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Original Article

Phytochemical screening, antimicrobial activity, *in vitro* and *in vivo* antioxidant activity of *Berberis lycium* Royle root bark extract

Triagem fitoquímica, atividade antimicrobiana, atividade antioxidante *in vitro* e *in vivo* do extrato de casca de raiz de *Berberis lycium* Royle

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Abstract

Antioxidants are materials that scavenge or remove free radicals from living systems. The oxidation process ends in the production of free radicals. These free radicals are the chief birthplace of cancerous cells. Antioxidizing agents remove free radical intermediates by terminating oxidation processes by being oxidized themselves. On the other hand, infectious diseases affect the world on a large scale. To fight these diseases several synthetic compounds have been used. Plant based medications play important role in this regard. So, the current research aimed to investigate the antibacterial and antioxidant effect of *Berberis lycium* Royle root bark (BLR) extract. *Berberis lycium* Royle was used for phytochemical analysis and also as antimicrobial and antioxidant agents. The antimicrobial activity was evaluated by the agar well diffusion method. Current study revealed that BLR was rich in phytochemicals and toxic against tested pathogenic bacteria. BLR showed the highest activity against *S. pyogenes* (13.3±0.8 mm). The lowest antibacterial activity was reported against *E. coli* (0±0 mm). In case of minimum inhibitory concentration, it was observed that BLR with 10 µg/mL concentration showed the highest activity while 2.5 µg/mL of BLR showed the least inhibitory activity. The highest *In vitro* antioxidant activity was recorded as 65% at 100 µg/mL. In case of *in vivo* antioxidant activity level of CAT, GSH and SOD were decreased while that of MDA was enhanced in groups treated with CCl_4 as compared to the control group. BLR extract treatment reversed all these changes significantly. Current results indicate that BLR is effective against bacterial pathogens and also has antioxidant potential.

Keywords: *Berberis lycium* Royle, root bark extract, phytochemicals, antibacterial, antioxidant.

Resumo

Os antioxidantes são materiais que eliminam ou removem os radicais livres dos sistemas vivos. O processo de oxidação termina na produção de radicais livres. Esses radicais livres são o principal local de nascimento das células cancerosas. Os agentes antioxidantes removem os intermediários dos radicais livres ao encerrar os processos de oxidação ao serem eles próprios oxidados. Por outro lado, as doenças infecciosas afetam o mundo em grande escala. Para combater essas doenças, diversos compostos sintéticos têm sido utilizados. Os medicamentos à base de plantas desempenham um papel importante a este respeito. Assim, o objetivo da pesquisa atual é investigar o efeito antibacteriano e antioxidante do extrato da casca da raiz de *Berberis lycium* Royle (BLR). *Berberis lycium* Royle foi utilizado para análises fitoquímicas e também como agentes antimicrobianos e antioxidantes. A atividade antimicrobiana foi avaliada pelo método de difusão em ágar em poço. A partir do estudo atual, observou-se que o BLR era rico em fitoquímicos e tóxico contra bactérias patogênicas testadas. BLR apresentou maior atividade contra *S. pyogenes* (13,3 ± 0,8 mm). A menor atividade antibacteriana foi relatada contra *E. coli* (0 ± 0 mm). No caso de concentração inibitória mínima, observou-se que BLR com concentração de 10 µg / mL apresentou maior

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atividade, enquanto BLR 2,5 µg / mL apresentou menor atividade inibitória. A maior atividade antioxidante *in vitro* foi registrada como 65% a 100 µg / mL. No caso do nível de atividade antioxidante *in vivo* de CAT, GSH e SOD diminuiu, enquanto o de MDA aumentou nos grupos tratados com CCl4 em comparação com o grupo controle. O tratamento com extrato de BLR reverteu todas essas mudanças significativamente. Os resultados atuais indicam que o BLR é eficaz contra patógenos bacterianos e também tem atividade antioxidante.

Palavras-chave: *Berberis lycium* Royle, extrato da casca da raiz, fitoquímicos, antibacteriano, antioxidante.

1. Introduction

Bacterial infections cause millions of infection-based morbidities and mortalities annually and became the worldwide open health matter (Ji et al., 2016). Microorganisms are cosmopolitan and cause various types of infectious diseases, hence they are termed pathogens. Every microbe has a different potential for pathogenicity. The efficacy of available antibiotics is endangered by pathogen resistance (Tong et al., 2018) which is a global health problem today. This peril is increasing day by day and breeds the requirement for innovative antibiotics with value-added bactericidal activities. Phyto-based medications are the need of the hour because of their eco-friendly nature. Medicinal plants are potential source of antibacterial drugs. Many of the synthetic drugs were discovered either directly or indirectly from the plant source (Swaroopa et al., 2017).

Antioxidants are, the substances that deal with the free radicals. Free radicals are the cause of numerous diseases like atherosclerosis, cancer, diabetes and liver cirrhosis (Slatern, 1987). Antioxidants involve in protecting the human body against the damage caused by free radicals. The antioxidants play their role as reducing agents, being oxidized themselves (Sies, 1997). Antioxidants, natural and artificial, have hydroxyl groups involved in the redox reactions. Antioxidants that occur naturally include vitamins (B, C, E), phytochemicals (mostly flavonoids) and some minerals having cupper, iron, zinc, manganese and selenium metals (Hamid et al., 2010). Phenolic compounds involved in the capturing and scavenging of free radicals act as synthetic antioxidants (Ansari et al., 2021).

Berberis lycium Royle (family: Berberidaceae), a plant, called barberry in English, the word "kashmal" and "Darhald" are used for fruit and roots respectively. *B. lycium* Royle is usually present in the Himalayas area (Kaur and Miani, 2001). Intestinal colic, diarrhea, jaundice, piles, ophthalmia, internal wounds, rheumatoid arthritis, gingivitis, diabetes, back pain, throat pain, scabies, pustules, remittent fever, broken bones, sun blindness, menorrhagia and intermittent fever are treated by using *B. lycium* Royle. *B. lycium* Royle is also playing its role as a diaphoretic, diuretic, febrifuge, and expectorant (Shabbir et al., 2012).

In many traditional preparations, different parts of this plant have been used. Dry powder of bark is used to treat throat pain, dysentery and wounds. Root bark aqueous extract is used for the treatment of diabetes, scabies and pustules. Root bark powdered paste has been used in the treatment of bone fracture, gingivitis and rheumatism (Ahmed et al., 2004). Traditional medicine systems involved in the development of modern medicines for curing numerous diseases.

According to various studies, many newly known targets of the drug are moderated by plant components. Traditional drugs should be renewed concerning the recent understanding of modern or allopathic medications. *B. lycium* Royle has been used as an anti-diabetic medicine for various times. Recent research work has mounted that *B. lyceum* Royle act as insulin, involves in reducing hyperglycemia. The major alkaloid in *B. lycium* Royle, Berberine, involved in the anti-inflammatory effect. To treat wounds, *B. lycium* Royle is suggested by local consultants. *B. lycium* Royle plays its role in healing wounds by increasing the deposition of collagen and epithelialization area. For the treatment of different diseases, new drug targets and modern medicines are being developed from the knowledge of traditional medicines (Aggarwal et al., 2006).

The current study aimed to find out the alternatives of the already available antibacterial and antioxidant agents. The extracts of *B. lycium* Royle were prepared and analyzed for antibacterial and antioxidant activity.

2. Materials and Methods

2.1. Ethical statement

Animal trials were carried out in accord with indigenous (law of Government College University, Lahore, Pakistan) and international law (Wet op de dierproeven, Wod, Article 9 of Dutch Law as mentioned in our former studies (Dar et al., 2019; Mughal et al., 2019; Mumtaz et al., 2019; Ali et al., 2020; Mughal and Ali, 2020; Mughal et al., 2020a; Mughal et al., 2020b).

2.2. Collection of medicinal plant

Berberis lycium Royle plant was collected from Battangi Mughalan, Chinari, District Jhelum valley, Azad Kashmir (Figure 1). The plant was recognized by ethno-phytologist, Department of Botany, University of Azad Jammu and Kashmir (UAJK) Muzaffarabad. The plant was washed with tap water to confiscate dust. Shade dried plant parts were creased into fine powder.

2.3. Preparation of plant extract

Plant powder (10 g) was boiled with distilled water (100 mL). The extract was filtered using Whatman No. 1 filter paper and intended by a rotary evaporator (R-200 Buchi, Switzerland). Plants extract was dried in a vacuum oven (Vacucell, Einrichtungen GmbH) at 40 °C (Figure 2) as mentioned in our previous studies (Mughal et al., 2020a; Mughal et al., 2020b; Mughal and Ali, 2020).

2.4. Phytochemical screening

The aqueous extract of BLR root bark was tested for the presence of alkaloids, steroids, tannins, saponins, glycosides,

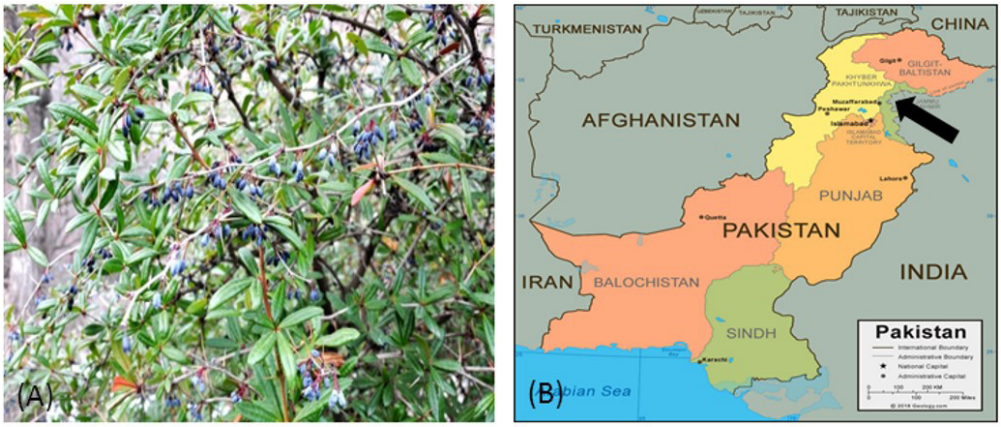

Figure 1. (A): *Berberis lycium* Royle, (B): Map of Pakistan. Arrow indicates the localization of plant collection.

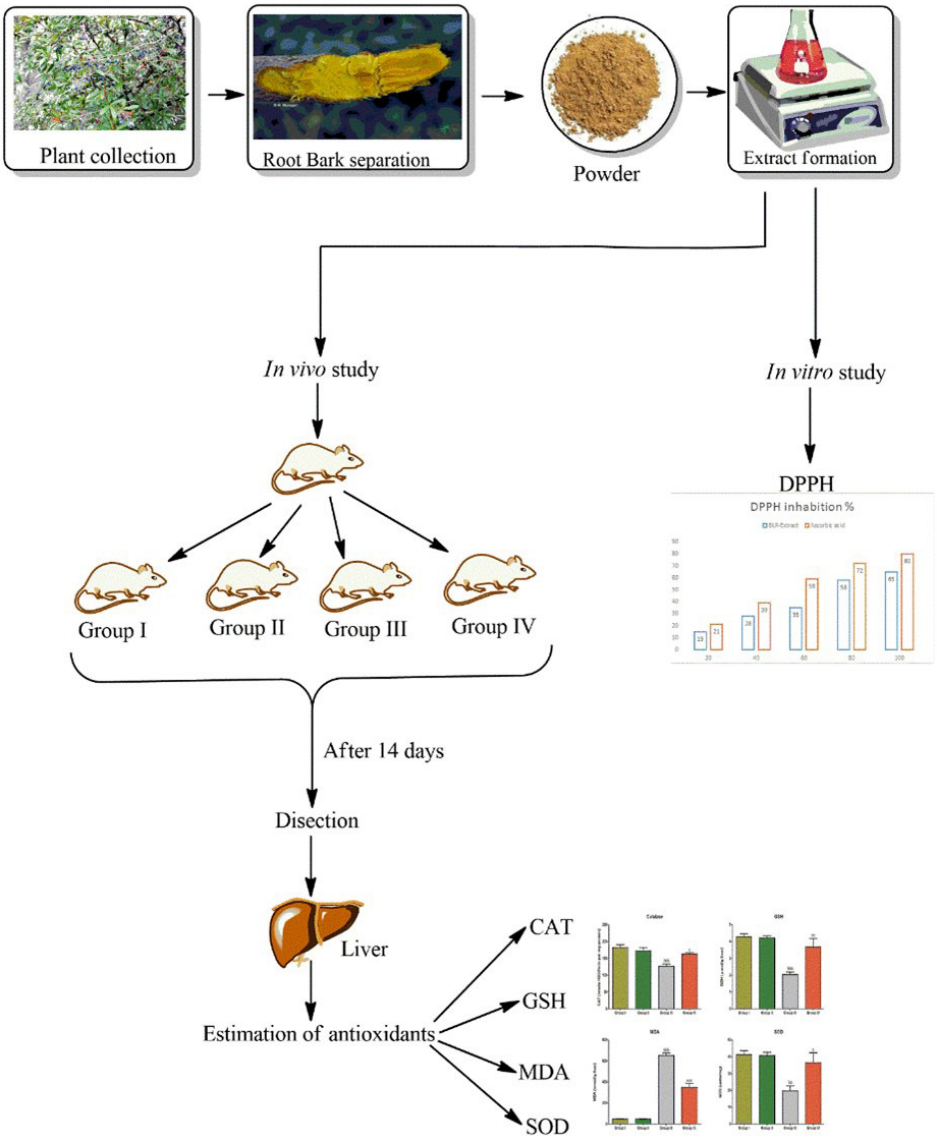

Figure 2. Schematic illustration of research plan.

terpenoids, proteins, free amino acids, carbohydrates, phenols and flavonoids according to a method mentioned in Souza et al. (2020) as shown in Table 1.

2.5. Antibacterial activity

2.5.1. Pathogenic bacteria tested

Seven clinical pathogens such as *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Serratia marcescens* (Gram-negative bacteria) *Staphylococcus epidermidis, Staphylococcus aureus* and *Streptococcus pyogenes* (Gram-positive Bacteria) were used.

2.5.2. Agar well diffusion assay

The antimicrobial activity was evaluated by the agar well diffusion method (Valgas et al., 2007).

2.5.3. Minimum Inhibitory Concentration (MIC)

The bactericidal activity of BLR was experienced using the standard microdilution method, which concludes the minimum inhibitory concentration (MIC) leading to the inhibition of microbial growth. MIC of BLR extract was determined by the agar well diffusion method. Different concentrations of extract (2.5 µg, 5 µg and 10 µg) per 1 ml of DMSO were used for antimicrobial activity. The least concentration that did not show growth of tested microorganisms was considered as MIC (Eloff, 1998).

2.6. In vitro antioxidant activity

2.6.1. DPPH radical-scavenging activity

The radical scavenging activity of the BLR extract was measured in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH (Kwon et al., 2007). 1.0 ml of the prepared DPPH (0.1 mM in ethanol) was added to different concentrations (0.5, 1 and 1.5 ml) of BLR-Extract. Reaction mixture was shaken and then incubated for 30 minutes in the darkness. The absorbance was recorded at 517 nm after thirty minutes. For the positive control, ascorbic acid was used. Higher radical scavenging activity was the indication of lower absorbance. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the subsequent formula (Equation 1):

Table 1. The test for different phytochemical constituents.

S. No.	Phytochemical constituents	Test	Methodology	Positive indications
1	Alkaloids	Wagner	i. Potassium iodide (2 g) + iodine (1.27 g) +Distilled water (5 ml)	Reddish brown and white
			ii. This solution was diluted with distilled water up to 100 ml.	Dull precipitate
			iii. Few drops of this solution + Extract	
2	Steroids	Salkowski	Extract (100 mg) + chloroform (2 ml) + con. H_2SO_4 (2 ml)	Formation of brown ring
3	Terpenoids	Salkowski	Extract (100 mg) + chloroform (2 ml) + con. H_2SO_4 (2 ml)	Reddish brown coloration
4	Tannins	Ferric chloride	Extract (0.5 gm) + distilled water (10 mL) +5% ferric chloride (few drops)	Black or blue-green coloration
				or precipitate
5	Saponins	Foam	Extract (0.5 g) + distilled water (10 ml)	Formation of frothing
6	Glycosides	Keller-Killiani	Extract (0.5 gm) + distilled water (5 ml) + glacial acetic acid (2 ml containing few drops of ferric chloride) + H_2SO_4 (1 ml)	formation of brown ring
7	Proteins	Ninhydrin	i. Extract (1 ml) + 0.2% ninhydrin reagent (few drops)	Blue color
			ii. Heated for 5 minutes	
8	Free amino acids	Ninhydrin	Extract (1 ml) + ninhydrin reagent (few drops)	Purple, light brown color
9	Carbohydrates	Benedict's	i. Extract (1 ml) +Benedict's reagent (2 ml)	Formation of reddish brown colorcolor
			ii. Heated for few seconds	
10	Phenols	Folin ciocalteu	i. Extract (1 ml) + Folin ciocalteu reagent (few drops) + sodium carbonate aquous solution.	Formation of grey or black color
			ii. Mixture was allowed to stand for 10 minutes	
11	Flavonoids	Ferric	Extract (2 ml) + $FeCl_3$ (few drops) chloride	Blackish red/ Dark brown color

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100 \quad (1)$$

2.7. In vivo antioxidant activity

In vivo antioxidant activity was studied by using mice as the experimental model.

2.8. Animal management

Animals were managed according to the method discussed in different studies (Dar et al., 2019; Mughal et al., 2019; Ali et al., 2020; Mughal and Ali, 2020; Mughal et al., 2020a; Mughal et al., 2020b). Mice of various groups were kept in different polypropylene cages. *Ad libitum* conditions were provided to the mice (adequate food and water). The temperature of the experimental room was kept within a narrow range of 22 ± 3 °C. Light and dark cycle of 12 hours was maintained in the room and the relative humidity was controlled to be within 70%.

2.9. Chemicals

Carbon tetrachloride was bought from Sigma, Aldrich (USA).

2.10. Experimental animals grouping

Twenty Swiss albino mice were obtained with an average weight of 43.6±1.5 g from Foot and Mouth Disease Research Center (FMDRC), Veterinary Research Institute (VRI), Lahore, Pakistan. The mice were treated gently, placed in cages in the hygienic and ventilated conditions in animal house. *Ad libitum* conditions were provided to the mice (adequate food and water). The lethal dose (LD_{50}) of CCl_4 was taken as 1 ml/kg body weight (b. w.). These animals were divided into 4 groups, each group having five mice namely I, II, III and IV. Group I was a control group administered with olive oil (0.4 ml/kg b. w.). Group II was given *B. lycium* Royle root bark extract (200 mg/kg b. w.). Group III was administered with CCl_4 (0.4 ml/kg b. w) intra-peritoneal for a single time and group IV was given CCl_4 (0.4 ml/kg b. w) and treated with *B. lycium* Royle root bark extract (200 mg/kg b. w.) for 14 days. All treatments were given orally. After the treatment period of 14 days, anesthesia was given to the animals after 24 hours of the last dosing, sacrificed and liver was taken for antioxidant study (Figure 2).

2.11. Estimation of antioxidants

2.11.1. Estimation of catalase

Sinha (1972) method determined the catalase activity in the liver. When dichromate in acetic acid was heated with H_2O_2 then firstly it was converted to perchromic acid which was further converted to chromic acetate. The product was then measured at the wavelength of 620 nm. The catalase preparation was then allowed to catalyze H_2O_2 for various periods. Dichromate-acetic acid is added to the reaction mixture at different time intervals, stoped the reaction for sometimes and the leftover H_2O_2 as chromic acetate was evaluated calorimetrically.

2.11.2. Estimation of Reduced Glutathione (GSH) (non-enzymatic antioxidant)

Using the standard protocol, the activity of reduced glutathione was measured (Ellman, 1959). 0.5 of Elman reagent i.e., 19.8 mg DTNB dissolved in 100 mL of 0.1% sodium nitrate is added to the aliquot of 1 ml liver tissue supernatant. Following the addition of Elman reagent, phosphate buffer i.e., 3 ml was added. Then, the absorbance was calculated at 412 nm.

2.11.3. Estimation of Malondialdehyde bis-(dimethyl acetal) Tetra Ammonium (MDA)

Okkawa et al. (1979) described the following method. The tissues of the liver were homogenized in the solution of aqueous KC1 followed by incubation with a thiobarbituric acid reagent for about 1 hour at 90 °C. The mixture was then centrifuged after cooling. After the centrifugation, clear pink supernatant was obtained whose optical density value was determined at 532 nm. As an external standard, malondialdehyde bis-(dimethyl acetal) tetra ammonium was used.

2.11.4. Estimation of Superoxide Dismutase (SOD)

The standard method with small modifications was used to estimate the superoxide dismutase (Chia et al., 2010). By the addition of 1 ml of 50 mM sodium carbonate, 0.4 ml of 25 µM nitroblue tetrazolium and 0.2 ml of 0.1 mM freshly prepared hydroxylamine hydrochloride, the reaction mixture was prepared. To the reaction mixture, liver homogenate's clear supernatant (0.1 ml, 1:10 w/v) was added. The absorbance of the sample was determined at 560 nm and changes in the value were observed.

2.12. Statistical analysis

All statistical analyses were performed by Graph Pad prism. All the values were expressed as mean ± SEM. The statistical difference among different groups was assessed by one-way ANOVA with the Bonferroni test. Values were considered statistically significant at $p \leq 0.05$.

3. Results

3.1. Phytochemical screening

The plant extract was tested for the presence of different phytochemicals. Results are shown in Table 2.

The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals.

3.2. Antibacterial activity

The study was started to investigate the antibacterial effect of *Berberis lycium* Royle root bark aqueous extract (BLR-Ex) against various disease-causing bacteria. From the current study, it was observed that BLR extract was toxic against tested pathogenic bacteria.

Table 3 shows the zones of inhibition of BLR extract at 2.5 µg/ml, 5 µg/ml and 10 µg/mL. BLR-extract of 10µg/mL displayed antibacterial activity (*E. coli:* 9.3±0.7 mm*; K.*

pneumoniae: 9.7±0.16 mm*; S. aureu:* 10±1.5 mm; *S. pyogenes:* 13.3±0.8 mm; *P. seudo:* 8.5±0.2 mm; *S. marcesscens:* 9.8±0.4 mm*; S. epidermidis:* 10±0.6 mm). The highest activity was shown against *S. aureu* (10.0±1.5 mm) and *S. epidermidis* (10.0±0.6 mm). 2.5 µg/ml showed minimum activity (*S. aureu:* 3.3±0.3 mm; *S. pyogenes:* 5.3±0.3 mm; *P. seudo:* 2±0.5 mm; *S. marcesscens:* 2.7±0.3 mm) and in some cases, it showed no activity at all (*E. coli: 0*±0 mm*; K. pneumoniae: 0*±0 mm*; S. epidermidis: 0*±0 mm) as shown in Figure 3.

3.3. In vitro antioxidant activity

By the use of the DPPH test, the antioxidant capacity of extract was evaluated. Ascorbic acid was considered as the positive control. The results in Figure 4 show that antioxidant activity was increased by increasing the BLR-extract concentration. At 20, 40, 60, 80 and 100 µg/mL it was 15, 28, 35, 58, and 65% respectively (Figure 4).

3.4. In vivo antioxidant activity

3.4.1. Effect on Catalase (CAT)

CCl_4 (0.4 mL/kg body weight) injection through intraperitoneal route caused a highly significant decrease in levels of Catalase (CCl_4: 126.8±5.67 mmol/min/g liver)

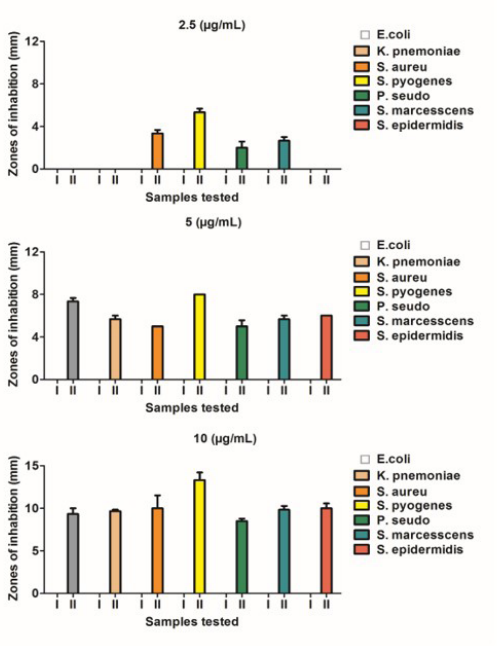

Figure 3. Zones of inhibition at 2.5, 5 and 10 µg/mL concentration of (I) DMSO and (II) BLR extract.

Table 2. Phytochemical screening.

Sample No.	Phytochemical tested	Results
1	Alkaloids	+
2	Steroids	+
3	Tannins	+
4	Saponins	+
5	Glycosides	+
6	Carbohydrates	+
7	Proteins	+
8	Free Amino acids	+
9	Terpenoids	+
10	Phenols	+
11	Flavonoids	+

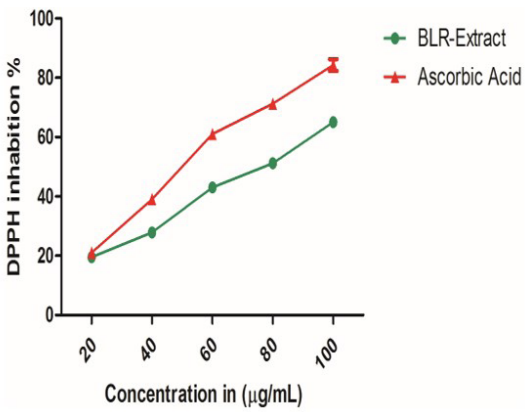

Figure 4. *In vitro* antioxidant activity at 20-100 µg/mL.

Table 3. Antibacterial activity of *Berberis lycium* Royle extract against bacterial pathogens.

Agents	Conc. (µg/ml)	*E. coli*	*K. pneumoniae*	*S. aureus*	*S. pyogenes*	*P. aeruginosa*	*S. marcesscens*	*S. epidermidis*
Dimethyl sulfoxide (DMSO)	2.5	0	0	0	0	0	0	0
	5	0	0	0	0	0	0	0
	10	0	0	0	0	0	0	0
BLR extract	2.5	0	0	3.3±0.3	5.3±0.3	2±0.5	2.7±0.3	0
	5	7.3±0.3	5.7±0.3	5±0	8±0	5±0.6	5.7±0.3	6±0
	10	9.3±0.7	9.7±0.16	10±1.5	13.3±0.8	8.5±0.2	9.8±0.4	10±0.6

as compared to control (182±7.76 mmol/min/g liver). When BLR extract was given alone it caused no significant change (172.0±8.34 mmol/min/g liver) as compared to control (182±7.76 mmol/min/g liver). When BLR extract was given intraperitoneally (150 mg/kg body weight) to CCl_4 treated mice, a significant change in the amount of Catalase (CCl_4 + BLR extract: 163.2±3.29 mmol/min/g liver) was observed (Figure 5).

3.4.2. Effect on Reduced Glutathione (GSH)

CCl_4 (0.4 mL/kg body weight) intraperitoneal administration resulted in a significant decrease in levels of GSH (control: 4.28±0.15 µ mol/g liver; CCl_4: 2.04±0.12 µ mol/g liver). When BLR extract was given alone it caused no significant change (4.22±0.1 µ mol/g liver) as compared to control (4.28±0.15 µ mol/g liver). When BLR extract was given intraperitoneally (150 mg/kg body weight) to CCl_4 treated mice, a highly significant increase in the amount of GSH (CCl_4 + BLR extract: 3.68±0.42 µ mol/g liver) was observed (Figure 5).

3.4.3. Effect on Malondialdehyde bis-(dimethyl acetal) Tetra Ammonium (MDA)

Intraperitoneal administration of CCl_4 (0.4 mL/kg body weight) resulted in a significant increase in levels of MDA (control: 46.2±4.45 mmol/g liver; CCl_4: 652.2±20.19 mmol/g liver). When BLR extract was given alone it caused no significant change (45.8±4.95 mmol/g liver) as compared to control (46.2±4.45 mmol/g liver). When BLR extract was administered intraperitoneally (150 mg/kg body weight) to CCl_4 treated mice, a highly significant decrease in the level of MDA (CCl_4 + BLR extract: 349.6±31.75 mmol/g liver was observed (Figure 5).

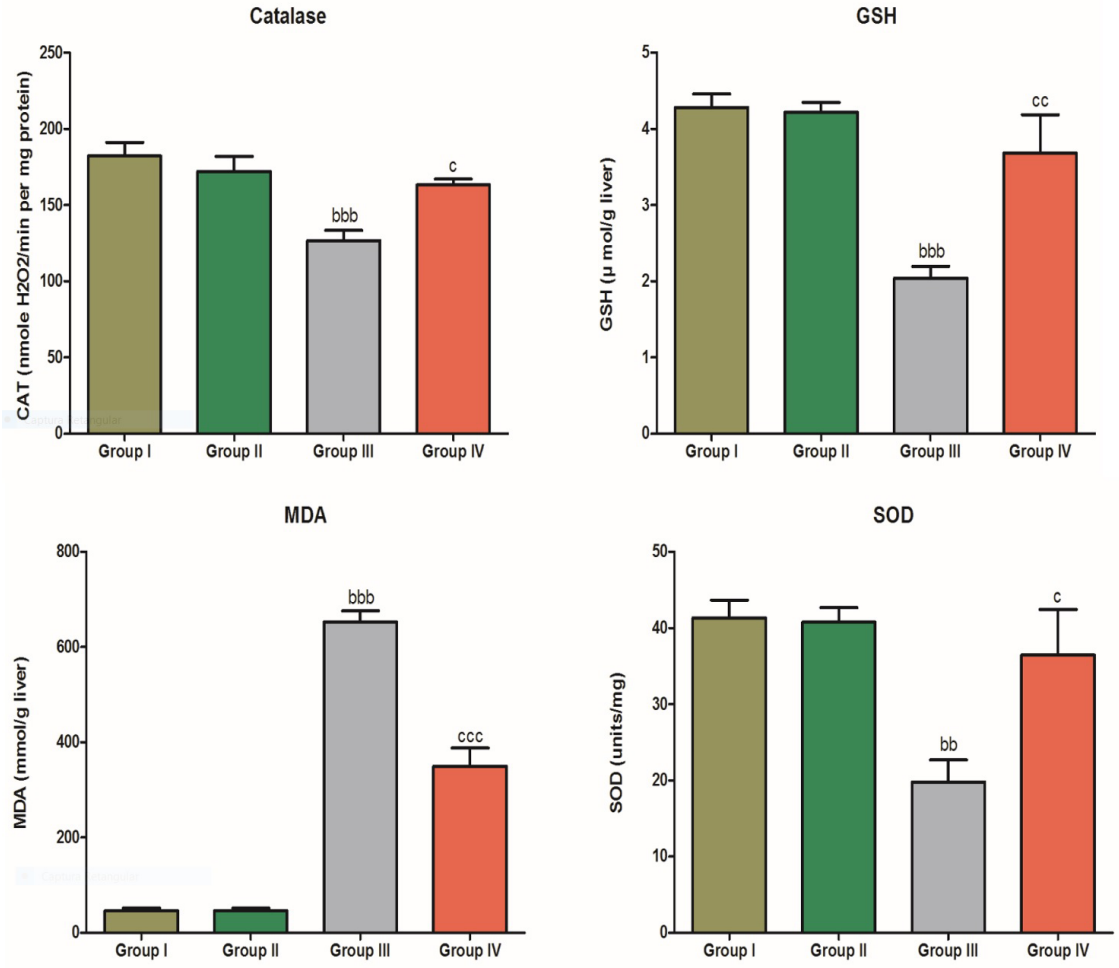

Figure 5. In vivo antioxidant activity. Analysis of catalase, GSH, MDA and SOD in the liver of Swiss albino mice. Group I: control group; Group II: B. *lycium* Royle root bark extract treated group; Group III: CCl4 treated group; Group IV: CCl4 plus B. *lycium* Royle root bark extract treated group. Keys: (b) indicates the significant difference between group I and group III. (c) indicates a significance difference between group III and group IV. Each bar signifies the mean value of five replicates and SEM. Statistical icons: c = $p \leq 0.05$, bb, cc = $p \leq 0.01$, bbb, ccc = $p \leq 0.001$.

3.4.4. Effect on Superoxide Dismutase (SOD)

Intraperitoneal administration of CCl_4 (0.4 ml/kg body weight) produced a highly significant decrease in the SOD level of the CCl_4 treated group (19.8±2.41 U/mg) as compared to the control (41.35±1.97 U/mg). When BLR extract alone was given to mice they caused no significant change in SOD level (40.8±1.59 U/mg). When BLR extract was injected intraperitoneally (150 mg/kg body weight) to CCl_4 treated mice, the significant increase in the amount of SOD (CCl_4 + BLR extract: 36.4±5.096 U/mg) was observed (Figure 5).

4. Discussion

Plant parts have been used for a long time to defeat microbes. Local populations were aware of plant's medicinal uses. Available antibiotics face resistance to microbes. This problem is increasing day by day. Hence, the development of new antimicrobial drugs is the necessity of time. Plants are enriched with molecules that have antimicrobial activity. Plants are being scrutinized as possible sources to explore new bactericidal mediators to manage bacterial infections. Lamiaceae and Rutaceae family plants are broadly used in numerous medicinal practices to treat wide-ranging infections (Sharma et al., 2013; Venkateshappa and Sreenath, 2013). Sasikala et al. (2014) testified aqueous extract of *P. barbatus* against *B. cereus*, *E. coli*, *S. aureus*, *S. epidermidis*, and *S. pneumonia*. This extract showed antimicrobial activity against all these pathogens. Our results are in line with these results. A lot of previous studies confirmed that plants act as antibacterial agents. Results of investigations by Vaťák et al. (2014), Erecevit and Kirbağ (2017), Zoysa et al. (2019), Manandhar et al. (2019), Nguta and Kiraithe (2019) and Othman et al. (2019) also support our findings.

It is already known that *Berberis lycium* bark contains an alkaloid namely Berberine which has great antimicrobial potential. The rise in the concentration of root bark extract increases the inhibitory activity (Figure 3). The poposed antibacterial mechanism of plant extract is diagrammatically expressed below (Figure 6).

In the current study, experiments were also designed to unveil the antioxidant potential of aqueous extract of *Berberis lycium* Royle's root bark. Antioxidant activity was measured *in vitro* and *in vivo* conditions. *In vitro* activity was measured by the DPPH method. Antioxidant capacity relates to the reducing capability of a component. The antioxidant compounds are based on the reduction of the free radicals. DPPH is a stable free radical, is often involved in the determination of radical scavenging activity in the chemical analysis (Duh et al., 1999). DPPH radicals possess a single electron and at 517 nm demonstrates the highest absorption. The DPPH results are shown in Figure 4. Color removal of the DPPH solution indicates its reduced state as a result of the interaction of electrons with the reducing agents. After receiving electrons or protons DPPH free radicals were reduced (Muniyappan and Nagarajan, 2014). When the concentration of BLR extracts raised from 20 µg/mL to 100 µg/mL, the antioxidant activity increased up to 65% from 15%. The maximum antioxidant activity was observed as 65% at 100 µg/mL.

In vivo antioxidant activity of BLR extract was evaluated by comparing hepatic CAT, GSH, MDA and SOD levels of CCl_4 intoxicated mice and BLR treated mice. In present

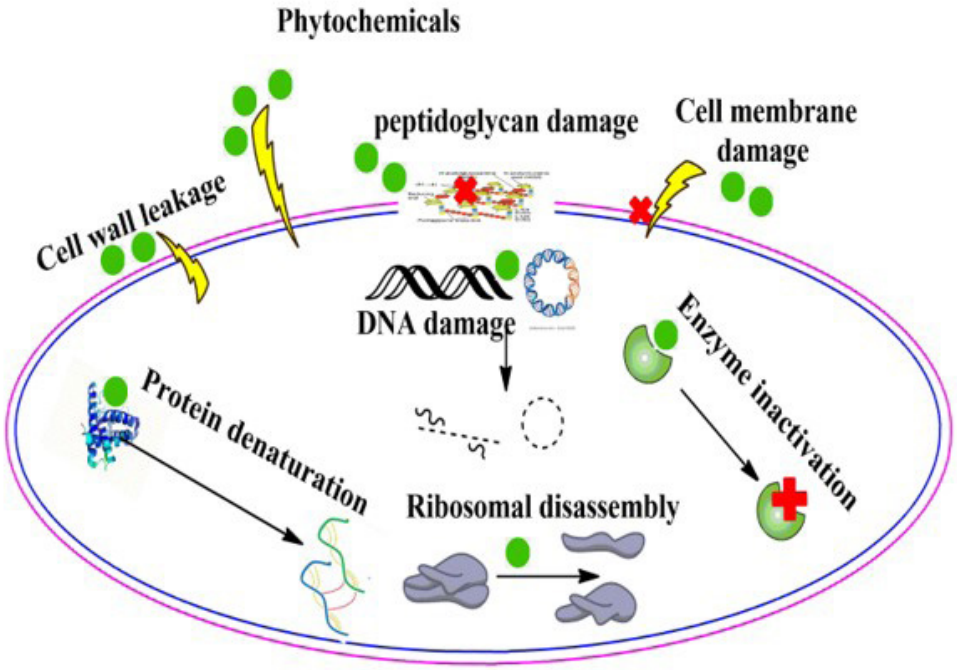

Figure 6. Proposed bactericidal mechanism of plant extract.

research in CCl_4 intoxicated mice level of MDA was increased while that of catalase, GSH and SOD decreased. These conditions were reversed on treatment with BLR-extract (Figure 5). These results are in line with Al-Snafi (2017) who stated that when rats were treated with thioacetamide to induce the oxidative stress significant abate in CAT, GSH and SOD level was found while, when these mice were treated with seed extract of *Dacus carota*, significant elevation in the CAT, GSH and SOD level was determined according to the thioacetamide group. Rahate and Rajasekaran (2015) had found that when female rats were intoxicated with tamoxifen to induce hepatotoxicity significant increase in the MDA level was measured according to the control group. Whereas, a polyphenolic fraction of *Desmostachia bipinnata* root brought down the level of MDA and elevated the total protein, SOD and GSH concentration in serum of female rats.

5. Conclusion

The present study validated that *B. lycium* Royle is enriched with alkaloids, steroids, tannins, saponins, glycosides, terpenoides, proteins, free amino acids, carbohydrates, phenols and flavonoids. This study also validated that *B. lycium* Royle root bark extract exhibits antibacterial activity. Results also revealed that hepatic damage was caused by the administration of carbon tetrachloride in Swiss albino mice. CAT, GSH, MDA and SOD levels were disturbed that were normalized by BLR extract. The present study validated that *B. lycium* Royle root bark extract has a potential to cope with infectious diseases and also exhibit antioxidant activity as estimated *in-vivo* and *in-vitro*.

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