108 ± 4.06	66.31 ± 5.05
Bark	Alkaloids, Flavonoids, Saponin, Steroids, Terpenoids, Glycosides, oils, fats, reduced sugar	Yellowish brown	200	20	10	83.30 ± 4.07	6 ± 0.57
Root	Alkaloids, Flavonoids, Saponin, Steroids, Terpenoids, Glycosides, oils, fats, reduced sugar	Yellow	200	20	10	40.794 ± 1.51	4.49 ± 1.54

The highest TPC was measured in the leaf sample extract, whereas the lowest TPC was in the root extract (Fig. 4a). TFC was calculated by quercetin equivalent per gram of dry weight of the sample. All samples tested for TFC showed flavonoid content, however, the quantity differed. The leaf extract had the greatest TFC, while the least flavonoid content was reported in roots. The TFC (mean ± standard error) was reported to be 4.49 ± 1.54, 6 ± 0.57, and 66.31 ± 5.05 mg QE/g for root, bark, and leaf, respectively (Fig. 4b). Evaluation of antioxidant properties of the plant extracts revealed highest percentage inhibition by aqueous fraction (93.14%), followed by EtOAc (92.36 %) (Fig. 4c).

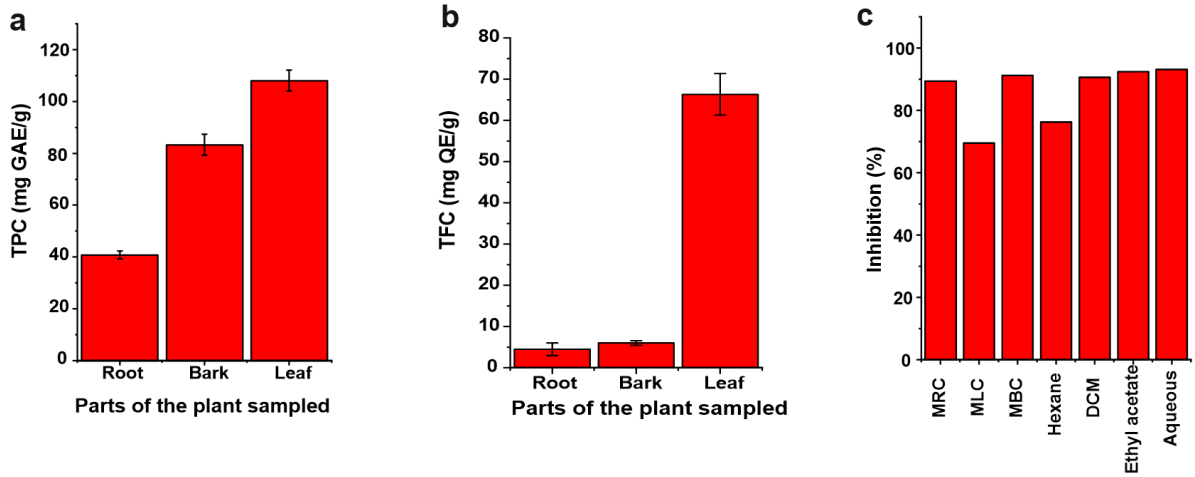

Figure 4. Analysis of different parameters of *B. aristata* plant. a) Total phenolic content of different extracts of *B. aristata*. b) Bar graph showing TFC (mg QE/g) of different extracts. c) The percentage inhibition of methanolic crude extract and the fractional sample of bark at a particular concentration (MRC: Methanolic root crude, MLC: Methanolic leaf crude, MBC: Methanolic bark crude, DCM: DCM fraction).

Biological activities

Antioxidant properties

During this assay, quercetin was taken as a standard and 30% DMSO was taken as a negative control. The crude extract which had an inhibition percentage above 50 was used to calculate the IC_{50} value (Fig. 5a). The antioxidant capacity of plant extracts was calculated in the form of a 50% inhibitory concentration (IC_{50}). The ethyl acetate fraction showed the highest antioxidant capacity even in the minimal concentration. Among the different plant parts sampled, crude extract from leaves showed the highest DPPH radical scavenging activity, while the bark showed the lowest. In addition, hexane as an extracting solvent was shown to have the highest DPPH value compared to the standard compound quercetin and other extracting solvents (Fig. 5a).

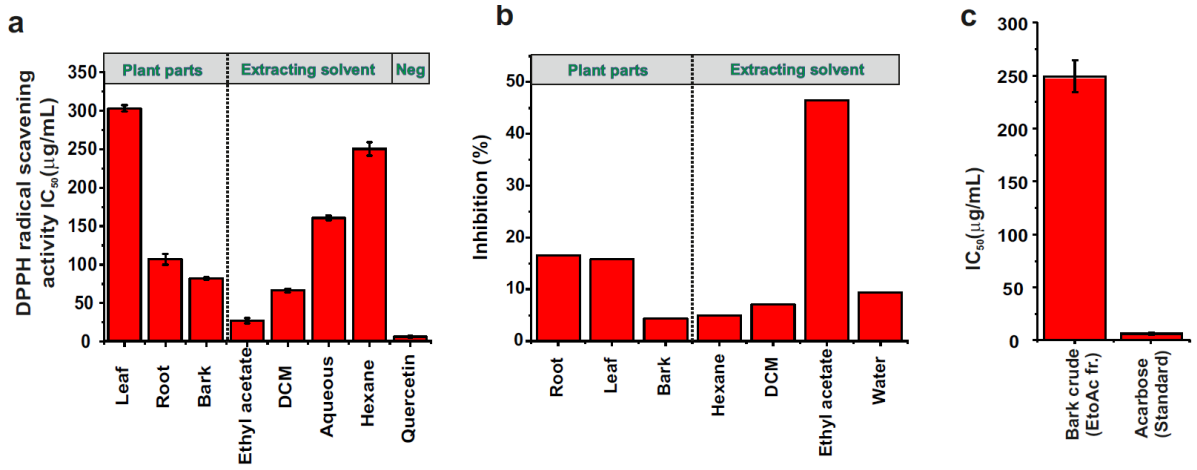

Figure 5. Different activities of *B. aristata* plant extract. a) IC_{50} values of different crude extracts and fractions of *B. aristata* and standard quercetin. b) The screening of α-amylase assay of methanolic crude samples and fractional samples (500 µg/mL). c) IC_{50} value of EtOAc fraction of bark crude.

α-amylase enzyme assay

The *in-vitro* α-amylase enzymatic assay performed for the crude extract of different parts of *B. aristata* and its fractional sample derived from methanolic extraction revealed that at a concentration of 500 µg/mL, the crude extract of different parts of the plant displayed percentage inhibitions below 50%. Consequently, the IC_{50} calculation was not performed due to the insufficient inhibition potential at half concentration. Next, the α-amylase assay was carried out on different solvent fractions namely hexane, DCM, ethyl acetate, and water of fractionated sample of bark. Among them, the ethyl acetate (EtOAc) fraction demonstrated the highest efficacy compared to other solvent fractions. As a result, the IC_{50} value was calculated. The substrate $CNPG_3$ was used during the screening of enzyme inhibition activity. The root extract showed higher inhibition potential (4.32%) than the bark extract (16.52%) at 500 µg/mL concentration. Likewise, the EtOAc fraction of bark crude has an inhibition value of 46.47% at 500 µg/mL, and IC_{50} value is 248.7 ± 14.25 µg/mL and that of Standard Acarbose is 6.02 ± 1.04 mg/mL. This enhanced inhibitory activity could be attributed to the presence of bioactive constituents within the EtOAc fraction, which potentially contributes to the reduction of blood sugar levels (Fig. 5b and c).

Antimicrobial activity

Screening for antimicrobial activity as performed in various Gram-positive and Gram-negative bacteria revealed crude leaf extract not to have any antimicrobial activity against any tested bacteria. However, the crude root and bark extract displayed some antagonistic activity against *S. aureus* with the ZOI of 15 mm and 16 mm, respectively. The bark extract showed a ZOI of 11 mm against *K. pneumoniae*. Notably, none of the crude extracts showed antagonistic activity against *E. coli* and *S.* Typhi (Table 3).

Table 3. Antimicrobial activity of some tested microorganisms

Pathogens	Concentration of the sample used (mg/mL)	Extracts obtained from	Diameter of zone of inhibition (ZOI, mm)
Staphylococcus aureus (ATCC 25293)	50	Root	15
	50	Bark	16
	50	Leaf	-
Escherichia coli (ATCC 25922)	50	Root	-
	50	Bark	-
	50	Leaf	-
Salmonella Typhi (ATCC 14028)	50	Root	-
	50	Bark	-
	50	Leaf	-
Klebsiella pneumoniae (ATCC - 13883)	50	Root	-
	50	Bark	11
	50	Leaf	-

Note: '-'represents an absence of the ZOI or any antagonistic effects of the plant extracts against the respective pathogens

MIC and minimal bactericidal concentration

The screening for MIC and MBC of all sample extracts did not actively inhibit microbial growth, however, the bark extract displayed a good ZOI against *S. aureus* and *K. pneumoniae*, root extract showed ZOI against *S. aureus*. Notably, the leaf extract displayed not any ZOI against any pathogens tested (Table 4).

Table 4. MIC and MBC of bark and root extracts against different pathogens

Tested microorganisms	Sample	MIC (mg/mL)	MBC (mg/mL)
S. aureus	Bark extract	1.562	6.25
	Root extract	1.562	3.125
K. pneumoniae	Bark extract	6.25	12.5
S. aureus and *K. pneumoniae*	Neomycin	0.125	1.562

Here, neomycin and DMSO were used as positive and negative controls, respectively. MIC values for bark and root for *S. aureus* were 1.562 mg/mL and that of positive control was reported at 0.125 mg/mL, while MBC for bark and root extracts were 6.25 mg/mL and 3.125 mg/mL, respectively. Similarly, MIC and MBC of bark crude extract against *K. pneumoniae* were revealed to be 6.25 mg/mL and 12.5 mg/mL, respectively. MIC and MBC values for root crude extract did not show a significant effect against *K. pneumoniae* and thus were not considered.

Analysis of Fourier Transform Infra-Red (FT - IR) Spectra

The subfractions 8-10 (10 %) and 23-24 (60 % ethyl acetate in hexane) fractions obtained from column eluent of hexane fraction of bark crude were used for the identification of various functional groups present in the active fraction. A sharp peak at 1750 cm^{-1} indicated the presence of a carbonyl functional group. The alcoholic -OH functional group peak was observed at 3200 -3600 cm^{-1} (Fig. 6a and b). The C-O stretch was given a stretching at 1100 -1200 cm^{-1} (Table 5).

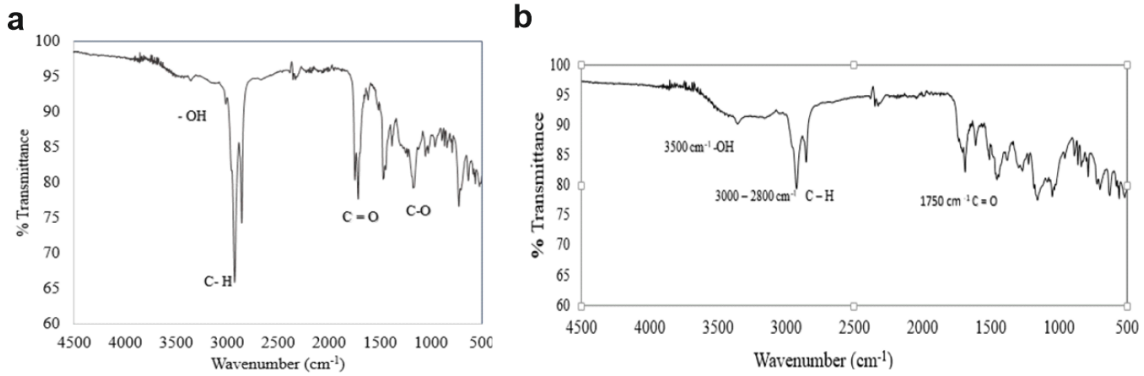

Figure 6. Fourier Transform Infra-Red (FT - IR) spectra of different fractions. a) FT-IR spectra of sub-fraction 8-10 (10 % EtOAc in hexane). b) FT- IR spectra of sub-fraction 23-24 (60% EtOAc in hexane)

Table 5. Functional group present in the single spot obtained from column chromatography.

Functional group	Assignment regions (cm^{-1})
-OH	3230 - 3550
O - H (carboxylic)	3300 - 2500
-C=O	1690 - 1760
C - H (stretching of alkane)	3000 - 2840
C - O Stretching	1260 - 1100

Discussion

Plant diversity

Nepal embraces a diverse and abundant plant population because of its distinct geographic and climatic characteristics. Nepal's geography located in the eastern Himalayas, varies from the low-lying Terai plains to the high Himalayan peaks. A staggering variety of plant species is supported by this varied geography, including subtropical forests, temperate woods, alpine meadows, and dry areas. More than 6,000 different vascular plant species, many of which are indigenous and unusual, can be found in Nepal. The diverse flora of the nation supports a wide range of biological processes, including the provision of fundamental ecological services like carbon sequestration and water purification as well as acting as a critical resource for

traditional medicine, agriculture, and livelihoods. Threats to Nepal's plant variety, nevertheless, include habitat loss, deforestation, climate change, and overuse of resources. To protect this unique plant variety for future generations and to ensure the resilience of Nepal's ecosystems in the face of increasing environmental problems, conservation efforts—both national and international—are essential (Bhaila *et al.* 2022).

Due to Nepal's extensive heritage of using plant-based treatments and the potential for finding new therapeutic agents, ethnomedical use of medicinal plants in Nepal is of great scientific significance. Over the course of many years, Nepal's traditional healers have amassed a wealth of knowledge about medicinal plants, employing them to cure a variety of illnesses. Scientific research into these folk medical practices has found that many plant species have bioactive chemicals that have pharmacological effects, such as antibacterial, anti-inflammatory, and antioxidant capabilities (Baral *et al*. 2011, Baral & Maharjan 2011). Additionally, ethnobotanical research has uncovered a variety of plant species with unrealized therapeutic potential, offering a useful resource for the discovery and development of new drugs (Bhaila *et al.* 2022). Discovering new bioactive chemicals is made more likely by Nepal's various ecosystems, which range from subtropical to alpine areas and support a large variety of plant species. Utilizing the scientific potential of ethnomedical knowledge in Nepal not only aids in preserving local customs but also presents prospects for the improvement of evidence-based healthcare practices and the creation of novel medications (Bhattarai *et al.* 2022). Therefore, the ethnomedical use of medicinal plants in Nepal constitutes an important research frontier with enormous promise for both traditional and contemporary medicine (Bhaila *et al.* 2022).

Traditional uses and ethnopharmacology

Despite its use reports being mentioned from 32 districts (Kunwar *et al.* 2022), we reported the extensive uses of *B. aristata* only from 24 districts, particularly from the rural areas of mid-hills and mountains. As it was used for the treatment of 20 types of ailments, the use for digestive system disorders and musculoskeletal diseases was found in most districts. Moreover, the use of the plant parts to cure jaundice and eye complaints was found highly consented to as the species was found to be used in 11 districts out of 24 districts reported. Even though its wider application in Nepal has not yet been thoroughly researched, the phytochemical, pharmacological, and molecular studies are underemphasized.

B. aristata is found distributed throughout Nepal from the mid-hills to the high hills. However, the regions of central and eastern Nepal are enriched, offering scopes of extensive indigenous uses. The species is common in areas where large numbers of ethnic groups reside, particularly in Koshi, Bagmati, and Gandaki. Gandaki province and Koshi province have over 90 ethnic groups.

As observed in the analysis, various local ethnic communities utilize *Berberis* for several reasons as mentioned in the results. The uses of this plant date back several decades when this plant was being used to cure different ailments. Though the historical archives and specialized research databases regarding Nepal's flora and botanical studies for the first usage of this plant as therapeutic implications are under-emphasized, it has still been an integral part of traditional medicine in South Asia for centuries. The exact date of the first usage of *B. aristata* in Nepal is difficult to determine due to the oral nature of traditional knowledge transmission and the lack of written records. This phenomenon is quite challenging to preserve as most information that is supposed to be transmitted to the next generation is not readily acquired and adjusted by the next generation. Indeed, their utilization is still unrecorded apart from some research reviews.

Nepalese indigenous communities have long employed *Berberis* as a remedy for diabetes mellitus, improving glucose metabolism, enhancing insulin sensitivity, and protecting pancreatic β-cells. Despite being unaware of the hypoglycemic compound berberine present in these plants, they have utilized it for low blood pressure. Moreover, berberine stimulates signaling pathways involved in glucose homeostasis, such as AMP-activated protein kinase. Additionally, it shows signs of possible inhibition of crucial enzymes involved in glucose metabolism, pointing to potential antidiabetic effects. In addition, *Berberis* exhibits antidiabetic and hepatoprotective properties by reducing liver enzyme levels, attenuating oxidative stress markers, and preventing liver cell damage (Ashrafizadeh *et al*. 2020, Jahan *et al.* 2022). Indigenous knowledge of various ailments remains unrecorded and lost over generations. Collaboration among traditional healers, pharmacologists, and researchers is imperative to validate its traditional use and fully utilize its potential in modern healthcare practices.

Phytochemicals, bioactive compounds, and biological properties

Plants' bioactive molecules and their therapeutic properties rely on their ecological niches. *Berberis* is rich in berberine, particularly in its roots (Chander *et al.* 2017, Furrianca *et al*. 2015), along with other bioactive compounds, such as aromoline, berbamine, berlambine, columbamine, palmatine, jatrorrhizine, karachine, oxyberberine, taxilamine, and oxyacanthine

(Bhardwaj & Kaushik 2012, Watanabe *et al*. 2006). The plant also contains carbohydrates, tannins, phytosterols, flavonoids (including quercetin, meratin, and rutin), and volatile oils (Furrianca *et al.* 2015).

Our result of free radical scavenging propensity supported by the TPC and TFC values is consistent with earlier investigations as done by Bhatt *et al*. (2018) and Chaudhari & Mahajan (2015). However, some parameters, such as the antioxidant efficacy of the extracts may differ from previous studies because of several factors to be taken into consideration, for instance, DPPH, experimental conditions, harvesting time, and geographical location. Support for the bark extract and root extract to have significant antidiabetic activity from various solvent extractions has also been supported by previous studies.

Considering the enzyme inhibition activity using the substrate $CNPG_3$, the root extract displayed higher inhibition potential than the bark extracts at 500 µg/mL concentration, and the EtOAc fraction of bark crude had a higher inhibition value compared to Standard Acarbose. The polyphenolic compound present in the polar solvent extract may be able to show inhibition potential of EtOAc fraction than crude extract. In a similar experiment, the methanolic bark extract showed the inhibition of 7.6% at 10 mg/mL concentration (Chakrabarti *et al.* 2014) and an antihyperglycemic effect was reported in Alloxan-induced diabetic rats by lowering the blood glucose level in a dose-dependent manner (Semwal *et al.* 2009). A remarkable effect was reported for root extract in antidiabetic activity which safely lowers the blood glucose level by acting like insulin or triggering the formation of insulin (Akhtar *et al.* 2008). The leaf extract was not found to be potent for antimicrobial activity, which may be due to the absence of bioactive metabolites. However, the root and bark extracts were found effective against gram-positive microorganisms which could be due to the cell structure and working mechanism (Cowan 1999). The bark and root extracts showed effective action against *S. aureus* bacteria. Their MIC was higher i.e., 1.562 mg/mL compared to the standard neomycin antibiotics, which have an MIC of 0.125 mg/mL. The MBC for the extracts was 6.25 mg/mL and 3.125 mg/mL, respectively, whereas, for the positive control, it was 1.562 mg/mL. These results indicate bark and root samples required a higher concentration to kill and inhibit microbial growth than available standard neomycin antibiotics. The bark extract had a good ZOI for *K. pneumonia* and its MIC and MBC values were 6.24 mg/mL and 12.5 mg/mL, respectively, while for positive control, they were reported to be 0.125 mg/mL and 1.562 mg/mL, respectively. The MICs of the root extract were found at low for *E. coli* and *S. aureus* while the stem extract showed a strong tendency against *Berberis cereus* and *Streptococcus pneumoniae* (Mazumder *et al*. 2011). The root and bark extracts showed strong pharmacological activities, such as antioxidant, antidiabetic, and antimicrobial, the reason which we argue may be the presence of active bioactive phytochemical compounds (Akhtar *et al.* 2018, Khan *et al.* 2020). Similar results have also been observed by Sood *et al.* (2019). The major functional groups such as -OH, O-H carboxylic stretch, -C=O, C-H, C-O stretching have been observed in IR analysis, indicating the presence of bioactive metabolites, such as berberine, chloroberberi, palmitin, etc.

Comparative assessment of traditional medicine and biological properties of *Berberis*

For decades now, conventional techniques and cultural perspectives regarding the use of medicinal plants have been incorporated into ethnomedicine and indigenous plant knowledge, along with larger ecological and sustainable approaches. Modern pharmacological research, in contrast, uses scientific approaches to identify bioactive molecules, elucidate mechanisms of action, and generate standardized drugs. Combining these strategies may generate complete, culturally competent healthcare solutions by metabolites of plant origin that balance conventional knowledge with empirical evidence.

B. aristata is traditionally employed as a blood purifier and as a tonic (Watanabe *et al.* 2006). The bark, wood, and roots of the plant are utilized to treat various ailments, including jaundice, malarial fever, diabetes, diarrhea, and skin diseases (Chapagain *et al*. 2018, Manandhar 2002, Rajbhandari 2001, Watanabe *et al*. 2006). In specific areas, such as Baglung and Terhathum districts, it is used as a laxative and for the management of backache, fractures, rheumatism, eye ailments, fever, and weakness. In Kaski (Bhasin *et al.* 2022) and Rasuwa (Shrestha *et al.* 2014) districts, it is utilized to control fever, jaundice, malaria, diarrhea, swellings, eye problems, and rabies. The Gurung, Tamang, and Sherpa communities use *B. aristata* for the management of skin disorders, jaundice, and gastric problems. Apart from medicinal uses, the ripe fruits of *Berberis* are consumed both fresh and in the preparation of alcoholic beverages (Dangol *et al.* 2017). The inner bark of the stem and roots is used to produce a yellow dye. Additionally, the spiny branches of the plant are occasionally utilized to construct fences around fields in village settings (Manandhar 2002).

The traditional utilization of *Berberis* for therapeutic purposes is attributed to the presence of the bioactive compound berberine. Despite the lack of awareness among local Indigenous people regarding the specific bioactive molecules present in the plant, they have been utilizing it for generations, passing down the knowledge through the years. Through scientific validation, we have confirmed the presence of these compounds and their biological roles. Berberine has been scientifically shown to possess antimicrobial, anti-inflammatory, antioxidant (Gaurav *et al.* 2020, Jahan *et al*. 2022), anticancer, and

hepatoprotective properties (Gilani & Janbaz 1995), rendering it a sought-after compound in both traditional and modern medicine. Berberine exhibits antagonistic activity against a broad spectrum of pathogens, further enhancing the value of this compound and the plant itself (Bhaila *et al.* 2022). Moreover, berberine shows efficacy in managing chronic inflammatory ailments, including rheumatoid arthritis and inflammatory bowel disease (Habtemariam 2016, Jahan *et al*. 2022). Additionally, the antioxidative, chemo-preventive, and anticancer effects of various *Berberis* extracts have increased the demand for this botanical in both traditional and contemporary medical practices.

We argue that the experiment that has been done in one place may not reflect the overall status of this plant used in various parts of Nepal. For proper documentation and preservation, it is crucial to continue documenting and preserving indigenous knowledge regarding the traditional uses of *Berberis* in various parts of the country. This involves documenting how *Berberis*-based treatments are prepared, dosed, and administered as described by the native populations. Such efforts can ensure the preservation and transmission of valuable traditional knowledge to future generations. In addition, collaborative efforts between scientists and indigenous communities can lead to the development of standardized formulations derived from *Berberis*. This involves optimizing extraction methods, developing quality control protocols, and exploring novel delivery systems to ensure the safe and effective utilization of *Berberis*-based products. Moreover, sustainable harvesting practices and rigorous clinical trials should be undertaken to evaluate the safety and efficacy of *Berberis* extracts or isolated compounds in specific health conditions. These trials can provide evidence-based data on the effectiveness of *Berberis*-based interventions and their potential integration into modern medical practices. Moreover, encouraging collaboration between indigenous healers, traditional medicine practitioners, and scientific researchers can foster a fruitful exchange of knowledge and ideas. This collaboration can help bridge the gap between traditional and modern medicine systems, leading to the development of integrated healthcare approaches.

Overall, a multidisciplinary approach that values and incorporates indigenous knowledge while utilizing scientific methods can further our understanding of the traditional medicine and biological properties of *Berberis*. This approach has the potential to unlock new therapeutic possibilities and promote the sustainable use of *Berberis* for the benefit of both indigenous communities and global healthcare practices.

Conclusions

This study offers accounts that conform to the traditional uses of *B. aristata* with its biological properties in treating various disorders, including diabetes and microbial infections. Our study identified 14 distinct ethnic communities residing in 24 districts that utilize *B. aristata* for diverse therapeutic purposes. The Tamang indigenous group emerged as the primary users. Most of the ethnic communities employing *B. aristata* for medicinal reasons are concentrated in Bagmati and Koshi Province, followed by Gandaki Province. As it was used for the treatment of 20 types of aliments, the use for jaundice and eye complaints was found highly consented as the species was found to be used in 11 districts out of 24 districts reported. Phytochemical analyses revealed the ethyl acetate fraction to be the most effective for free radical scavenging activity followed by DCM, and water fractions. The α-amylase inhibition activity of different fractions seems quite deplorable. Only the ethyl acetate fraction had shown certain inhibition properties. The methanolic extract of bark showed significant antimicrobial activity against *S. aureus* and *K. pneumoniae*.

Moreover, traditional uses of *Berberis* in Nepal for gastrointestinal problems, skin conditions, diabetes, and infections are supported by current research demonstrating the antimicrobial, anti-inflammatory, and antioxidant effects of crude extracts of *Berberis*. These traditional uses, particularly for gastrointestinal problems, diabetes, and skin diseases draw attention to the potential of *B. aristata* as an evidence-based treatment that fuses conventional wisdom with cutting-edge research and motivates further study of its bioactive constituents and clinical applicability. This convergence highlights the integration of traditional knowledge and phytochemical analyses that could potentially lead to the development of novel therapeutic agents based on *Berberis* and its constituents.

Declarations

List of abbreviations: AST: Antimicrobial susceptibility testing, CFU: Colony-forming units, DCM: DCM fraction, DPPH: 1, 1-diphenyl-2-picrylhydrazyl, ELISA: Enzyme-linked Immunosorbent Assay, EtOAc: Ethyl acetate, IC_{50}: 50% inhibitory concentration, MBC: Methanolic bark crude, MBC: Minimal bactericidal concentration, MIC: Minimal inhibitory concentration, MLC: Methanolic leaf crude, MRC: Methanolic root crude, NA: Nutrient agar, TFC: Total flavonoid content, TLC: Thin-layer chromatography, TLC: Thin-layer chromatography, TPC: Total phenol content, ZOI: Zone of inhibition.

Ethics approval and consent to participate: Informed consent was taken from all the participants.

Consent for publication: No personal data is published.
Availability of data and materials: All data collected in the study has been presented and visualized in the manuscript.
Conflicts of interest: The authors declare to have no conflict of interest.
Funding: Applicable.
Authors' contributions: IP, KB, RB, KB, and BB carried out laboratory experimentation and chemical analysis. KB and BB performed a thorough ethnobotanical survey, data collection, and detailed literature survey. RK provided critical comments and suggestions to improve the manuscript. BB supervised the research, prepared figures, performed statistical analysis, and wrote the manuscript with the help of KB, IP, and RB. All authors approved the final version of the manuscript.

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