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 shows 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), arsagnha (cure for piles and hemorrhoids), and kandughna (curative of pruritus) in Charka Samhita 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, sapopins, terpenoids, coumarins, quinines, anthraquiones, and cardiac glycosides. For qualitative analysis, 50 mg of extract was dissolved in...
The highest phenolic content was exhibited by the methanol extract (409 mg GAE/g) and the lowest by the ethyl acetate extract (0.04%). Our analysis was in agreement with George et al. [20] who reported the results of the chemical tests for the screening which revealed the presence 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 (39.572 g GAE/g) followed by water extract (39.532 g GAE/g), ethyl acetate (28.517 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 (311 mg 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
assay was carried out in duplicates. The antioxidant potential of samples was expressed in terms of IC\textsubscript{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 (IC\textsubscript{50} 10.96 µg/mL) followed by water (IC\textsubscript{50} 56.44 µg/mL), ethyl acetate (IC\textsubscript{50} 103.94 µg/mL), chloroform extract (IC\textsubscript{50} 120.97 µg/mL) and least by the acetone extract (IC\textsubscript{50} 149.27 µg/mL) (Fig. 3). Pet ether extract of \textit{B. lycium} showed maximum antioxidant activity (82% inhibition) followed by methanol (49.7% inhibition) [21].

FRAP assay of \textit{B. lycium} extracts

In the present study, the standard curve of ferrous (Fe\textsuperscript{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 \textit{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 \textit{B. lycium} extracts

The hydrogen peroxide scavenging activity of extracts of \textit{B. lycium} was evaluated and compared with ascorbic acid. The minimum IC\textsubscript{50} were calculated of extracts of \textit{B. lycium} after the evaluation of percentage inhibition. Our analysis is in accordance with Lamichhane et al., 2014 who reported that \textit{B. aristata} have an effective H\textsubscript{2}O\textsubscript{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\textsubscript{50} 433.98 µg/mL), followed by water extract.
Table 3: Antibacterial assay of Berberis lycium extracts

<table>
<thead>
<tr>
<th>Extracts (100 µg/mL)</th>
<th>Zones of Inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td>Bacillus subtilis</td>
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<tr>
<td></td>
<td>Achromobacter denitrificans</td>
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<td></td>
<td>Klebsiella pneumoniae</td>
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<td></td>
<td>Micrococcus luteus</td>
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<td></td>
<td>Bacillus cereus</td>
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<tr>
<td>BLC (mm)</td>
<td>15±1.34</td>
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<tr>
<td>BLEA (mm)</td>
<td>10±0.43</td>
</tr>
<tr>
<td>BLAC (mm)</td>
<td>12±3.64</td>
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<tr>
<td>BLM (mm)</td>
<td>20±0.86</td>
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<tr>
<td>BLW (mm)</td>
<td>18±0.66</td>
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<tr>
<td>Ampicillin (30 µg/mL) (positive control) (mm)</td>
<td>30±0.05</td>
</tr>
</tbody>
</table>

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

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±0.86 mm) 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 IC₅₀ value of 673.43 µg/mL (Fig. 6). The methanolic extract showed the best antifungal activity against B. specifera with IC₅₀ value of 687.32 µg/mL (Fig. 7). Further against A. niger, the highest antifungal activity was demonstrated by methanolic extract followed by 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.
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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.

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REFERENCES