ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 17, Issue 6, 2024

Online - 2455-3891
Print - 0974-2441

Research Article

PHYTOCHEMICAL SCREENING, ANTIMICROBIAL, AND ANTIOXIDANT ANALYSIS OF *BERBERIS LYCIUM* ROYLE CRUDE ROOT EXTRACTS

MONICA SANGRAL, DIVYA GUPTA, PARAMVEER SINGH, ROSHANI LOCHAN, NAZLI BHAT, KIRANBALA, MADHULIKA BHAGAT*

School of Biotechnology, University of Jammu, Jammu, Jammu and Kashmir, India.
***Corresponding author: Madhulika Bhagat; Email: madhulikasbt@gmail.com**

Received: 22 March 2024, Revised and Accepted: 05 May 2024

ABSTRACT

Objective: This particular study was designed to evaluate the qualitative, quantitative, anti-oxidant, and antimicrobial potential of *Berberis lycium* root extracts.

Methods: The plant root extracts were prepared using a sequential extraction method using different solvents with increasing polarity. Further extracts were evaluated qualitatively for the presence of different phytochemicals and quantitatively for the presence of total phenols and flavonoid, antioxidant potential using DPPH, ferric reducing anti-oxidant power, and H_2O_2 radical scavenging assay. Biological potential of these extracts was established for antibacterial effect against different Gram-negative and Gram-positive bacteria and antifungal potential against fungal strains.

Results: Qualitative analysis of the root extracts showed the presence of phytochemicals such as saponins, tannins, phenols, and flavonoids. Among all the evaluated extracts in quantitative analysis, methanolic extracts showed the highest content of phenolic with 39.75 mg gallic acid equivalence/g and flavanoid with 456 mg QE/g, highest antioxidant potential with half-maximal inhibitory concentration (IC_{50}) value of 10.96 µg/mL in DPPH assay. Similarly, the highest antibacterial effect against *Bacillus cereus* (20±0.86 mm) and antifungal against *Aspergillus niger* (IC_{50} 399.64 µg/mL) was observed.

Conclusion: The plant root extracts of *B. lycium* have shown substantial content of phytochemicals with appreciable antioxidant and antimicrobial activities comparable to the currently prescribed modern drugs tested. Accordingly, further studies on clinical efficacy trial, safety, and toxicity analyses have to be instigated promptly, so as to head to the final step to synthesize precursor molecules for new effective antimicrobials.

Keywords: *Berberis lycium*, Root extracts, Phytochemicals, Antioxidant, Antibacterial, Antifungal.

 DOI: http://dx.doi.org/10.22159/ajpcr.2024v17i6.51305. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Berberis lycium of the family *Berberidaceae* is an evergreen herb also known by the name berberry, kashmal, and Ishkeen. Each part of the plant has medicinal value such as root, bark, stem, and fruits that are used for various ayurvedic preparations [1]. It is extensively used for the treatment of various diseases such as liver disorders, abdominal disorders, skin diseases, cough, and diabetes mellitus. [2]. The plant can be located covering the belt from Jammu and Kashmir, Himachal Pradesh, Uttar Pradesh, Sikkim, Madhya Pradesh, and Tamil Nadu. The local practice involves the use of the root part of *B. lycium* for the treatment of several human infections [3]. The plant has been exploited for the isolation of many compounds out of which the major alkaloid, berberine which is an isoquinoline dominates [4]. Other compounds, such as berbamine [5], ascorbic acid, chinabine, malic acid, karakoramine, acetic acid, and palmatine [6], are known to be present in various other parts of the plant. Interestingly, the distribution of berberine has been studied which showed its localization primarily in the roots and stem cortical tissues. The upper stem part is known to have low concentration of alkaloids while in leaves alkaloids could not be detected [7]. In Indian species of *Berberis*, the histological distribution of berberine is well examined [8,9]. The tannins, malic acid, tartaric, and citric acid are present in the fruit part of the plant [10]. *B. lycium* found an important place in Ayurveda for its wide range of application. It is classified as lekhaniya (i.e. reducing obesity and scarifying), arsaghna (cure for piles and hemorrhoids), and kandughna (curative of pruritus) in Charka Samhitta whereas it is used for the treatment of dysentery, uterine and vaginal disorders, quick healing of wounds and indigestion in Sushruta Samhitta [11]. The present study aims to screen *B. lycium* root extracts collected from the Rajouri region of J and K for their phytochemical and antimicrobial potential.

METHODS

Sample collection

Roots of *B. lycium* were collected in a sterilized bag from the Rajouri district of Jammu. The plant was identified by morphological features. Further, the identity of the plant was authenticated by an expert taxonomist from the Department of Botany, University of Jammu.

Sequential extraction

The extracts were prepared using sequential extraction methods, that is, solvents from low polarity to high polarity. 150 g of powder was dissolved in 300 mL chloroform and kept for 1 day. It was then filtered and the obtained residue was further extracted 2 times using the same solvent. Then the residue left as the solvent evaporated was collected in the vials and weighed. The Same procedure was followed for other extracts [12]. Other solvents which were used sequentially after chloroform were ethyl acetate, acetone, methanol, and water, as this progress from low polarity to high polarity. Finally, the extracts obtained from each solvent were correctly labeled and stored in glass vials at −20°C for further use.

Qualitative screening of root extracts of *B. lycium*

The extracts were subjected to phytochemical screening to test the presence or absence of phytochemical constituents such as tannins, saponins, terpenoids, coumarins, quinines, anthraquinones, and cardiac glycosides. For qualitative analysis, 50 mg of extract was dissolved in

1 mL of dimethyl sulfoxide (DMSO). It was kept as a stock solution of extracts and used for various tests [13].

Quantitative phytochemical analysis

Total phenols were estimated using Folin–Ciocalteu assay described by [14]. This is a colorimetric assay and is also called as gallic acid equivalence (GAE) method. The total flavonoid content of the samples was estimated using the colorimetric method given by [15]. The presence of flavonoid was detected by the formation of yellow colored complex. Quercetin was used as a standard.

Determination of antioxidant activity

The DPPH radical-scavenging activity

It was determined using the method of [16]. Ascorbic acid and Trolox were used as positive controls; methanol as negative control and extract without DPPH was used as blank. Results were expressed as percentage reduction of the initial DDPH absorption in relation to the control. The concentration of extract leading to 50% reduction of DPPH (half-maximal inhibitory concentration [IC_{50}]) was also determined.

Ferric reducing antioxidant power (FRAP) assay

This method is used for assessing total antioxidant power [17]. It is based on the principle that the reduction of ferric to ferrous ions at low pH causes the formation of a colored ferrous-tripyridyl triazine complex. The more the color more is the antioxidant power. Ferrous sulfate was taken as standard, and the results were expressed as μg Fe (II)/g of extract.

Hydrogen peroxide scavenging assay

Hydrogen peroxide can cross cell membranes rapidly once inside the cell, H_2O_2 can probably react with Fe_2+, and possibly Cu_2+ ions to form hydroxyl radical and this may be the origin of many of its toxic effects. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. Ascorbic acid was used as a positive control compound.

The percentage inhibition was calculated using the following equation=([AO−AS]/AO) ×100.

Antimicrobial screening

Test microorganisms

Three Gram-positive bacteria: *Bacillus subtilis* (MTCC No. 441), *Bacillus cereus* (MTCC No. 492), *Micrococcus luteus* (MTCC No. 2470), and *Staphylococcus epidermidis*; two Gram-negative bacteria: *Alcalygenes denitificans* (MTCC No. 5710), *Klebsiella pneumoniae* (MTCC No. 530) and three fungi: *Fusarium oxysporum* (MTCC No. 1755), *Bipolaris specifera* (MTCC No. 2769) and *Aspergillus niger* (MTCC No. 1344) were used in the study.

Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB) for bacteria and Sabouraud dextrose broth (SDB) for fungi that were incubated without agitation for 24 h at 37°C and 25°C, respectively. To 5 mL of MHB and SDB, 0.2 mL of culture was inoculated and incubated till it reached turbidity.

Antibacterial screening

The anti-bacterial activity of the crude extracts was determined by the agar well diffusion method [18] with brief modifications. Agar well diffusion method was performed for screening of antibacterial activity and the antibiotic streptomycin (10 μg/mL) was used as standard. The media was prepared and poured at a rate of 15 mL each in a petri dish and allowing for solidification for about 5 min. A loopful of inoculums was swabbed uniformly onto the media and left them drying. The different concentration of root extract of (20 μg/mL, 40 μg/mL, 60 μg/mL, and 80 μg/mL) and standard (10 μg/mL) was loaded into each well and allowed diffusion of extract. Plates are incubated overnight at 37°C zone appeared after the incubation period and the percent of incubation was determined in millimeters using scale. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded.

Antifungal screening

Antifungal activity of the root crude extract was determined by the poisoned food technique [19] with some modification. Stock extracts (1 mg/mL) were prepared by dissolving in 1% DMSO. Plant extract concentrations of 5%, 10%, and 15% were obtained by adding an appropriate quantity of the corresponding solvent to a standard concentration (1 mg/mL). Potato dextrose agar (PDA) medium with various concentrations of root extracts was sterilized and put on labeled petri plates. The plant extract concentrations were combined with PDA medium and then solidified on sterilized petri plates in a laminar airflow setting. The Petri plates were infected after solidification by putting 5 mm mycelial discs of the specific fungus at the center of each plate. The discs were collected from colonies that were rapidly growing. Triplicates of each dosage were retained. At a temperature of 24±2°C, the Petri plates were inspected for mycelial development after 7 days of incubation. As a control, PDA plates without root extracts (1% DMSO) were utilized and hexaconazole (1 mg/mL) was considered as the positive control. The inhibition of growth (as percentage) caused by different treatments at various dosages was calculated using the below equation:

$$\% \text{ inhibition} = (C - T)/C \times 100$$

where *C* signifies the fungal colony's average diameter (mm) in the control and *T* denotes the fungal colony's average diameter (mm) in the test.

Statistical analysis

The collected data were subjected to the analysis of variance (ANOVA), and the means were separated using the least significant difference at $p \leq 0.05$ and at $p \leq 0.01$.

RESULTS AND DISCUSSION

Percentage yield of *B. lycium* extracts

The highest percentage yield was exhibited by the water extract with 0.70% followed by methanol (0.633%), chloroform (0.12%), acetone (0.046%), and least by the ethyl acetate extract (0.04%) (Table 1).

Qualitative analysis of phytochemicals of *B. lycium* extracts

The extracts of *B. lycium* show the presence of nearly all the categories of phytochemicals, that is, saponins, terpenoids, flavonoids, anthocyanins, coumarins, cardiac glycosides, and phenols (Table 2). Our analysis was in agreement with George *et al*., [20] who reported the results of the chemical tests for the screening which revealed the presences of flavonoids, alkaloids, tannins, carbohydrates, glycosides in the methanolic extract of *B. lycium* root.

Total phenolic and flavonoid content of *B. lycium* extracts

The highest phenolic content was exhibited by the methanol extract (39.752 mg GAE/g) followed by water extract (39.532 mg GAE/g), ethyl acetate (28.571 mg GAE/g), chloroform (23.269 mg GAE/g) and least by the acetone extract (7.912 mg GAE/g) (Fig. 1). The highest flavonoid content was exhibited by the methanolic extract (456 mg quercetin equivalents [QE]/g) followed by the water (409 mg QE/gm), ethyl acetate(311mg QE/g), chloroform (277 mg QE/g) extract and least by the acetone extract (199 mg QE/g) (Fig. 2). Our analysis was in accordance with [21] who reported high total phenolic content in methanol extract of *Buxus microphylla* of family *Berberidaceae* (64.22 mg GAE/g dry wt).

DPPH radical scavenging assay of *B. lycium* extracts

The radical scavenging activity was measured as the decrease in absorbance of the samples versus the DPPH standard solution. This

Table 1: Percentage yield of *Berberislycium* root sex tracts

Plant material	BLC (chloroform extract)	BLEA (ethyl acetate extract)	BLAC (acetone extract)	BLM (methanol extract)	BLW (water extract)
150 g powder	0.12	0.04	0.046	0.633	0.70

BLC: *Berberis lyceum* chloroform, BLEA: *Berberis lyceum* ethyl acetate extract, BLAC: *Berberis lyceum* acetone extract, BLM: *Berberis lyceum* methanol, BLW: *Berberis lyceum* water

Table 2: Qualitative analysis of phytochemicals of extracts of *Berberis lycium*

Extracts	Saponins	Terpenoids	Coumarins	Cardiac glycosides	Flavonoids	Phenols	Anthocyanins
BLC	++	++	+	++	++	++	++
BLEA	+	+	+	+	++	+	+
BLAC	+	++	+	+	++	+	++
BLM	+	+++	++	++	++	++	++
BLW	++	++	+	++	++	+	++

+: Slight amount, ++: High amount, +++: Very high amount, −: Absent. BLC: *Berberis lyceum* chloroform, BLEA: *Berberis lyceum* ethyl acetate extract, BLAC: *Berberis lyceum* acetone extract, BLM: *Berberis lyceum* methanol, BLW: *Berberis lyceum* water

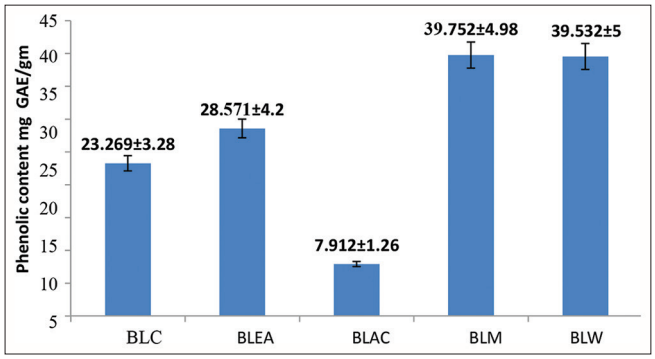

Fig. 1: Total phenolic content of *Berberis lycium* extracts

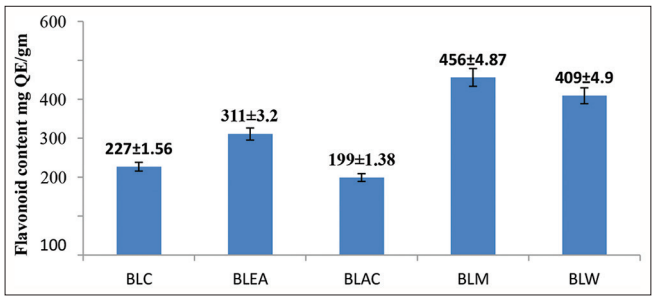

Fig. 2: Total flavonoid content of *Berberis lycium* extracts

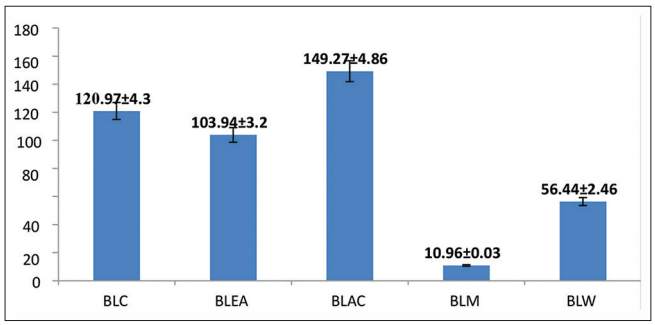

Fig. 3: The DPPH assay of *Berberis lycium* extract

assay was carried out in duplicates. The antioxidant potential of samples was expressed in terms of IC_{50} which is defined as the concentration of substrate necessary to scavenge 50% of DPPH free radicals. The highest antioxidant potential was demonstrated by the methanolic extract

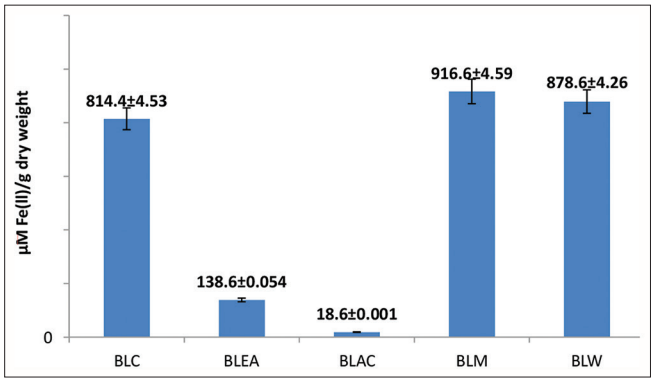

Fig. 4: Ferric reducing antioxidant power assay of *Berberis lyceum*

(IC_{50} 10.96 µg/mL) followed by water (IC_{50} 56.44 µg/mL), ethyl acetate (IC_{50} 103.94 µg/mL), chloroform extract (IC_{50} 120.97 µg/mL) and least by the acetone extract (IC_{50} 149.27µg/mL) (Fig. 3). Pet ether extract of *B. lycium* showed maximum antioxidant activity (82% inhibition) followed by methanol (49.7% inhibition) [21].

FRAP assay of *B. lycium* extracts

In the present study, the standard curve of ferrous (Fe^{2+}) sulfate was plotted using different concentrations (100–1500 µM) and the results were expressed as µM Fe (II)/g dry weight of the plant material. The FRAP value of extracts ranged from 18.6 µM Fe (II)/g to 916.6 µM Fe(II)/g dry weight of the plant material. According to the antioxidant power, all the extracts were divided into four groups as per the classification given by [23]; extremely high (>500 µM Fe(II)/g), high (100–500 µM Fe(II)/g), medium 10–100 µM Fe(II)/g) and low (≤10 µM Fe(II)/g). As per estimated by our analysis, it was observed that the methanolic extract of *B. lycium* showed the strongest antioxidant capacity to reduce ferric ions with FRAP value 916.6 µM Fe(II)/g dry weight followed by water, chloroform, ethyl acetate extract, and least by the acetone extract (Fig. 4).

Hydrogen peroxide scavenging assay of *B. lycium* extracts

The hydrogen peroxide scavenging activity of extracts of *B. lycium* was evaluated and compared with ascorbic acid. The minimum IC_{50} were calculated of extracts of *B. lycium* after the evaluation of percentage inhibition. Our analysis is in accordance with Lamichhane *et al*., 2014 who reported that *B. aristata* have an effective H_2O_2 scavenging activity. The percentage scavenging activity on hydrogen peroxide radical is 81.8% of extract at the concentration 100 µg/mL is similar to that of ascorbic acid standard showing an inhibition percentage of 86.7%. The highest hydrogen peroxide scavenging activity was demonstrated by the methanolic extract (IC_{50} 433.98 µg/mL), followed by water extract

Table 3: Antibacterial assay of *Berberis lycium* extracts

Extracts (100 µg/mL)	Zonesofinhibition (mm)				
	Bacillus subtilis	*Achromobacter denitrificans*	*Klebsiella pneumoniae*	*Micrococcus luteus*	*Bacillus cereus*
BLC (mm)	15±1.34	8±0.87	7±1.75	11±2.38	10±0.05
BLEA (mm)	10±0.43	10±0.74	9±1.65	19±0.8	18±2.26
BLAC (mm)	12±3.64	10±0.35	10±2.47	11±3.5	12±1.37
BLM (mm)	20±0.86	14±1.45	15±0.37	17±1.33	19±0.37
BLW (mm)	18±0.66	7±0.05	8±1.47	14±0.48	16±0.35
Ampicillin (30 µg/mL) (positivecontrol) (mm)	30±0.05	25±0.03	23±0.32	21±0.043	23±0.054

BLC: *Berberis lyceum* chloroform, BLEA: *Berberis lyceum* ethyl acetate extract, BLAC: *Berberis lyceum* acetone extract, BLM: *Berberis lyceum* methanol, BLW: *Berberis lyceum* water

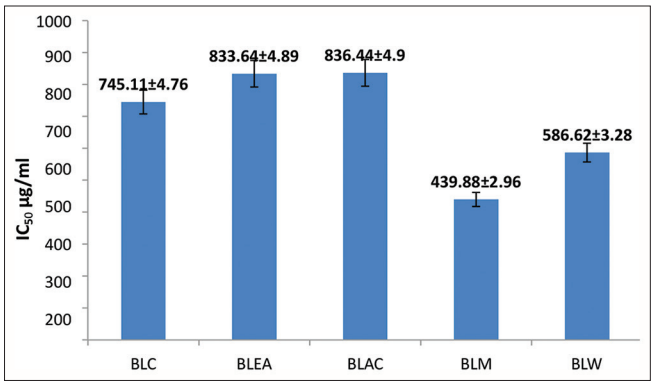

Fig. 5: Hydrogen peroxide scavenging assay of *Berberis lycium* extracts

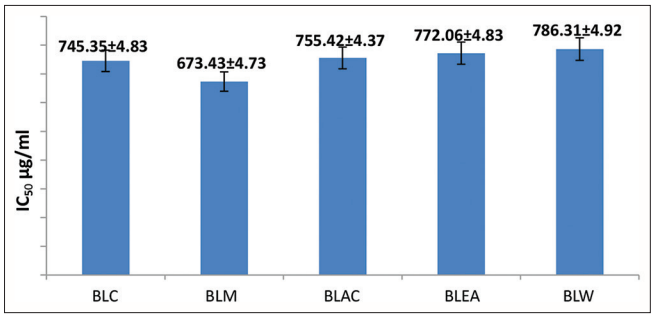

Fig. 6: Antifungal assay of *Berberis lycium* extracts against *Fusarium oxysporum*

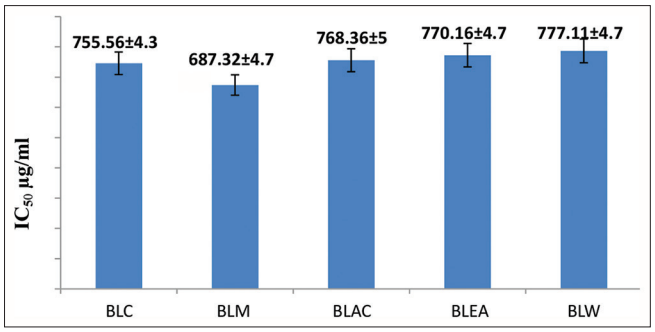

Fig. 7: Antifungal assay of *Berberis lycium* extracts against *Bipolaris specifera*

(IC_{50} 586.62 µg/mL), chloroform extract (IC_{50} 745.11 µg/mL), ethyl acetate extract (IC_{50} 833.64 µg/mL), and least by the acetone extracts (IC_{50} 836.44 µg/mL) (Fig. 5). There is an evidence that genus *Berberis* have an effective H_2O_2 scavenging activity [22].

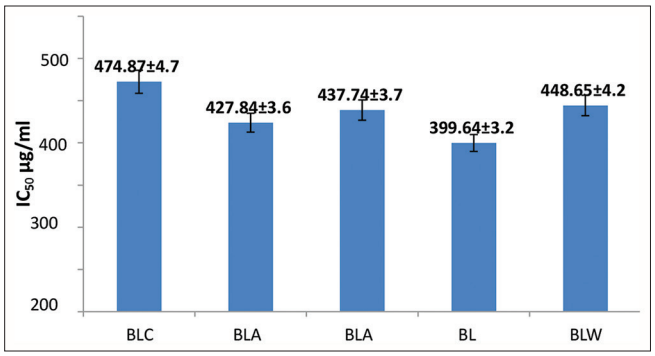

Fig. 8: Antifungal assay of *Berberis lycium* extracts against *Aspergillus niger*

Antibacterial assay of *B. lycium* extracts

The results were analyzed by measuring the diameter of the zone of inhibition. The value of this zone ranges from 7.0 mm to 20.0 mm. The antibacterial assay showed that nearly all the extracts possess antibacterial activity against all the strains tested. The methanolic extract showed the highest activity against *B. subtilis* (20±0.86mm) and *B. cereus* (19±0.37 mm) (Table 3). Further, it was concluded that all the extracts of *B. lycium* showed good activity against *K. pneumonia, Alcaligenes denitrificans* and *B. subtilis.* The results are in accordance with [25] who reported that both alcoholic and aqueous extract showed antimicrobial activity against the tested bacteria

Antifungal assay of *B. lycium* extracts against *F. oxysporum*, *B. specifera* and *A. niger*

The methanolic extract of *B. lycium* showed the best antifungal activity against *F. oxysporum* with IC_{50} value of 673.43 µg/mL (Fig. 6). The methanolic extract showed the best antifungal activity against *B. specifera* with IC_{50} value of 687.32 µg/mL) (Fig. 7). Further against *A. niger*, the highest antifungal activity was demonstrated by methanolic extract followed ethyl acetate, acetone, water extract, and least by the chloroform extract (Fig. 8). Thus, methanolic root extracts of *B. lycium* was having potent antifungal activity. Since alkaloids are the secondary metabolites, whose function is known to be defensive to the plants. The antifungal activity of *Berberis* species may be due to presence of the major alkaloid berberine.

CONCLUSION

In this study, root extracts of *Berberis lycium*, a plant grown in Rajouri area of Jammu was analysed for various phytochemical studies. The biological potentials such as free radical scavenging, antioxidant activity and antimicrobial activities were evaluated. Different assays were employed for assessing the antioxidant. On the basis of results obtained it was concluded that *Berberis lycium* methanolic root extracts showed the best activity. Further these findings could be attributed to the presence of berberine, a potential phytochemicals in the methanolic extract.

ACKNOWLEDGMENT

The authors wish to acknowledge the DBT grant of Monica Sangral, JK Science Technology and Innovation Council (JKST&IC/J/14/2022/174-78), Seed Grant (University of Jammu), Advanced instrumentation facility of School of Biotechnology, University of Jammu.

AUTHORS CONTRIBUTIONS

All authors contributed for the study. Monica sangral designed the experiment, analyzed and compiled the results, and prepared the manuscript. Divya Gupta and Param Vir Singh contributed to the experiments. Madhulika Bhagat supervised the research.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

AUTHORS FUNDING

DBT, GOI.

REFERENCES

1. Mokhber-Dezfuli N, Saeidnia S, Gohari AR, Kurepaz-Mahmoodabadi M. Phytochemistry and pharmacology of *Berberis* species. Pharmacogn Rev. 2014;8(15):8-15. doi: 10.4103/0973-7847.125517, PMID 24600191
2. Ahmed MM, Alamgeer A, Sharif T, Muhammad Zabta CH, Akbar A. Effect of *Berberis lycium* royle on lipid profile in alloxan induced diabetic rabbits. Ethnobotanical Leafl. 2009;13:702-708.
3. Sun Y, Xun K, Wang Y, Chen X. A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. Anticancer Drugs. 2009;20(9):757-69. doi: 10.1097/CAD.0b013e328330d95b, PMID 19704371
4. Singh J, Kakkar P. Antihyperglycemic and antioxidant effect of *Berberis aristata* root extract and its role in regulating carbohydrate metabolism in diabetic rats. J Ethnopharmacol. 2009;123(1):22-6. doi: 10.1016/j.jep.2009.02.038, PMID 19429334
5. Huang WY, Su CH, Sheu SJ. Separation and identification of the constituents in fangchi radix of different origins. J Food Drug Anal. 2006;14(4):357-67.
6. Khare CP, editor. Rational Western Therapy. Ayurvedic and other Traditional Usage, Botany. Indian Herbal Medicine. Berlin: Springer; 2004. p. 98.
7. Greathouse GA, Rigler NE. Isolation of the alkaloids berberine, and berbamine from *Mahonia swaseyi*. Plant Physiol. 1940;15(3):563-4. doi: 10.1104/pp.15.3.563, PMID 16653655
8. Chatterjee R. Plant alkaloids. J Indian Chem Soc. 1952;28:225-8.
9. Chatterjee R. Studies on Indian *Berberidaceae* from botanical, chemical and pharmacological aspects. Recent Bot Survey India. 1953;16:1-86.
10. Sharma SK, Singh L, Singh S. A review on medicinal plants having antioxidant potential. Indian J Res Pharm Biotechnol. 2013;3:404-9.
11. Dev S. A Selection of Prime Ayurvedic Plant Drugs Ancient and Modern Concordance. New Delhi: Anamaya Publishers; 2006. p. 103-5.
12. Bhagat M, Gupta S, Jamwal VS, Sharma S, Kattal M, Dawa S, *et al*. Comparative study on chemical profiling and antimicrobial properties of essential oils from different parts of *Eucalyptus lanceolatus*. Indian J Tradit Knowl. 2016;15(3):425-32.
13. Wani IA, Sogi DS, Gill BS. Physicochemical properties of acetylated starches from some Indian kidney bean (*Phaseolus vulgaris* L.) cultivators. Int J Food Sci Technol. 2012;47(9):1993-9. doi: 10.1111/j.1365-2621.2012.03062.x
14. Chang ST, Wu JH, Wang SY, Kang PL, Yang NS, Shyur LF. Antioxidant activity of extracts from *Acacia confusa* bark and heartwood. J Agric Food Chem. 2001;49(7):3420-4. doi: 10.1021/jf0100907
15. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. J Food Chem. 1999;64(4):555-9.
16. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958;181(4617):1199-200. doi: 10.1038/1811199a0
17. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay. Anal Biochem. 1996;239(1):70-6. doi: 10.1006/abio.1996.0292, PMID 8660627
18. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Test. Approved Standard M2-A6. Wayne, PA: National Committee for Clinical Laboratory Standards; 1997.
19. Bhagat M, Anand R, Datt R, Gupta V, Arya S. Green synthesis of silver nanoparticles using aqueous extract of *Rosa brunonii* Lindl and their morphological, biological and photocatalytic characterizations. J Inorg Organomet Polym Mater. 2019;29(3):1039-47. doi: 10.1007/s10904-018-0994-5
20. George M, Joseph L, James S. Phytochemical and pharmacological screening of anti-inflammatory activity of *Berberis lycium* root extract. Pharmacophore. 2016;7(1):1-5.
21. Sabir S, Tahir K, Rashid N, Naz S, Masood B, Shah MA. Phytochemical and antioxidant studies of *Berberis lyceum*. Pak J Pharm Sci. 2013;26(6):1165-72.
22. Lamichhane B, Adhikari S, Shreshtha P, Govinda Shreshtha BG. Study of phytochemical, antioxidant, antimicrobial and anticancer activity of *Berberis asiatica*. J Trop Life Sci. 2014;4(1):1-7. doi: 10.11594/jtls.04.01.01