

## Article

# Quantitative Ethnomedicinal Status and Phytochemical Analysis of *Berberis lyceum* Royle

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**Citation:** Nazir, N.; Rahman, A.; Uddin, F.; Khan Khalil, A.A.; Zahoor, M.; Nisar, M.; Ullah, S.; Ullah, R.; Ezzeldin, E.; Mostafa, G.A.E.; et al. Quantitative Ethnomedicinal Status and Phytochemical Analysis of *Berberis lyceum* Royle. *Agronomy* **2021**, *11*, 130. <https://doi.org/10.3390/agronomy11010130>

Received: 26 November 2020

Accepted: 6 January 2021

Published: 11 January 2021

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**Abstract:** *Berberis lyceum* Royle has such pronounced medicinal values that it is used as cure of many diseases and has exhibited great therapeutic effects among the local communities throughout the world. The present research was carried out to evaluate the quantitative ethnobotanical status and phytochemical analysis of *B. lyceum*. Regular field trips were arranged to the study area (Shangla District) in August 2017 to October 2019 and interviews with 100 residents (age range: 30 to 50 years) were conducted. The approach adopted for ethnobotanical data was semi-scientific as the inhabitants were not aware about the modern names of some diseases and therefore physician prescriptions were also consulted. Ethnobotanical data were examined using relative frequency of citation and % use value. The % use value of *B. lyceum* indicates that the people of District Shangla mostly used it for curing of different diseases. In spite of tremendous uses the plant still survived in this area mainly due to the non-accessibility for humans of the mountain tops; otherwise, increase in anthropogenic activities even in these hilly areas poses a threat of the eradication of this plant. To correlate the folkloric uses with its phytochemical composition, HPLC (high performance liquid chromatography) analysis was performed and a total of six phenolic compounds (quercetin, chlorogenic acid, berberine, rutin, mandelic acid, and hydroxy benzoic acid) were identified in its root. As most of the health complications are correlated to oxidative stress therefore in vitro antioxidant activity were also performed using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azinobis-3-ethyl benzo thiazoline-6-sulfonic acid) assays. The observed antioxidant potential may most probably be due to berberine and chlorogenic acid that were present in highest concentration in the analyzed extract. The effectiveness of the selected plant as remedy for a number of diseases (that were pointed out by the local community) may be due to its phytochemical composition especially berberine and chlorogenic acid as oxidative stress is the root cause of many diseases. The plant extract exhibited high antioxidant potential (DPPH IC<sub>50</sub> = 165 µg/mL; ABTSIC<sub>50</sub> = 110 µg/mL) in relation to the detected concentration of berberine and chlorogenic acid. It can be inferred from experimental results that the ethnopharmacological efficacy of this endangered species may be due to its phytochemical composition and antioxidant activities. This case study helps to revive the importance of *B. lyceum* in local communities and emphasizes the need for its conservation.

**Keywords:** antioxidant activity; *Berberis lyceum* Royle; conservation status; ethnobotanical status; HPLC-UV

## 1. Introduction

Ethnobotany is branch of botany that correlates the importance of a plant with its uses as remedy for different health complications in the past as well in the present by local communities. In short it is a biological, economic, and cultural inter-relationship between the people and plants of the studied area. Ethnobotanical studies enhance the plant biodiversity knowledge as well as human awareness about their uses, applications, and ensure its natural conservation [1]. The World Health Organization (WHO) has estimated that due to poverty and lack of access to modern medicine almost 65–80% of the world's populace of developing countries mostly use medicinal plants for their primary healthcare and medication [2].

Even in developed countries, these days natural products are the first priority of people as they are associated with no or insignificant side effects. They may be obtained from animals, plants and microorganisms; however, amongst them plants products are more reliable and green renewable sources [3]. It is obvious from human history that mankind has used herbal products to cure different diseases [4,5]. Almost all plants contain bioactive compounds capable of curing a number of diseases. However, some of them contain higher amounts of certain phytochemicals capable of being used as medicine. Such plants have been characterized as medicinal plants and according to the WHO the term medicinal plants could be used for plants containing ingredients with great therapeutic and remedial functions or it can be used as a substrate in the synthesis of beneficial drugs [6,7].

Plants in general, and high-valued medicinal plants specifically, have a long history of use as a source of cheap and effective remedy for various ailments. Like other developing countries, a significant portion of the population in Pakistan also uses medicinal plants traditionally for therapeutic purposes [8]. In spite of their uses in folkloric medicines, the modern pharmaceutical industry is also dependent on plant phytochemicals. Despite the fact that about 25% of the modern drugs are originated from plants, the use of plants in modern medicine has considerably increased recently [9–11].

The *Berberis* is a major dicotyledonous genus belonging to the Berberidaceae family; the plants of this family are commonly woody with evergreen spiny leaves and grow in the form of bushes. According to recent reports [12,13], this family is comprised of 17 genera and 650 species. The *Berberis* genus has wide distribution all over the world mainly in Asia, Europe, Africa, and America. In Pakistan, species belonging to this genus are found across most of the mountainous areas at 1400–3500 m above the sea level (in Baluchistan, Khyber Pakhtunkhwa, and Punjab provinces, especially in northern areas, Swat and Azad Kashmir) and its chemical constituents are used both in traditional and modern medicines [12,13].

Among *Berberis* species, *Berberis lyceum* Royle has a specific position in numerous traditional medicines worldwide. *B. lyceum* is commonly known as Indian lycium, Indian barberry, and English barberry, while in Pakistan it is known as “Zyarah larghai” in Pashtu and “Kashmal” or “Kasmal” in Urdu [14]. The tribal people of Kashmir valley mostly used its roots for treating hemorrhagic dysentery. Every part of *B. lyceum* has great medicinal value [15]. The roots of *Berberis* species are used as an antipyretic, diaphoretic and anti-periodic while its bark is used as a tonic and anti-periodic. The plants belonging to this genus have also demonstrated activities like hepatoprotective, cardiovascular, anti-cancerous, and antimicrobial activities, and as a safer remedy for gastrointestinal disorders in humans [15]. The root powder of *B. lyceum* is used to relieve muscular and rheumatic pain while stem powder is used for curing diarrhea, and jaundice, while its leaves are used as a tea substitute. The plant extract is used to cure spleen diseases, cough, chest, throat, and intestinal problems, burning of the eye, diarrhea, and healing of fractured bones [15–17]. Fruits of *B. lyceum* are used as coagulant, to treat stomach-ache, hypo-

glycemic, anti-carcinogenic, anti-inflammatory, and antipyretic agents. The whole plant is used by the local inhabitants as a remedy for sore eyes, broken bones, ulcer, jaundice, and rheumatism [18–20]. The stem bark, root bark and fruit extract have also exhibited antihyperglycemic, antihyperlipidemic, anticancer, antioxidant, antitumor, antiurolithic, anthelmintic, immunity enhancing and wound healing effects in animal models [15,21,22].

Being a woody plant in hilly areas like District Shangla where there is no natural gas supply, this species is considered an endangered species. In spite of its medicinal uses the local communities are using it for burning to cook food. However, with the advancement of communication systems and education facilities, the local communities have started to rely on modern approaches and in the near future the traditional knowledge about this plant might be totally replaced with modern knowledge [14]. Therefore, it is necessary to preserve the traditional knowledge and correlate it with the modern approaches. *B. lycium* contains important phytoconstituents like berberine, plamitine, berbamine, baluchistanamine, karakoramine, gilgitine, jhelumine, punjabine, sindamine, chinabine, and umbellatine. Among them the major phytochemical is berberine which is an isoquinoline alkaloid isolated from the roots and bark. Berberine and palmatin alkaloids extracted from *B. lycium* were reported to have strong antioxidant, proapoptotic effect, and growth inhibitory activities against microorganisms including bacteria, fungi, and protozoa [23].

The aim of present study is therefore to revive the importance of this endangered plant species [24,25]. The collected ethnopharmacological data have been correlated with its phytochemical composition and antioxidant potentials [21,26]. This ethnobotanical study in form of a case report will help researchers to recognize the medicinal importance of *B. lyceum* and will open a new dimension for further studies on its chemical profile and biological activities.

## 2. Materials and Methods

All experimental protocol/field work was carried out according to research and ethical committee of the Department of Botany, University of Malakand, Pakistan (BOT/2020/1).

### 2.1. Geographical Overview of District Shangla (Study Area)

District Shangla is a hilly area comprising Besham, Shahpur, Aloch, Chakesar, Damorai and Olander sub tehsils [27] with; 34, 31 to 33°, 08° N latitude and 72, 33 to 73°, 01° E longitude, at an elevation of 3164 m above sea level with a total area of 1586 square kilometers, located in the north of Peshawar the capital of Khyber Pakhtunkhwa province Pakistan (Figure 1). District Shangla have renowned mountain tops like Spinghar (4464 m), Takhtghar (4332 m), Dobandighar (4063 m), Yakhghar (4179 m), Koparsar (3278 m) and Spinsar (3181 m) [28,29]. The extreme temperature of the area is 38 °C in summer however in winter season it goes down up to −2 to −5 °C. The soil is sandy loam to clay. Rainy season starts from July to September while snow falls from December to February [30].

### 2.2. Field Interviews and Ethno Medicinal Data Collection

To study the ethno-medicinal status of *B. lyceum* Royle in District Shangla, regular field visits were organized from August 2017 to October 2019. This ethnobotanical survey was planned to gather the folkloric knowledge about the *B. lyceum* and inform the youngsters of the local community about its importance. A semi-structured questionnaire was used to collect the data from inhabitants. The study was based on direct communication with the local people through corner meetings and group discussions according to the method reported by Martin [31].



**Figure 1.** Geographical location of the District Shangla (study area), Khyber Pakhtunkhwa, Pakistan (adopted from Tabani, et al., 2016).

A total of 100 well aware inhabitants were interviewed with ages ranging from 30 to above 51 years. Informed consent was taken in written from all participants. They were asked several questions about their traditional knowledge, diseases for which it is used as treatment, plant portion used in their preparation and mode of administration, about its conservation and causes of eradication. Conversations were generally conducted in local language (Pashto) and then translated into English. The documented data were analyzed for variables like age-wise percentage of folklore uses, way of use, reason of extinction/need for survival, and its existence etc. using Microsoft excel 2007. As the inhabitants of this area are scarcely educated and were not aware about modern scientific terminologies like antibiotics, antifungal etc., physician prescriptions (in case when a patient was also examined by a doctor in hospital) were also consulted.

### 2.3. Quantitative Ethno-Medicinal Data Analysis

According to the reported method [32] quantitative ethno-medicinal data were analyzed. The Relative Frequency Citation (RFC) index was calculated to check the indigenous importance of this pharmacologically important plant species. RFC was calculated as

follows from Equation (1), from the frequency of citation (FC, the number of informers mentioning the usage of the species) divided by the total number of informers (N) in the study [33]:

$$RFC = FC/N \quad (0 < RFC < 1) \quad (1)$$

Note: RFC values generally range from 0–1 where 0 = nobody quotes the plant species as useful and 1 = all the informers mentioned the plant species as useful. FC—Frequency of citation.

The use value that describes the relative significance of locally known plant was determined [31] using the following formula:

$$\text{Use value} = \frac{\sum U_i}{N} \quad (2)$$

where  $\sum U_i$  is the total number of uses mentioned by each informant while N is the total number of informants.

#### 2.4. *Berberis lyceum* Royle Collection and Extraction

*B. lyceum* was collected from District Shangla Khyber Pakhtunkhwa, Pakistan in the years 2017–2019. The plant was identified by plant taxonomist Prof. Mehboob-UR-Rahman, PGC. Swat, KP, Pakistan. The plant specimens were deposited in the Botanical-Garden-Herbarium, University of Malakand, Pakistan with voucher number of B.G.H-UOM-169. The extraction process was carried out using the previously reported method [34]. About 3.5 kg of dried *B. lyceum* root were crushed through a mechanical grinder into fine powders which were then macerated in 80% methanol for 14 days with periodical shaking. Filtration was carried out through muslin cloth followed by Whattman filter paper. The filtrates were converted into a semisolid mass under reduced pressure in the rotary evaporator (Schwabach: 4000; Heidolph-Laborota-Germany) at 40 °C. The semisolid mass was then solidified (2.5 g end yield) in open air.

#### 2.5. HPLC-UV Characterization of *Berberis lyceum*

For the preparation of *B. lyceum* root methanolic extract (Me. Ext), 1 g root powder was mixed with methanol and water (20 mL) in ratio of 1:1 v/v. This sample mixture was heated for 1 h at 70 °C in a water bath and finally centrifuged for 10 min at 4000 rpm and then filtered through Whattman filter paper after cooling. Finally, 2 mL of each sample was transferred into HPLC vials. Detection and identification of phenolic compounds were performed using the reported method [35,36]. For the identification of phenolic antioxidants, the UV (ultraviolet) detector was set at 320 nm. The identification of bioactive compound was done by comparing its retention time with those of standards used (Quercetin, chlorogenic acid, berberine, mandelic acid and hydroxy benzoic acid (Sigma Aldrich; London, UK)). The peak area of the sample and standards reference were inserted in following formula to calculate the concentration of a given ingredient:

$$C_x = \frac{A_x \cdot C_s \left(\mu \frac{\text{g}}{\text{mL}}\right) \cdot V(\text{mL})}{A_s \cdot \text{Sample weight (g)}} \quad (3)$$

where  $C_x$  = concentration of sample;  $A_s$  = peak area of standard reference;  $A_x$  = peak area of sample (g);  $C_s$  = concentration of standard reference (0.09  $\mu\text{g/mL}$ ).

#### 2.6. Antioxidant Scavenging Assays

##### 2.6.1. DPPH (2,2-diphenyl-1-picrylhydrazyl) Free Radical Scavenging Activity

The chemicals used in antioxidant tests were DPPH (Sigma Aldrich; St. Louis, MO, USA), 2,2'-azinobis-3-ethyl benzo thiazoline-6-sulfonic acid (ABTS) and ascorbic acid (Sigma Aldrich; Darmstadt, Germany). Antioxidant activity of *B. lyceum* root Me. Ext was determined against DPPH by Brand-Williams et al.'s method [37]. DPPH (24 mg) was added to 100 mL of methanol to prepare its stock solution. Me. Ext (1 mg/mL) solution was

also prepared in methanol and then dilutions 1000, 500, 250, 125 and 31.05 µg/mL were prepared from it. About 0.1 mL of each dilution was added to 3 mL DPPH solution and then incubated for 30 min at 25 °C. Finally absorbance was recorded at 517 nm by UV spectrophotometer. As a negative control a methanolic solution of DPPH was used. Ascorbic acid was used as a positive control. The same procedure mentioned above was used for the reaction mixture of the positive control and absorbance was measured at 517 nm. All results were recorded in triplicate as the mean ± SEM (standard error of mean). Percent DPPH scavenging activity was measured using the formula:

$$\% \text{ Free radicals Scavenging potential} = \frac{\text{Blank sample absorbance} - \text{sample absorbance}}{\text{Blank sample absorbance}} \times 100 \quad (4)$$

### 2.6.2. ABTS (2,2'-azinobis-3-ethyl benzo thiazoline-6-sulfonic acid) Free Radical Scavenging Activity

ABTS scavenging activity of *B. lyceum* root Me. Ext was carried out using Re et al.'s method [38]. ABTS free radicals were prepared by taking 7 mM ABTS and thoroughly mixing it with 2.45 mM potassium per sulfate solution then keeping it in the dark overnight for incubation. Me. Ext (300 µL) were mixed with ABTS (3.0 mL) solution and then absorbance was recorded 745 nm after 5 min via a double beam spectrophotometer. Ascorbic acid was used as the positive control in this assay as well. All results were recorded in triplicate. Percent ABTS free radicals scavenging activity was calculated by Equation (4).

### 2.7. Statistical Analysis

All in vitro experiments were performed in triplicate by applying two-way ANOVA followed by the Bonferroni Post-test to determine the values of *p*. The *p*-value less than 0.05 ( $p \leq 0.05$ ) was considered as statistically significant. The results were represented as the mean with SEM. For quantitative analysis of ethno medicinal data, the Relative Frequency Citation (RFC) index was estimated to check the indigenous importance of pharmacologically significant plant species. Linear regression was used to calculate the IC<sub>50</sub> from % inhibition of data of DPPH and ABTS by different concentrations of test samples using the Excel program 2007.

## 3. Results and Discussion

### 3.1. Informant Demographic Status

The age wise groups interviewed were: 22% belonging to the 30–40 years age group, 62% from the 41–50 years age group, while above fifty years were 16%.

### 3.2. Folk Uses of *B. lyceum* Royle

The opinions of District Shangla peoples were different and interesting about *B. lyceum* Royle. Their responses about the medicinal uses of *B. lyceum* among different age groups are summarized in Table 1. Almost all age groups have used this plant for the treatment of various diseases like hypertension, diabetes, stomach diseases/ulcers, dry cough, throat diseases, malaria, gall bladder stones and inflammation, wounds and damage bones, kidney stone removal, enhancement of liver function, arthrosclerosis, cholesterol control, neurological diseases, digestive disorders, urinary bladder diseases, eye burning sensation, diarrhea, dysentery, arthritis, jaundice, urinary tract disorders, bile duct disorders, respiratory tract diseases, cardiovascular dysfunction, cholera, dental/toothache, epilepsy, hepatitis, heart-burn, skin diseases, burning sensation of urine, and lung diseases. Some other therapeutic uses like anti-cancer, anti-bacterial, anti-fungal, anti-parasitic, anti-depressant, antiseptic, anti-pyretic/pylori, as a laxative and blood purifier were also pointed out by some informers.

**Table 1.** Response of different age groups regarding the folk uses of *Berberis lyceum* Royle for curing different diseases.

Folk Use	FC (30–40)	FC (41–50)	FC (≥51)	∑Ui	%Use Value
Hypertension	7	5	3	15	12.2
Anti-diabetic	6	6	2	14	11.4
Stomach diseases/Ulcers	2	4	2	8	6.5
Dry cough and throat diseases	3	3	0	6	4.8
Anti-malarial	6	6	2	14	11.4
Gall bladder stones and inflammation	2	4	2	8	6.5
Wounds and injury of bones	0	2	0	2	1.6
Kidney stone removal	6	6	2	14	11.4
Enhancement of liver function	1	1	0	2	1.6
Arthrosclerosis	0	2	0	2	1.6
Cholesterol control	6	6	2	14	11.4
Neurological diseases	0	1	2	3	2.4
Digestive disorders	0	2	3	5	4.1
Urinary bladder diseases	6	6	2	14	11.4
Eye burning sensation	2	4	2	8	6.5
Diarrhea	1	3	1	5	4.1
Dysentery	0	2	0	2	1.6
Anti-arthritic	1	3	1	5	4.1
Jaundice	6	6	2	14	11.4
Urinary tract disorders	0	2	0	2	1.6
Anti-pyretic/ <i>H. pylori</i>	0	2	0	2	1.6
Bile duct disorders	6	6	2	14	11.4
Respiratory tract diseases	0	2	0	2	1.6
Anti-microbial/Anti-septic	0	4	0	4	3.3
Cardiovascular dysfunction	0	1	1	2	1.6
Laxative	0	3	0	3	2.4
Anti-cancer	0	3	4	7	5.7
Cholera	6	6	2	14	11.4
Dental/toothache	0	2	0	2	1.6
Anti-bacterial	0	2	0	2	1.6
Anti-fungal	1	4	0	5	4.1
Epilepsy	0	4	0	4	3.3
Anti-parasitic	0	3	4	7	5.7
Anti-depressant	0	2	0	2	1.6
Blood purifier	1	4	0	5	4.1
Hepatitis	0	2	0	2	1.6
Heart-burn	0	3	4	7	5.7
Skin diseases and wounds	1	4	0	5	4.1
Burning sensation of urine	0	2	0	2	1.6
Lung diseases	0	3	4	7	5.7
Antibiotic	2	0	2	4	3.3

FC = Frequency of citation, ∑Ui = The total number of uses mentioned by each Informer while  $n = 3$  which is the total number of informers.

Table 1 indicates that the first age group (30–40 year) used *B. lyceum* mostly for hypertension and as anti-diabetic and least for stomach ulcer, dry cough and throat diseases. The second age group (41–50 year) used this plant as remedy for hypertension, diabetes, stomach ulcer, dry cough, throat diseases, gall bladder stone removal, inflammation and as anti-microbial/anti-septic, laxative, anti-cancer and to cure skin diseases and for wounds healing. The third age group (≥51 years) have used it as a remedy for hypertension, cancer, digestive disorders, neurological disorders, anti-diabetic, and stomach ulcers. This group have rarely used it as anti-malarial, heart-burn and for lung diseases.

### 3.3. Conservation Indicator of *Berberis lyceum*

Table 2 shows the conservation of *B. lyceum* in District Shangla, Khyber Pakhtunkhwa, Pakistan. According to the opinions of most people this plant is not endangered as it is safeguarded by humans (31.7%) and is non-accessible to human (47.1%) as it grows on hill tops while a lesser number of participants give other reasons like the plant having no storage of water due to its growth on slopes (1.9%) and the presence of thorns and spikes (1.9%) that is why it is not eaten by grazing animals, in opinion of few, there being no grazing (6.7%) in this area, the area being less populated (0.9%) with no sliding and natural disasters (4.8%). A few of them were of the opinion that this plant cannot be replaced by other plants (1.9%) and will therefore survive in this area. The % use value also indicated that the main reason of survival is due to the non-accessibility for humans.

**Table 2.** Reasons for survival of *Berberis lyceum* Royle in District Shangla, Khyber Pakhtunkhwa, Pakistan.

Reason for Survival	FC 30–40	FC 41–50	FC ≥51	∑Ui	%Use Value
Safety from humans	5	22	6	33	31.7
Non accessibility for humans	16	26	7	49	47.1
No storage of water in this plant	2	0	0	2	1.9
Presence of thorns and spikes	1	0	1	2	1.9
No grazing	1	5	1	7	6.7
Less population	2	0	0	2	1.9
Not eliminated	0	5	0	5	4.8
No human activities	1	5	1	7	6.7
No sliding and natural disasters	0	5	0	5	4.8
No farming	2	0	0	2	1.9
No replacement	0	1	1	2	1.9

FC = Frequency of citation, ∑Ui = the total number of use mentioned by each Informer while  $n = 3$  which is the total number of informers.

### 3.4. Eradication Indicator of *Berberis lyceum*

In the present case study, the root causes of extinction of this medicinally important plant *B. lyceum* were also investigated. The main reasons for its extinction are anthropogenic activities like deforestation, house making, artificial disasters like fire, the accessibility for human being and also natural disasters like sliding, quicksand etc. (Table 3) as pointed out by participants. About 50% of the participants were of the opinion that the anthropogenic activities in this area are the main reason for its extinction. Other reasons like sliding/disaster, population growth, flood, replacement by grasses and shrubs, construction, agriculture and farming lands, roads, lack of knowledge about medicinal use, selling in market, replacement by other ornamental plants and crops, invasive species, cutting for fuels, replacement by highly yield flowering plants, highly expensive varieties, being eliminated for grasses, digging of mountains for farming lands, exporting purposes, eradication by herbicides, soil erosion, more rainfall, unsustainable use and deforestation were also pointed out by some participants. The informants were of the view that the main reason of the eradication is anthropogenic activities that need to be minimized otherwise this high valued medicinal plant will be extinct from this area within a few decades.

### 3.5. Identification of Phenolic Compounds

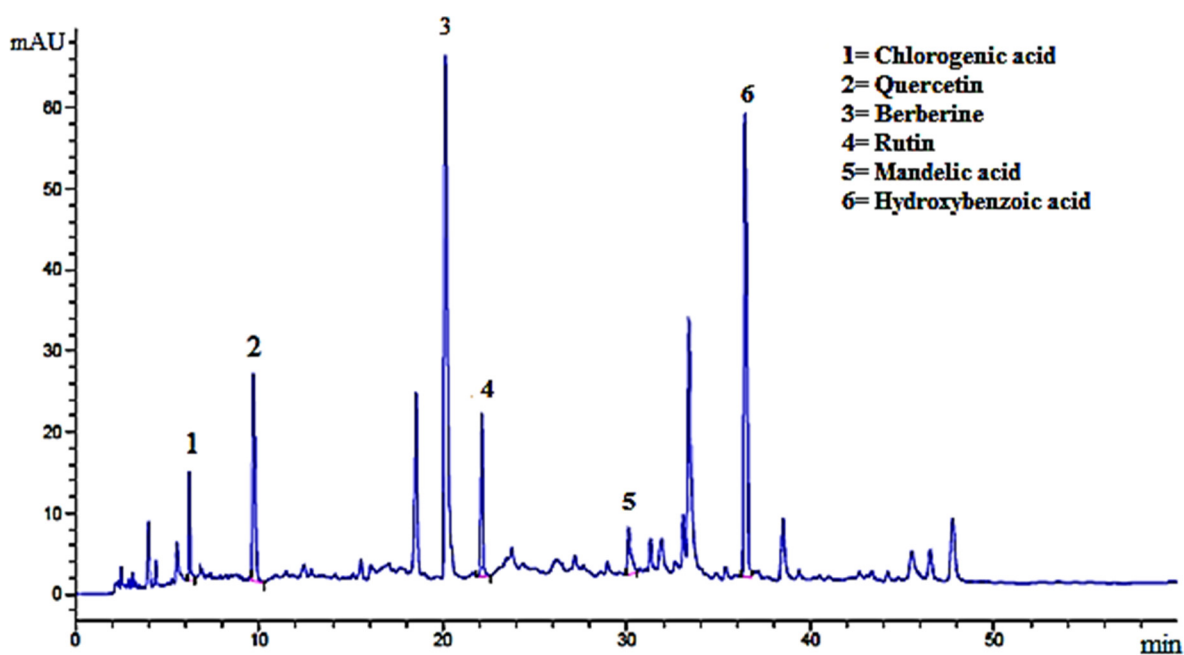
The HPL-UV chromatogram of *B. Lyceum* root Me. Ext is shown in Figure 2. A total of six phenolic compounds were identified when compared to the standard chromatogram. The respective peak position, retention time and concentration of identified phenolic compounds are given in Table 4. The identified compounds from the HPLC chromatogram as shown in Figure 2 were quercetin, chlorogenic acid, berberine, rutin, mandelic acid and hydroxy benzoic acids with retention times of 9.6, 6.2, 20.1, 22.2, 30.1, and 36.4 min, respectively.



**Table 3.** Reasons for extinction of *Berberis lyceum* from the selected locality.

Reason for Extinction	FC 30–40	FC 41–51	FC ≥51	∑Ui	% Use Value
Sliding/disasters	2	4	1	7	5.4
Agriculture activities	17	40	8	65	50.0
Population growth	1	0	4	5	3.8
Flood	1	1	0	2	1.5
Replaced by grasses and shrubs	1	0	2	3	2.3
Construction	2	0	0	2	1.5
Land use	1	0	4	5	3.8
Roads	1	0	2	3	2.3
Lack of knowledge about medicinal uses	1	0	4	5	3.8
Selling in market	0	4	1	5	3.8
Replacement by other ornamental/crops	0	5	3	8	6.2
Invasive species	1	0	4	5	3.8
Cutting for fuels	0	1	1	2	1.5
Flowering plants giving more yield	1	0	2	3	2.3
Highly expensive varieties	1	0	4	5	3.8
Eliminated for grasses	0	3	1	4	3.1
Digging of mountains for farm lands	1	0	2	3	2.3
Exporting purpose	1	0	4	5	3.8
Eradication by herbicides	0	3	1	4	3.1
Soil erosion	0	4	1	5	3.8
More rainfall	0	3	1	4	3.1
Unsustainable use	5	4	2	11	8.5
Deforestation	0	3	1	4	3.1

FC = Frequency of citation, Use value = the relative significance of locally known plant, Ui = number of use value of plant mentioned by each informer while  $n = 3$  which is the total number of informers.

**Figure 2.** HPLC-UV chromatogram of *Berberis lyceum* Royle root Me. Ext.

**Table 4.** Identified phenolic compounds in *Berberis lyceum* root Me. Ext.

Peak	Retention Time (min)	Antioxidant Identified	Sample Peak Area	Standard Peak Area	Concentration ( $\mu\text{g/mL}$ )
1	9.6	Quercetin	247.66753	7089.285	0.0314
2	6.2	Chlorogenic acid	28.94050	12.94050	2.0144
3	20.1	Berberine	85.10175	26.02970	2.9424
4	22.2	Rutin	28.60065	2241.215	0.00574
5	30.1	Mandelic acid	76.60439	7195.522	0.00958
6	36.4	Hydroxy benzoic acid	66.07519	40.1960	1.47944

### 3.6. DPPH Free Radical Scavenging Activity

The DPPH scavenging activity of *B. Lyceum* root Me. Ext is shown in Table 5. The highest percent inhibition ( $79.61 \pm 0.74$ ) was recorded for highest tested concentration of  $1000 \mu\text{g/mL}$  with an  $\text{IC}_{50}$  value of  $110 \mu\text{g/mL}$ . %DPPH inhibition potential of Me. The observed response was a concentration dependent one. Ascorbic acid exhibited percent inhibition of  $89.09 \pm 0.41$  at high concentration ( $1000 \mu\text{g/mL}$ ) with a calculated  $\text{IC}_{50}$  value of  $50 \mu\text{g/mL}$ .

**Table 5.** Percentage DPPH free radical scavenging activity of root extract.

Samples	Concentration ( $\mu\text{g/mL}$ )	%DPPH Scavenging Mean $\pm$ SEM	$\text{IC}_{50}$ ( $\mu\text{g/mL}$ )
Me. Ext	1000	$79.61 \pm 0.74$ ***	110
	500	$72.57 \pm 0.93$ ***	
	250	$67.76 \pm 0.79$ ***	
	125	$51.72 \pm 0.89$ ***	
	62.5	$44.11 \pm 1.22$ ***	
	31.25	$13.47 \pm 0.86$ ***	
Ascorbic acid	1000	$89.09 \pm 0.41$	50
	500	$84.80 \pm 0.63$	
	250	$69.21 \pm 0.55$	
	125	$67.52 \pm 0.56$	
	62.5	$52.32 \pm 0.91$	
	31.25	$31.91 \pm 0.21$	

Met. Ext, methanolic extract. Note: the data are represented as the mean  $\pm$  SEM, ( $n = 3$ ). Values are significantly (\*\*\*)  $p < 0.001$  different as compared to the positive control (Ascorbic acid) using the Student  $t$ -test.

### 3.7. ABTS Free Radical Scavenging Activity

Table 6 presents the ABTS scavenging activity of *B. lyceum* root Me. Ext. About  $84.09 \pm 1.15\%$  inhibition was recorded for highest tested concentration of  $1000 \mu\text{g/mL}$  with an  $\text{IC}_{50}$  value of  $165 \mu\text{g/mL}$ . For ascorbic acid the  $\text{IC}_{50}$  value was  $120 \mu\text{g/mL}$ .

A number of bioactive compounds have been reported from *B. lyceum*. Among them alkaloid-like berberine has been reported as the major one among all *Berberis* species. Isolation and characterization of berberine and other alkaloids like jatrorrhizine, palmatine, berbamine, karakoramine, berbaminine, chenabine,  $\beta$ -Sitosterol, 4,4-dimethyl hexadeca-3-ol, butyl-3-hydroxy propyl-phthalate, 3-(4<sup>1</sup>-(6-methyl-butyl)-phenyl) propan-1-ol, and 4-methyl-7-hydroxy-coumarin have been reported by many researchers [21,23,39–41]. *B. lyceum* fruit is also a good source of other phytochemicals like anthocyanin,  $\beta$ -carotene, ascorbic acid etc. [21,23,39–41]. In the present study flavonoids and phenolic acids like quercetin, chlorogenic acid, berberine, rutin, mandelic acid, and hydroxy benzoic acids were identified in which berberine and chlorogenic acid were present in the highest concentration which most probably are responsible for the observed antioxidant potentials. The antioxidant potentials of berberine and chlorogenic acid have been previously reported by other researchers as well [41–43]. Berberine has been reported as the major active constituent in almost all *Berberis* species [44–46]. Bhardwaj and Kaushik (2013) have reported

the phytochemical and pharmacological studies of the genus *Berberis*. They have also shown berberine as major constituent in *B. lyceum* [47].

**Table 6.** Percent 2,2'-azinobis-3-ethyl benzo thiazoline-6-sulfonic acid (ABTS) free radical scavenging activity of root extract.

Samples	Concentration ( $\mu\text{g/mL}$ )	%ABTS Scavenging Mean $\pm$ SEM	IC <sub>50</sub> ( $\mu\text{g/mL}$ )
Me. Ext	1000	84.09 $\pm$ 1.15 ***	165
	500	71.63 $\pm$ 0.88 ***	
	250	64.16 $\pm$ 1.27 ***	
	125	41.11 $\pm$ 0.55 ***	
	62.5	35.41 $\pm$ 0.56 ***	
	31.25	29.05 $\pm$ 1.02 ***	
Ascorbic acid	1000	88.38 $\pm$ 0.51	120
	500	74.49 $\pm$ 0.89	
	250	64.16 $\pm$ 1.01	
	125	50.39 $\pm$ 0.67	
	62.5	43.20 $\pm$ 1.16	
	31.25	36.20 $\pm$ 0.77	

Met. Ext, methanolic extract. Note: the data are represented as the mean  $\pm$  SEM, ( $n = 3$ ). Values are significantly (\*\*\*)  $p < 0.001$ ) different as compared to the positive control (Ascorbic acid) using the Student *t*-test.

#### 4. Conclusions

The phytochemical analysis and antioxidant evaluation of *B. lyceum* were carried out to inform the local people of District Shangla about the medicinal importance of this plant which was being used as a remedy for a number of health complications there. Being an endangered species, the reasons of its extinction were also sought. According to the participants' opinion the main reason of eradication of the selected plant in this area is anthropogenic activities which should be checked to ensure its survival. The plant methanolic extract was found to be a potent scavenger of DPPH and ABTS free radicals. The study will inform researchers to recognize the latent and potent potentials of *B. lyceum* which will encourage further studies on its chemical profile and other biological activities which will revive the importance of this plant on one hand while it will also help to improve the socioeconomic status of inhabitants of Shangla District on the other hand.

**Author Contributions:** Conceptualization, N.N., M.N. and M.Z.; methodology, M.N.; F.U., S.U. and R.U.; formal analysis, N.N., A.A.K.K., E.E., A.R. and G.A.E.M.; resources, E.E. and G.A.E.M.; writing—original draft preparation, N.N. and M.Z.; writing—review and editing, M.Z.; supervision, M.N. and M.Z. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research received no external funding.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable for this submission.

**Data Availability Statement:** The data presented in this manuscript belong to the research work of Abdur Rahman under the supervision of Nausheen Nazir and Mohammad Nisar.

**Acknowledgments:** This research was funded by the Research Supporting Project at King Saud University via grant number RSP/2020/45 and article processing charge (APC) was also supported by the Research Supporting Project (RSP/2020/45).

**Conflicts of Interest:** The authors declare that they have no conflicts of interests.

## Abbreviations

WHO	World Health Organization
<i>B. lyceum</i>	<i>Berberis lyceum</i>
DPPH	2,2-diphenyl-1-picrylhydrazyl
ABTS	2,2'-azinobis-3-ethyl benzo thiazoline-6-sulfonic acid
M	Meters
g	Gram
km <sup>2</sup>	Square kilometers
RFC	Relative Frequency Citation
FC	Frequency of Citation
HPLC	High-performance, liquid chromatography
Me. Ext	Methanolic extract

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