



EXTRACTION, PHYTOCHEMICAL INVESTIGATION AND ANTIOXIDANT ACTIVITY OF VARIOUS EXTRACTS FROM ROOT BARK OF *BERBERIS LYCIUM* ROYLE

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ABSTRACT

The aim of present study was to investigate the presence of phytochemicals and evaluation of the antioxidant property of various extract of *Berberis lycium* Royle. The plant belongs to the family Berberidaceae, found in subtropical and temperate regions from Kashmir to Uttaranchal on the outer Northern-western Himalayas. The coarse powder of roots bark was extracted successively with petroleum ether, chloroform, methanol and aqueous by Soxhlation method. The extracts showed the presence of alkaloids, glycosides, terpenoids, saponins and diterpenes. Chloroform, methanol and aqueous extracts revealed the presence of alkaloids while petroleum ether extract devoid in it. In antioxidant activity methanolic extract have strongest reducing power and DPPH Scavenging capacity as compared to the other extracts. These findings provide clue that *Berberis lycium* Royle root bark are potential source of natural antioxidants.

KEYWORDS: Phytochemical, *Berberis lycium*, antioxidant, alkaloids, root bark.

INTRODUCTION

Berberidaceae is a heterogeneous assemblage of angiosperms comprising 17 genera and 650 species in the world, which are distributed mostly in the northern hemisphere. One of them is *Berberis lycium* (Berberidaceae), a lesser-known plant is named in English as barberry (Anwar *et al.*, 1979), whereas, its fruit is called as “Kashmal” (Baquar, 1989; Usmanhane *et al.*, 1997) and roots are known as “Darhald” (Nadkarni, 1980). *Berberis lycium* is an evergreen shrub 2-3 m in height. The plant is found throughout the temperate and subtropical regions of the world. It occurs in subtropical and temperate regions from Kashmir to Uttaranchal on the outer Northern-western Himalayas (Sharma, 2003). Traditionally, *Berberis lycium* is used by the tribal peoples in J&K, India, since the time immemorial (Sharma *et al.*, 2016) Traditionally the plant has been used against diarrhea (Zaman and Khan, 1970), Intestinal colic (Kaur and Miani 2001), Diabetes (Ahmed *et al.*, 2004), throat pain, scabies, bone fractures, sun blindness, fever (Ahmed *et al.*, 2004; Kaur *et al.*, 2001) and internal wounds (Hamayun *et al.*, 2005). Antioxidants are the first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress and even exercise can increase free radical exposure. Cell damage caused by free radicals appears to be a major

contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction (Percival, DR. M. 1998; Sies, H. *et al.*, (1992). Antioxidants are the substances that delays, prevents or removes the oxidative damage from a target molecule either by stabilizing or deactivating free radicals before they interact with the cells. As antioxidant plays an important role in inhibiting and scavenging radicals, by providing protection against infection and degenerative diseases in human beings. Antioxidants are the critical component in the optimal maintenance of cell and systemic homeostasis, as well as preserving their health. (Halliwell, B. 2007; Kumar, *et al.*, 2007). The aims of the present study were to evaluate the phytochemical screening and also measure the antioxidant activity of various extracts from roots bark of *Berberis lycium* Royle by using 2, 2 diphenyl-1picrylhydrazyl (DPPH) and reducing power assay.

Taxonomic position		
Kingdom	:	Plantae
Phylum	:	Tracheophyta
Class	:	Magnoliopsida
Order	:	Ranunculales
Family	:	Berberidaceae
Genus	:	<i>Berberis</i>
Species	:	<i>lycium</i>
Binomial name	:	<i>Berberis lycium</i> Royle

A photograph of a Berberis lycium plant, showing its green leaves and clusters of small, round, blue-purple berries. The plant is growing in a natural, hilly environment.

Figure 1: *Berberis lycium* Royle plant.

MATERIALS AND METHODS

Collection and Authentication Plant

Berberis lycium Royle (Berberidaceae) roots were collected in the month of October- November 2014 from Ghambir Mughlan Village of district Rajouri, Jammu and Kashmir, India. The plant was identified and authenticated by an Associate Prof. Dr. Susheel Verma, Center for Biodiversity, School for Biosciences and Biotechnology, Baba Ghulam Shah Badshah (BGSB) University Rajouri, J&K, India. A voucher specimen of the plant (NO: CBS-BER. 1001) was kept in the herbarium of the said Department for future references.

Preparation of extract

The roots were uprooted from the soil, washed under running tap water then rinsed in distilled water. The bark was removed, shade dried at room temperature and subjected to size reduction to a coarse powder by using electronic grinder. This powder was packed into Soxhlet apparatus and extracted successively with solvents viz Petroleum ether, chloroform, methanol and aqueous. The extracts were dried (free of solvent) in a vacuum evaporator and stored in refrigerator at 4°C for further use.

Qualitative phytochemical screening: Phytochemical tests were carried out on all the extracts as per the standard methods (Tiwari *et al.*, 2011).

1. Detection of alkaloids: Extracts were dissolved individually in dilute Hydrochloric acid and filtered.

a) Wagner's Test: Filtrates were treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

b) Dragendorff's Test: Filtrates were treated with Dragendorff's reagent (solution of Potassium Bismuth

Iodide). Formation of red precipitate indicates the presence of alkaloids.

C) Hager's Test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate.

2. Detection of carbohydrates: Extracts were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates were hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars.

3. Detection of glycosides

Legal's Test: Extracts were treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides.

4. Detection of saponins

a) Froth Test: Extracts were diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins.

b) Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins.

5. Detection of phytosterols

Salkowski's Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of

Conc. Sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

6. Detection of phenols

Ferric Chloride Test: Extracts were treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols. .

7. Detection of flavonoids

a) Alkaline Reagent Test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.

b) Lead acetate Test: Extracts were treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids.

8. Detection of proteins

Xanthoproteic Test: The extracts were treated with few drops of conc. Nitric acid. Formation of yellow colour indicates the presence of proteins.

9. Detection of diterpenes

Copper acetate Test: Extracts were dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes

Table 1: Phytochemical Screening of various crude extracts of root bark of *Berberis lycium* Royle.

S. No	Tests	Observations for extracts			
		Petroleum Ether	Chloroform	Methanol	Aqueous
1	Test for Alkaloids				
	Wagner's test	-	++	+++	+
	Hager's test	-	++	+++	+
	Dragendroff's test	-	++	+++	+
2	Test for Carbohydrates				
	Fehling's test	-	-	-	-
3	Test for Glycosides				
	Legal's test	-	+	+	+
4	Test for Saponins				
	Froth test	-	+	++	+
	Foam test	-	+	++	+
5	Test for Terpenoids				
	Salkowski's test	+	-	-	-
6	Test for Phenols				
	Ferric chloride test	-	-	-	-
7	Test for Flavonoids				
	Lead acetate test	-	-	-	-
	Alkaline reagent test	-	-	-	-
8	Test for Proteins				
	Xanthoproteic test	-	-	-	-
9	Test for Diterpenes				
	Copper acetate test	+	+	+	+

Key: (-) Absent; (+) low; (++) Average; (+++) High

DPPH Free radical scavenging assay

DPPH free Radical scavenging assay (2, 2-diphenyl-1-picryl hydrazyl) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the whole molecule. The free radical scavenging activity of various extract and standard ascorbic acid was measured by using the method of Lu, Yinrong and Foo, L. Yeap (2000) with slight modifications. A 0.1 mM DPPH solution was prepared in methanol and 2 ml of

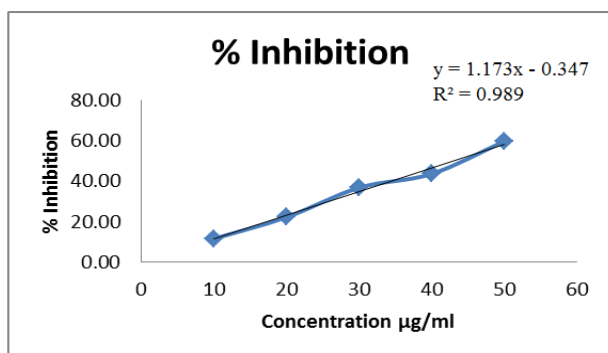
this Solution was added in 1 ml antioxidant solution of various extracts and standard ascorbic acid in different concentrations prepared in methanol. The control was prepared same as above but without the sample. The absorbance was measured at 517nm against methanol as blank after 30 minute of incubation in dark at room temperature. The assay was carried out in triplicate. The percentage (%) of inhibition was calculated by using the formula.

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

Table 2: Percentage inhibition of DPPH free radical by Ascorbic Acid

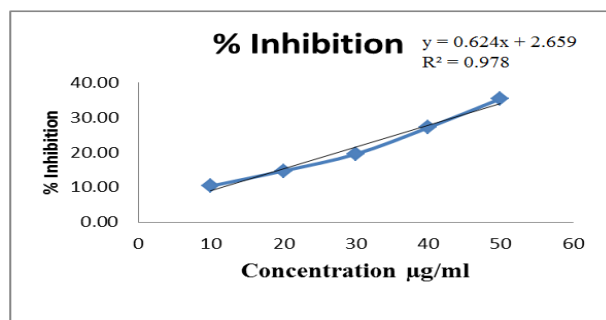
S. No	Concentration µg/ml	% Inhibition
1	10	11.70±0.001
2	20	22.34±0.002
3	30	36.88±0.002
4	40	43.69±0.001
5	50	59.72±0.002
	IC 50 Value	42.92µg/ml

Values are expressed as Mean±SD

**Fig 2: Regression curve of Ascorbic Acid by DPPH free radical assay.****Table 3: Percentage inhibition of DPPH free radical by Methanol extract.**

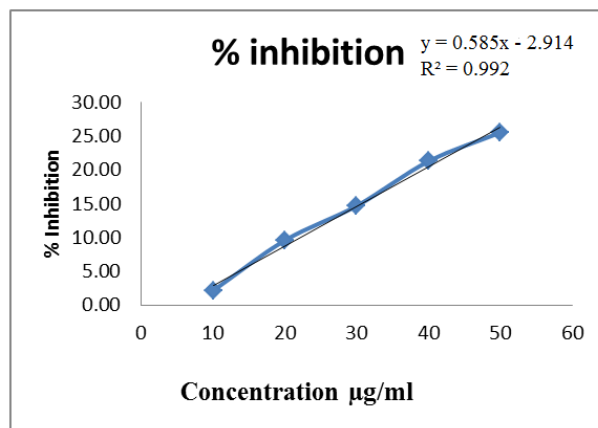
S. No	Concentration µg/ml	% Inhibition
1	10	10.35±0.002
2	20	14.68±0.001
3	30	19.50±0.002
4	40	27.09±0.003
5	50	35.39±0.002
	IC 50 Value	75.87µg/ml

Values are expressed as Mean±SD

**Fig. 3: Regression curve of Methanol extract by DPPH free radical assay.****Table 4: Percentage inhibition of DPPH free radical by Chloroform extract.**

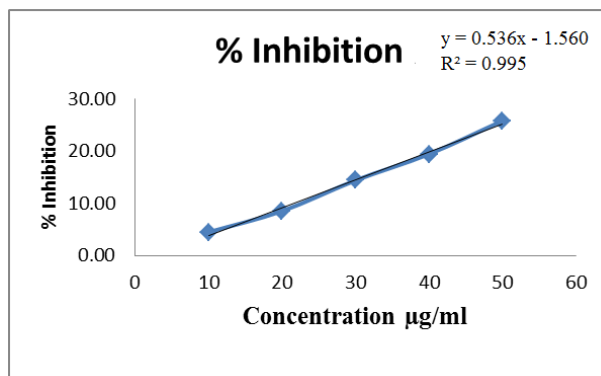
S. No	Concentration µg/ml	% Inhibition
1	10	2.13±0.002
2	20	9.57±0.001
3	30	14.68±0.004
4	40	21.28±0.001
5	50	25.53±0.002
	IC 50 Value	90.45µg/ml

Values are expressed as Mean±SD

**Fig. 4: Regression curve of Chloroform extract by DPPH free radical assay.****Table 5: Percentage inhibition of DPPH free radical by Aqueous extract.**

S. No	Concentration µg/ml	% Inhibition
1	10	4.47±0.001
2	20	8.51±0.001
3	30	14.40±0.002
4	40	19.43±0.001
5	50	25.82±0.002
	IC 50 Value	96.19µg/ml

Values are expressed as Mean±SD

**Fig. 5: Regression curve of Aqueous extract by DPPH free radical assay.****Table 6: Percentage inhibition of DPPH free radical by Petroleum ether extract.**

S. No	Concentration µg/ml	% Inhibition
1	10	5.25±0.002
2	20	6.17±0.001
3	30	8.16±0.002
4	40	9.50±0.001
5	50	12.13±0.001
	IC 50 Value	275.81µg/ml

Values are expressed as Mean±SD

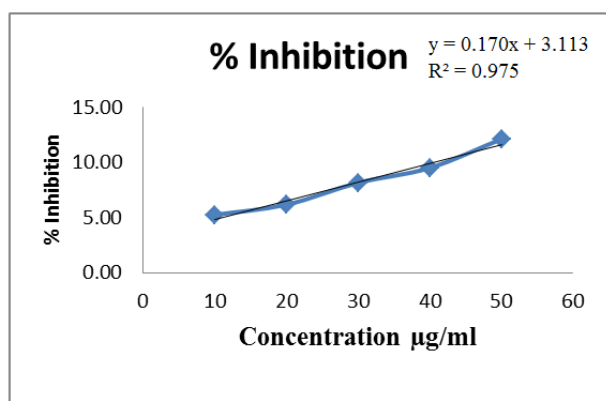


Fig. 6: Regression curve of Petroleum ether extract by DPPH free radical assay.

Table 7: IC₅₀ Values of Standard Ascorbic acid and extracts.

S.NO	Extracts	IC ₅₀ Value(µg/ml)
1	Standard Ascorbic Acid	42.92
2	Methanol Extract	75.87
3	Chloroform Extract	90.45
4	Aqueous Extract	96.19
5	Petroleum Ether Extract	275.81

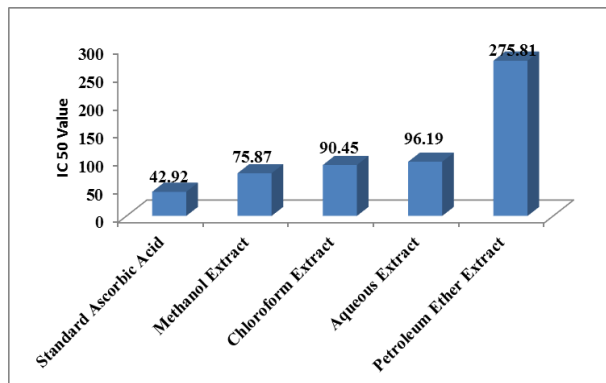


Fig. 7: Showing IC₅₀ Values of Standard Ascorbic Acid and extracts.

Reducing power assay

Reducing power assay was carried out by using the method of R. Jain and SK. Jain (2011). Stock solution of various extracts was prepared as 1mg/ml in methanol to obtain different concentrations (20, 40, 60, 80 and 100 µg/ml). Taking 0.5 ml from various concentrations of the extracts and was mixed with 0.5 ml phosphate buffer (0.2 M, pH 6.6) and 0.5 ml Potassium ferricyanide (1% W/v). The mixture was incubated at 50°C for 20 minutes. After cooling, 1.5 ml trichloro acetic acid (10% w/v) was added to terminate the reaction. After that 0.5 ml of freshly prepared ferric chloride (0.1% W/V) solution was added in the mixture and absorbance of the mixture in different concentrations was measured at 700 nm against methanol (as blank). Ascorbic acid was used as a standard reference at the same concentrations. The assay was carried out in triplicate.

Table 8: Reducing Power assay of Standard Ascorbic Acid and Various extracts.

S. No	Concentration µg/ml	Ascorbic Acid	Petroleum Ether	Methanol	Chloroform	Aqueous
1	20	1.120±0.0029	0.518±0.0012	0.838±0.0019	0.802±0.0024	0.783±0.0001
2	40	1.180±0.0019	0.612±0.0015	0.897±0.0012	0.857±0.0021	0.826±0.0023
3	60	1.240±0.0021	0.688±0.0013	0.938±0.0020	0.901±0.0012	0.878±0.0012
4	80	1.290±0.0012	0.721±0.0011	0.998±0.0001	0.943±0.0011	0.901±0.0020
5	100	1.330±0.0031	0.757±0.0021	1.140±0.0011	0.998±0.0004	0.936±0.0002

Values are expressed as Mean±SD

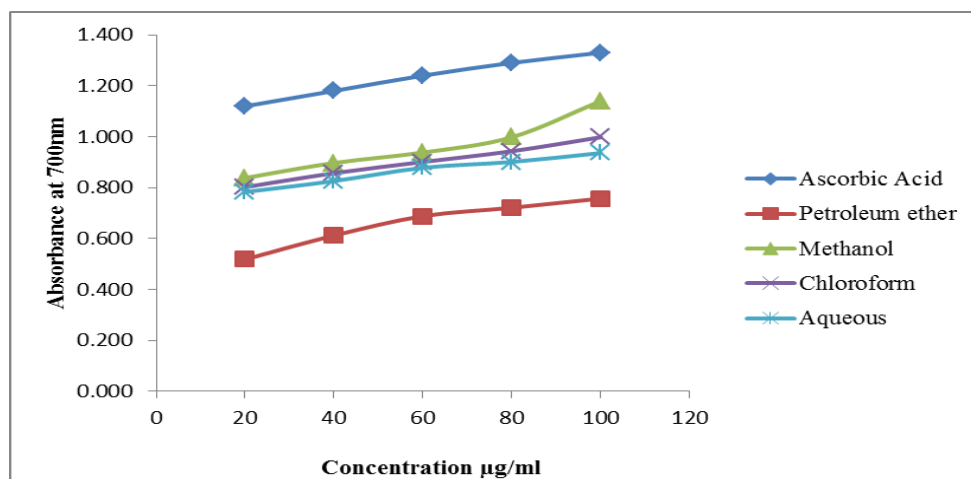


Fig. 8: Showing reducing power of Standard Ascorbic Acid, Petroleum ether, methanol, chloroform and aqueous extract.

RESULT AND DISCUSSION

The phytochemical analysis of various extracts of root bark of *Berberis lycium* revealed the presence of alkaloids, saponins, glycosides, and diterpenes (Table 1). The alkaloids were present only in chloroform, methanol and aqueous extract while petroleum ether extract devoid of it. Phenols and flavonoids were not detected in the extracts. The extracts possess concentration dependent scavenging activity on DPPH radicals as shown in (Table 2, 3, 4, 5, 6 and Fig. 2, 3, 4, 5, 6). The order of DPPH scavenging of standard ascorbic acid and various extracts were found to be in the order Ascorbic acid > Methanol > Chloroform > Aqueous > Petroleum ether. The IC₅₀ values were determined by the regression equation from the plotted graph of DPPH scavenging activity against various concentrations of extracts and standard ascorbic acid. The lowest IC₅₀ value indicates the strongest ability of extracts to act as DPPH radical scavengers. The methanol extract showed lowest IC₅₀ value 75.87 µg/ml as compared to Chloroform, aqueous and petroleum ether extracts, having 96.19 µg/ml, 90.45 µg/ml and 275.81 µg/ml respectively while standard ascorbic acid have 42.92 µg/ml IC₅₀ value (Table 7 and Fig. 7).

Reducing power is associated with antioxidant activity and serves as a significant reflection of the antioxidant activity (Oktay et al., 2003). Reducing power assay is used to evaluate the ability of an antioxidant to donate an electron (Yildirim et al., 2000). The increase in absorbance with the increasing concentrations, the higher would be the reducing power. The presence of antioxidant in the extract resulted into reduction of the ferric cyanide complex to ferrous cyanide form. Strong reducing agents formed Perls Prussian blue colour and absorbed at 700nm. The standard ascorbic acid and all extracts reducing power are shown in (Table 8 and Fig. 8). The reducing power is concentration dependent for all extracts but methanol extract exhibit more reducing capacity. It can be concluded that the methanolic extract have strongest antioxidant activity as compared to other extracts. It may be due to the presence of large quantities of phytoconstituents as compared to the other extracts.

CONCLUSION

The present study suggests that qualitative phytochemical screening of crude extracts of *Berberis lycium* Royle roots bark revealed the presence of bioactive chemical compounds such as alkaloids, terpenoids, saponins and diterpenes. These constituents support its strong antioxidant activity when compared to standard ascorbic acid. The methanolic extract shows less IC₅₀ value among the extracts which supports its strongest antioxidant activity as compared to the other extracts. So the *Berberis lycium* Royle roots bark could be a potential source of nature and have great importance in multi-therapeutic agents. Further research is recommended for better characterization and isolation of bioactive compounds for the development of new good efficacy drugs in future.

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