RESEARCH ARTICLE

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Berberis lycium: A Natural Source of Antioxidants and Bioactive Compounds

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Abstract

Berberis lycium (B. lycium) is a wild shrub, called barberry in English and *Simbulu* in Rajouri, Jammu and Kashmir. Traditionally, it is used as medicine for various ailments. The present study is aims to investigate qualitative, quantitative phytochemicals analysis and antioxidant activity of *B. lycium* root bark extracts. The coarse powder of root bark was successively extracted with petroleum ether, ethyl acetate and ethanol by maceration. Spectrophotometric quantification of total alkaloid content was determined by bromocresol green using atropine as standard. 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and reducing power methods were used for the determination of antioxidant activity using ascorbic acid as standard. In our finding, 76.88% was lost in weight on drying fresh root bark, 11.8% total ash and 0.97% moisture content was found. The weight and percentage yield of petroleum ether, ethyl acetate and ethanol solvent extracts were found to be 0.488g (0.06%), 17.568g (2.1%) and 79.935g (9.9%) respectively. The qualitative phytochemical screening showed the presence of alkaloids, terpenoids and saponins. Whereas, carbohydrates, glycosides, phenols, tannins, flavonoids and proteins were absent in the extracts. The ethanol extract showed high alkaloid contents 80.957mg and ethyl acetate extract 26.734mg AE/g in terms of atropine equivalent. While, ethanol extract's partitioned fraction (chloroform) contains 45.067mg and n-butanol 10.243mg AE/g. The reducing power and DPPH antioxidant activity were in order of ascorbic acid> ethanol extract> ethyl acetate extract> petroleum ether extract. Ethanol extract exhibit strong antioxidant activity among the extracts. The findings of the current study showed that the root bark of *B. lycium* contains such phytochemicals which have the potential to neutralize or reduce free radicals. So further chromatographic purification, identification of components and in vivo study of responsible constituents is required.

Keywords: Alkaloids- *Berberis lycium*- Maceration- Phytochemicals- Simbulu.

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Introduction

Berberis lycium (B. lycium) and their products have been used for food, shelter and medicine for the betterment of life since the origin of mankind [1, 2]. Across the world, Asian countries are well known for medicinal flora and indigenous implications which have played a significant role in pharmacognosy and pharmacology [3, 4]. *B. lycium* is a medicinal shrub, which is 2-3m in height and grows naturally in hilly areas. It is called Indian Barberry in English, Kashmal in Hindi and Ishkeen in Urdu. In Rajouri and Poonch districts of Jammu and Kashmir region people called Simbulu [5]. Traditionally, this shrub also used for the treatment of many diseases such as cough, throat pain, wound healing jaundice [5], diarrhoea, intestinal colic, scabies, bone fractures, sun blindness, fever and diabetes [6-10]. B. lyceum fruits are taken as food and are bitter in taste, those products which are bitter show the presence of alkaloids [11, 12]. Alkaloids are naturally occurring secondary metabolites that are basic and have heterocyclic nitrogen atoms which are widely used in medicine due to their remarkable physiological activities

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[13]. Free radicals are entities that lack electrons and require electrons to remain stable. Reactive oxygen species, which include singlet oxygen, superoxide ions, hydrogen peroxide radicals, and hydroxyl radicals, are free radicals that are derived from the oxygen atom. These free radicals are created by a chemical process called oxidation that sets off a chain reaction. Within seconds of the first reaction, an enormous number of free radical reactions take place [14, 15]. The production of free radicals is increased by pollution, smoking, drugs, illness, stress and even by exercise. These free radicals take electrons from the cells and cause cell damage that leads to degenerative diseases of aging such as a decline in immune system, cardiovascular disease, brain dysfunction, cataracts, and cancers [16-18]. In such conditions, there is a need of antioxidants, the molecules that stabilize or deactivate free radicals from a target molecule by preventing, delaying or removing the oxidative damage before interacting with the cells. Many antioxidants already exist in the human body but when the production of free radicals is high enough so it is difficult for natural antioxidants to neutralise them. So in such condition, there is a need for external antioxidants to overcome free radicals that we can get from medicinal plants. In this research, we aimed to examine phytochemical analysis, total alkaloid content and antioxidant activity of the root bark of *B. lycium*.

Materials and Methods

Collection and authentication of plant material

B. lycium roots were collected from a hilly village Ghambir Mughlan of district Rajouri in Jammu and Kashmir, India, during the month of November. *B. lycium* was identified and authenticated by Prof. (Dr.) Susheel Verma, Center for Biodiversity, School for Biosciences and Biotechnology, Baba Ghulam Shah Badshah University (BGSBU), Rajouri, J&K, India. A voucher specimen of the *B. lycium* (NO: CBS-BER. 1001) was kept in the herbarium of the BGSBU for future reference.

Preparation of plant material

B. lycium roots were washed thoroughly and dried for 2-3 hours. Then the bark was removed and shade-dried for 4 weeks at room temperature and the coarse powder was made with an electronic grinder. Further, the powder was observed for colour, odour, taste, texture and was packed in an airtight container until experiments.

Determination of loss on drying (LOD)

The weight of fresh and dried root bark was measured and the percentage loss due to drying was calculated.

LOD of root bark powder

An accurate quantity of about 2-4g of the powdered drug was taken in a petri dish and distributed evenly. The petri dish was kept open in a vacuum oven and the sample was dried at a temperature between 100-105oC for 2 hours until a constant weight was recorded. It was cooled in a desiccator at room temperature and weighed. The percentage of LOD was calculated using the following formula.

$$\% \text{ LOD} = \frac{\text{Loss in weight of the sample}}{\text{Weight of the sample}} \times 100$$

Determination of total ash

The total ash value was determined by placing 10g of ground air-dried powder in a previously ignited crucible of silica. The material was spread in an even layer and ignited by gradually increasing the temperature from 500-600°C until white, indicating the absence of carbon. It is cooled in a desiccator and weighs accurately. The percentage of ash was calculated with reference to the air-dried drug [19].

$$\% \text{ Ash content} = \frac{\text{Weight (Wt) of ash}}{\text{Wt of powdered sample used}} \times 100$$

Phytochemical extraction

The root bark of *B. lycium* was extracted [20]. The dried coarse powder was subjected to maceration successively with petroleum ether, ethyl acetate and ethanol. Each time before extracting with the next solvent, marc was dried. The obtained extracts were concentrated by using a rotary vacuum evaporator to get a powdery mass. The colour and consistency of the extract were also recorded. The percentage yield of extracts was calculated by using the following formula:

$$\% \text{ Yield} = \frac{\text{Weight of the extract}}{\text{Weight of the powder}} \times 100$$

Organoleptic evaluation and Solubility of extracts

All the extracts were investigated for their colour, taste and appearance and Solubility was checked in different solvents such as methanol, chloroform, Dimethyl sulfoxide (DMSO), acetone, ethanol and water.

Phytochemical screening of crude extracts

Phytochemical screening was done [21, 22] (Figure 1). Alkaloids were detected by four methods: Wagner's Test, Dragendroff's Test, Hager's Test and Mayer's Test. Detection of carbohydrates was carried out by Molisch's and Fehling's tests. Detection of glycosides, saponins, phytosterol, phenols, tannins, flavonoids, proteins, amino acids and diterpenes were carried out by Legal's froth test, Foam test, Salkowski's test, Ferric chloride test, Gelatin, Alkaline reagent test, Lead acetate test, Xanthoproteic test, Ninhydrin test and Copper acetate test respectively.

Determination of Total alkaloid content

Total alkaloid contents (TAC) were determined by procedure with slight modifications [23]. Atropine was used as the standard and TAC was expressed as atropine equivalent per gram of dry samples (mg AE/g).

Preparation of solutions

Bromocresol green (BCG) solution was prepared by heating the BCG with 2N sodium hydroxide and distilled water until completely dissolved and then the solution was diluted with distilled water. The phosphate buffer solution was prepared by adjusting the pH of 2M sodium phosphate to 4.7 with 0.2M citric acid in distilled water.

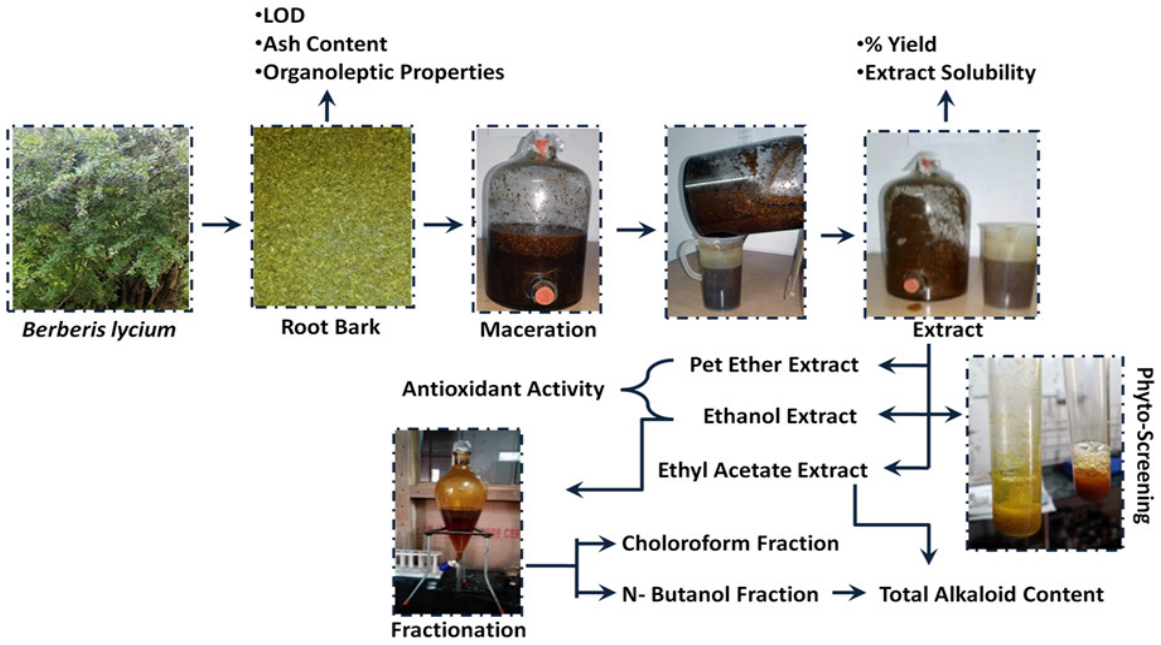

Figure 1. Phytochemical Extraction from the Root Bark of *B. lycium*

Atropine standard solution was prepared by dissolving 2.4mg atropine in 4ml distilled water.

Preparation of standard curve

Different concentrations (40, 60, 80, 100 and 120µg/ml) of atropine were prepared in methanol from the stock solution of atropine. About 1.5ml from these concentrations were transferred to different separatory funnels and then 2ml phosphate buffer solution of pH 4.7 and 2ml BCG solution were added and shaken further by adding 5ml chloroform. Furthermore, 5mg/ml of extract solutions were made in 2N, HCL and filtered. Then 1.5ml of this filtrate was transferred to the separatory funnel and 2ml of BCG and 2ml phosphate buffer solutions (pH 4.7) were added in the separatory funnel. A complex was formed by shaking after adding the 5ml chloroform in the separatory funnel mixture and after a few minutes of rest, chloroform formed a layer which was collected separately in the volumetric flasks and absorbance was measured at 470nm against the blank solution. The blank was prepared in the same way as described above but without atropine and extract.

Determination of in-vitro antioxidant activity

Reducing power and 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay were employed for the determination of antioxidant activity. Ascorbic acid was used as a standard reference. Percentage inhibition and inhibitory concentration (IC_{50}) were calculated by using the regression equation. The higher percentage inhibition and lowest IC_{50} values indicate strong antioxidant activity.

DPPH free radical scavenging assay

The DPPH assay of *B. lycium* root bark extracts (petroleum ether, ethyl acetate and ethanol) was determined with slight modifications [24]. The stock solution (1mg/ml in methanol) of the standard ascorbic acid and extracts were prepared. Various concentrations of 10, 20, 30, 40 and 50µg/ml were prepared from the stock. 0.1mM solution of DPPH was also prepared in methanol. DPPH solution 2ml and 1ml solution from different concentrations of extracts and standard ascorbic solution mixed, vortexed and left in the dark at room temperature for 30 minutes. The control solution was also prepared in the same way which contained 1ml methanol and 2ml DPPH solution. Thereafter, the absorbance of the mixture was measured spectrophotometrically at 517nm against methanol as a blank solution. All tests were performed in triplicates. The scavenging activity was calculated by the following formula.

$$\% \text{ Inhibition} = \frac{\text{Absorbance of Control} - \text{Absorbance of test sample}}{\text{Absorbance of Control}} \times 100$$

Reducing power assay

The method was used with slight modifications for the determination of reducing power [25]. Stock solution (1mg/ml in methanol) of standard ascorbic acid and extracts were prepared. Different concentrations (20, 40, 60, 80 and 100µg/ml) were prepared from the stock solution and 0.5ml from various concentrations were mixed with 0.5ml phosphate buffer (0.2M, pH 6.6) and 0.5ml (1% w/v) potassium ferricyanide solutions. The mixtures were incubated at 500C for 20 minutes. After cooling, 1.5ml trichloro acetic acid (10% w/v) and 0.5ml (0.1% w/v) freshly prepared ferric chloride solutions were added and the absorbance of mixtures were measured at 700nm against the methanol as blank solution.

Statistical analysis

The collected data was first subjected to descriptive analysis, where frequency methods were used to summarize

the data, and averages and standard deviations were calculated to provide measures of central tendency and variability. These analyses allowed for the identification of meaningful patterns and differences within the data. All statistical analyses were performed using SPSS software (IBM SPSS 20.0 version), ensuring a rigorous and systematic approach to data interpretation.

Results

Comparing the ethanolic extract from the root bark of *B. lycium* to ascorbic acid, the standard antioxidant, indicates less potential in reducing the activities of free radicals against various reactive oxygen species. The antioxidant qualities are partly attributed to the presence of several phytochemicals. The percentage loss due to drying and loss of water was calculated from the fresh sample and dried material and was found to be 76.88% (Table 1). The Organoleptic evaluations of coarse powder of roots bark of *B. lycium* show yellow in colour with a bitter taste that has no odour.

The total ash value percentage was 11.8% when placing 10 gm of ground air-dried powder in a previously ignited and crucible of silica (Table 2). Moisture content percentage was found to be 0.972% on drying powdered drug material at a temperature between 100 to 105oC for 2 hours and loss of weight was found 0.0389 from 4-gram powder that was placed in a vacuum oven.

Petroleum ether, ethyl acetate and ethanol extracts were obtained with each solvent and weighed and the percentage yield (Table 3) was calculated and found to be 0.488 gm (0.06%), 17.568 gm (2.1%) and 79.935gm (9.9%) respectively in terms of dried weight (800 gram) of the plant material.

Organoleptic properties

The organoleptic evaluations of root bark powder show a yellow appearance, no odour and have bitter taste. In the case of extracts, all extracts were observed to be olive in colour, however, petroleum ether and ethyl acetate extracts were woody in taste and showed a fine appearance but ethanol extract is bitter and crystal-like in appearance (Table 4).

Solubility of extracts

In solubility testing, petroleum ether extract was insoluble in water and partially soluble in all other solvents. Ethyl acetate extract was soluble in methanol and DMSO while partially soluble in other solvents but ethanol extract was partially soluble in chloroform and acetone and soluble in methanol and ethanol was completely soluble in DMSO and water (Table 5).

Phytochemical screening

Phytochemical screening of extracts showed the presence of alkaloids in ethyl acetate and ethanol extract while absent in petroleum ether. All extracts revealed the absence of carbohydrates, glycosides, phenols, tannins, flavonoids and proteins while the presence of terpenoids was seen in all the extracts. Saponins were seen only in the ethanol extract while absent in ethyl acetate and petroleum ether extract (Table 6).

Total alkaloid content

The regression line ($Y= 0.003x + 0.374$, $R^2 = 0.983$) from atropine was used for the estimation of alkaloid content (Figure 2) depicts the variation of mean absorbance with different concentrations of atropine (Table 7).

The quantitative analysis of TAC revealed that ethanol extract contains highest amount of TAC (80.957mgAE/gm.) followed by ethyl acetate extract (26.734mg AE/gm.). Liquid-liquid partition was carried out of ethanol extract due to highest contents of alkaloids presence in it by using the chloroform and n-butanol solvents. The partition parts of chloroform and n-butanol fractions

Table 1. Weight of Plant Material after Drying and Moisture Content

Wt. of root bark in fresh condition 4000g	Wt. after drying at room temp. 925g	% Loss 76.88%
Wt. of root bark	Loss of weight	Moisture
4 g.	0.0389	0.97%

Table 2. Showing Ash Content of *B. lycium*

Wt. of root bark	After burning in the crucible (Ash)	% Ash
10 g	1.18	11.80%

Table 3. Extraction Yield of Root Bark of *B. lycium*

Extracts	Vol. of solvent (L)	Wt. of extract (g)	Yield
Petroleum Ether	5	0.488	0.06%
Ethyl acetate	6	17.568	2.10%
Ethanol	7	79.935	9.90%

Table 4. Organoleptic Evaluation of Extracts of Roots Bark of *B. lycium*

Extract	Colour	Taste	Appearance
Petroleum Ether	Olive	Woody	Fine
Ethyl acetate	Olive	Woody	Fine
Ethanol	Olive	Bitter	Crystals

Table 5. Solubility of Extracts in Various Solvents

Solvents	Petroleum ether	Ethyl acetate	Ethanol
Methanol	Partially soluble	Soluble	Soluble
Chloroform	Partially soluble	Partially soluble	Partially soluble
DMSO	Partially soluble	Soluble	Completely soluble
Acetone	Partially soluble	Partially soluble	Partially soluble
Ethanol	Partially soluble	Partially soluble	Soluble
Water	Insoluble	Partially soluble	Completely soluble

Table 6. Phytochemical Screening of Crude Extracts

Test	Petroleum ether extract	Ethyl acetate extract	Ethanol extract
Test for Alkaloids			
Wagner's test	–	+	++
Hager's test	–	+	++
Dragendroff's test	–	+	++
Mayer's test	–	+	++
Test for Carbohydrates			
Molisch's test			
Fehling's test	–	–	–
Test for Glycosides			
Legal's test	–	–	–
Test for Saponins			
Froth test	–	–	+
Foam test	–	–	+
Test for Terpenoids			
Salkowski's test	+	+	+
Test for Phenols			
Ferric chloride test	–	–	–
Test for Tannins			
Gelatin test	–	–	–
Test for Flavonoids			
Alkaline test	–	–	–
Lead acetate test	–	–	–
Test for Proteins			
Xanthoproteic test	–	–	–
Test for Diterpenes			
Copper acetate test	+	+	+

(–), Absent; (+), Present; (++), High

Table 7.Absorbance of Atropine at 470nm

Conc. (µg/ml)	Absorbance
40	0.506±0.0198
60	0.577±0.0276
80	0.675±0.0141
100	0.730±0.0219
120	0.778±0.0198

Values are expressed as Mean±SD

Table 8. Extract Values of TAC

Extract/fraction	Conc. mg/ml	TAC mgAE/g sample
Ethyl Acetate	5mg/ml	26.734±0.334
Ethanol	5mg/ml	80.957±0.193
Chloroform fraction	5mg/ml	45.067±1.202
n-butanol Fraction	5mg/ml	10.243±0.167

Values are in triplicates and expressed as Mean±SD

were checked for further total alkaloids contents and found that chloroform fraction contains a high amount of TAC (45.067mgAE/gm.) followed by N-butanol fraction (10.243mgAE/gm) (Table 8).

DPPH free radical scavenging assay

The antioxidants provide protection against degenerative diseases and infection in the body of human beings by inhibiting and scavenging free radicals. An anti-oxident react with DPPH, a suitable free radical, which gets reduced to DPPH-H. Consequently, the absorbance gets decreased. The higher percentage inhibition and lowest IC_{50} values indicates the strongest antioxidant.

Table 9. Percent Inhibition of DPPH Radical by Ascorbic Acid and Extracts

Conc. µg/ml	Ascorbic acid	Ethanol	Ethyl acetate	Pet. Ether
10	8.574±0.0015	6.356±0.0015	4.582±0.0015	1.552±0.0010
20	19.734±0.0010	8.869±0.0010	6.652±0.0010	2.217±0.0010
30	34.959±0.0015	10.717±0.0010	9.313±0.0010	4.361±0.0015
40	41.833±0.0015	15.004±0.0006	13.452±0.0006	5.765±0.0010
50	58.980±0.0026	20.177±0.0010	14.412±0.0010	8.278±0.0015
IC_{50}	43.984µg/ml	142.17µg/ml	182.79µg/ml	298.029µg/ml

Values are expressed as Mean±SD

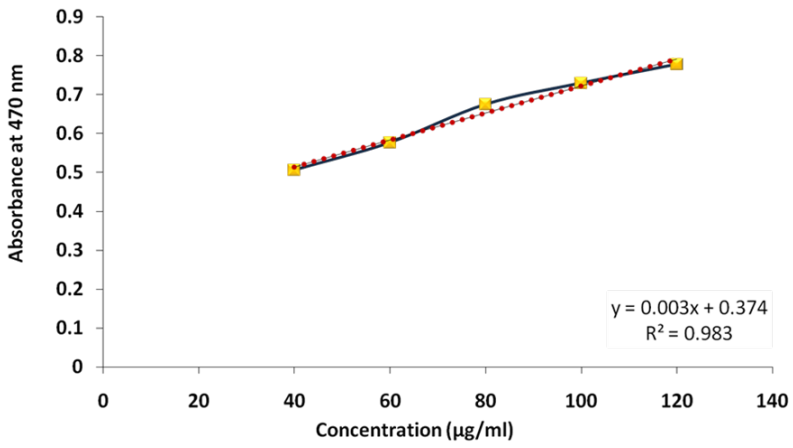

Figure 2. Regression Curve of Standard Atropine

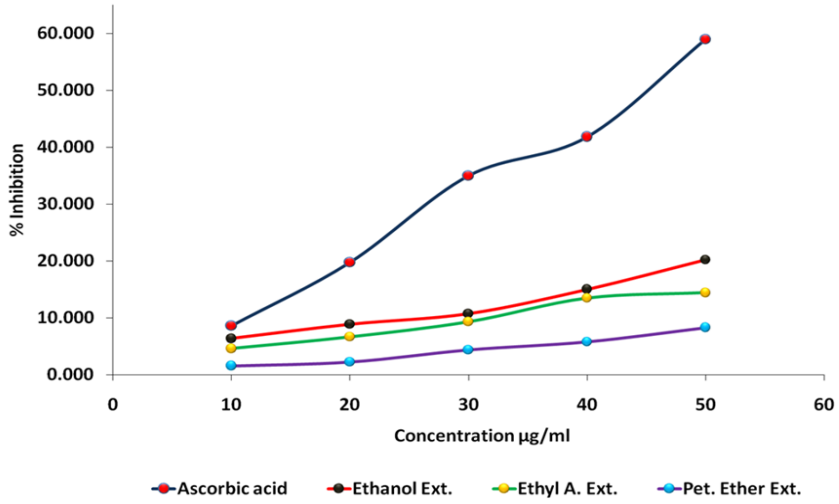

Figure 3. Represents Percent Inhibition of DPPH by Ascorbic Acid and Extracts

Table 10. Reducing Power Assay of Ascorbic Acid and Extracts

Conc. (µg/ml)	Absorbance			
	Ascorbic acid	Petroleum ether	Ethyl Acetate	Ethanol
20	2.514±0.0017	0.640±0.0025	0.784±0.0006	0.937±0.0021
40	2.546±0.0055	0.714±0.0020	0.934±0.0010	1.004±0.0006
60	2.645±0.0025	0.788±0.0020	0.954±0.0015	1.171±0.0006
80	2.653±0.0053	0.795±0.0015	0.980±0.0006	1.408±0.0012
100	2.686±0.0089	0.837±0.0011	1.003±0.0015	1.466±0.0010

Values are expressed as Mean±SD

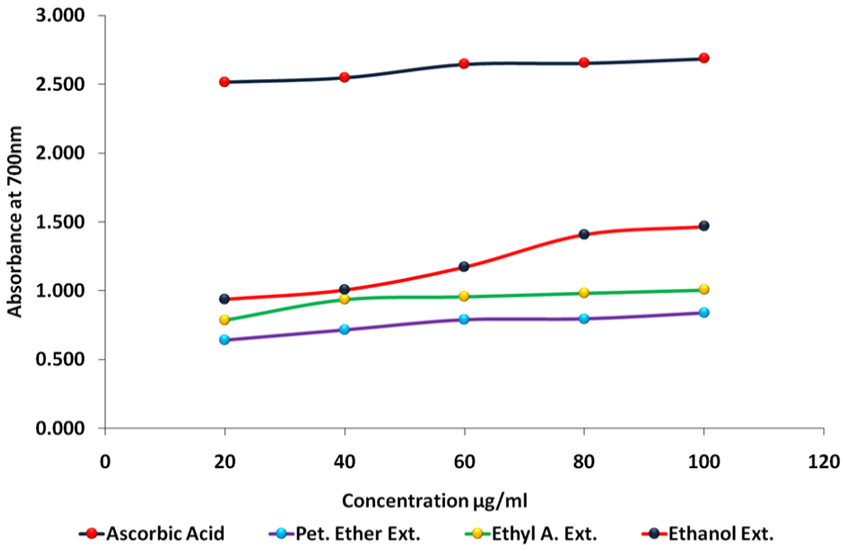

Figure 4. Reducing Power of Ascorbic Acid and Extracts

The highest inhibition and lowest IC_{50} value were found in ethanol extract as compared to other extracts which confirms its strongest antioxidant ability against DPPH radical. The results are compared with the IC_{50}values of standard ascorbic acid and found to be in the order of ascorbic acid (43.984µg/ml) >ethanol extract (142.17µg/ml) >ethyl acetate extract (182.79 µg/ml) >petroleum ether extract (298.029µg/ml) (Table 9 and Figure 3).

Reducing power assay

In reducing power assay, higher the absorbance of the reaction mixture, higher would be the reducing power which means strong antioxidant activity. In our results, the reducing power was found to be in the order as ascorbic acid >ethanol extract > ethyl acetate extract > petroleum ether extract. Ethanol extract showed the higher reducing power as compared to ethyl acetate and petroleum ether extract when compared to standard ascorbic acid. In both

DPPH and reducing power assay ethanolic extract shows highest antioxidant activity (Table 10 and Figure 4).

Discussion

In this research, solvents of different polarities were used for the extraction of root bark and obtained extracts were screened for phytochemical screening, TAC and antioxidant activity. As *B. lycium* contains various phytochemicals of different polarities and for their elution different polarities of solvent have to be used. In our research petroleum ether was used for the separation of non-polar components and ethyl acetate and ethanol were used for semi-polar and polar components. Ethanol extract yields more than ethyl acetate and petether extract indicating root bark contains such compounds which are more readily soluble in ethanol. However, the techniques of extraction and time duration also impact extraction such as a heating extraction technique like soxhlation can impact heat-sensitive compounds. Here we have used the cold maceration method. Solvents of the same polarities (n-hexane, chloroform and methanol) were used for extraction for the wide range of phytochemicals [26, 27].

Physiochemical methods such as moisture content, ash value, colour, organoleptic properties etc. are indicators of drug quality. The substance under investigation has a yellow hue and a bitter flavor product that have a bitter flavor also include alkaloids [11, 12]. In our phytochemical screening alkaloids were found in ethanol extract and ethyl acetate extract with terpenoids. All these constituents were also found in previous studies [28, 29]. There were no phenols or flavonoids found in any root bark extract. Alkaloids, tannins, glycosides, and saponins were present in the work, however reducing sugars and flavonoids were not discernible in the extracts [30, 31]. Total alkaloid content was estimated and found higher quantity in ethanol extract than ethyl acetate extract. Similar results were also estimated [32]. These components have been associated with various degrees of antioxidant activities [33-36].

B. lycium have rich source of antioxidants and acts at different stages and involves different mechanisms such as donating hydrogen atoms by scavenging reactive oxygen and by deactivating metal ions. The DPPH assays are mostly used to determine the antioxidant capacities of plant extracts. All of the extracts showed higher percentage of scavenging against DPPH free radicals and thus can be effectively utilized in diseases arising from radical attack. *B. lycium* has antioxidant properties [5] nearest to ascorbic acid [37]. Various biological activities are due to the presence of several alkaloids [38]. The extract colour was changed from less purple to colourless and this degree of colour change represents the antioxidants capacity of the extract which is found in order as ethanol extract > ethyl acetate extract > petroleum ether extract in our study. Due to the presence of antioxidants (phytochemicals) in the extract, its colour changed from less purple to colourless found in the study [39]. In the reducing power method, the increased absorbance indicates increased reducing power and same order was found as in previous DPPH method. *B. lycium* is a potential source of natural antioxidants and therapeutically useful for various health complications [31, 40] and ethanolic extract was more effective in biological activities [41-43] and is used in folk medicine because of ethnomedicinal properties [44-48]. The preliminary chemical examination of alcoholic extract has shown the presence of alkaloids, terpenoids and saponins which may be responsible of the antioxidant activities. Further studies on the isolation of these compounds are in progress. Ethanol extract activity may be related to the high amounts of alkaloids compounds leading to an antioxidant activity in the extract.

In ccnclusion, the *B. lycium* root bark extracts are rich in bioactive phytochemicals and exhibit significant antioxidant activity. These findings suggest the potential of *B. lycium* as a natural source of antioxidants for pharmaceutical and nutraceutical applications. The study is limited by the potential variability in phytochemical composition due to environmental and seasonal factors affecting *B. lycium*. Moreover, these assays only measure specific aspects of antioxidant activity, which may not capture the full range of interactions in a biological system. Further studies are needed on the isolation and identification of active phytochemicals and to clarify the in vivo potential of this plant in the management of diseases and this is a subject of investigation.

Author Contribution Statement

J.I. & M.Y. conceptualized and contributed to the study design, J.I. conducted experiments, J.I & M.Y drafted the manuscript, C.A, G.M., & S.K. edited the manuscript, pictures and table. A.G & B. M edited the final manuscript and P.K. finalized the manuscript.

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Availability of Data

For data contact to corresponding author (younisgenetic@gmail.com)

Conflict of Interest Statement

The authors declare that they have no conflict of interest.

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