

## Antimicrobial Activity of the Crude Root Extract of *Berberis Lycium* Royle

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### ABSTRACT

The present work was carried out to study the antimicrobial activity of the aqueous, petroleum ether and ethanolic extracts of the root of *Berberis lycium* Royle (Family Berberidaceae), against Gram(+) bacteria viz. *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*, Gram (–) viz *Salmonella typhi*, *E. coli* and a fungal strain *Candida albicans*. The antimicrobial activities were determined by using Paper-Disc method, described by Casal [7] and Chung *et al.*, [8]. The ethanolic and aqueous crude root extract were found to be most effective antifungal and antibacterial agents while no significant activity was shown by the petroleum ether extract against test organisms. The results were compared with the inhibition caused by commercially available standard reference antibiotic disc (Tetracycline). The inhibitory effects of all the crude extracts on the growth of both Gram (+) and Gram (–) organisms are very close and identical in magnitude and are comparable with standard antibiotic disc used.

### Key words:

### Introduction

The importance, necessity and potentiality of medicinal plants in the practice of medicine today is well established and can not be overlooked. The Indo-Pak subcontinent is very rich in having resources of medicinal plants. A large number of these plants are used in the form of powder, decoction and infusion for the treatment of various diseases including the infection caused by microbes with fair amount of success by hakims and vaidas. Several workers throughout the world have carried out anti-microbial studies on some medicinal plants including *Datura metel*, *Ageratum houstonianum* [5]. A number of other studies on anti-microbial activity of plants have been carried out in different parts of the world [4,17,10]. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the

plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils [13] as well as in tannin [23]. The antimicrobial properties of plants have been investigated by a number of researchers world wide. In Argentina, researcher tested 122 known plant species used for therapeutic treatments [1]. It was documented that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococcus aureus*, ten inhibited *Escherichia coli*, and four inhibited *Aspergillus niger* and also reported that the most potent compound was one extracted from *Tabebuia impetiginosa*. Many studies have been conducted in Brazil. The inhibitory activity of *Vatairea macrocarpa* on *Klebsiella spp.* and *S. aureus* was observed [20] and the inhibitory activity of extracts from *Eucalyptus spp.* against soil fungi [6]. A more detailed study on antimicrobial compounds was done evaluating

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extracts from 120 plant species from 28 different families [22]. It was documented that 81 extracts obtained from 58 plants were active against *S. aureus*, and five extracts from four other plants inhibited the growth of *P. aeruginosa*. Another study [18] detected the antibacterial and antifungal (*C. albicans*) activity of essential oils obtained from *Croton triangularis* leaves. Extracts from *Lippia gracilis* and *Xylopia sericea* showed antifungal activity. The antimicrobial activity from *Mikania triangularis*, known as “thin leaf guaco”, was tested against five genera of bacteria and three genera of yeast, and showed it had activity against *Bacillus cereus*, *E. coli*, *P. aeruginosa*, *S. aureus* and *S. epidermidis* [9]. Effects of phytochemical were conducted [12,13] and it was observed the antimicrobial activity of anacardic acid on *S. aureus*, *Brevibacterium ammoniagenes*, *Streptococcus mutans* and *Propionibacterium acnes*. Later, it was tested the bactericidal activity of anacardic acid and totarol on methicillin resistant strains of *S. aureus* and the synergistic effect of these compounds associated with methicillin [21].

*Berberis lyceum* Royle, (Berberidaceae) commonly known as Barberry is an evergreen shrub usually 1.2 – 1.8 m. high, but attaining 3.6m. height and 10cm. diam. Twigs pale yellowish, glabrous or minutely pubescent. Bark rough and rather deeply furrowed. Blaze 5mm, bright yellow with coarse reticulate fiber. Leaves 2.5-7.5 by 0.7-1.8 cm., lanceolate or narrowly obovate-oblong, coriaceous, entire or with a few large spinous teeth, dull green above, pale and glaucous beneath, secondary nerves not prominent on the upper surface. Petiol are distinct up to 2.5mm. Inflorescence a simple raceme 13 – 38mm. Long, often with a few long - stalked flowers at the base.

Root bitter with an unpleasant taste; used in splenic trouble; tonic, a good febrifuge; intestinal astringent; good for cough, chest and throat troubles, eye-sores and itching of the eye, piles, and manorrhagia; useful in chronic diarrhea; always thirst; as a gargle strengthens the gums; a good application to boils.

An extract prepared by digesting in water sliced pieces of the root, stem and branches is called rusot, and is used in cases of ophthalmia.

The leaves are administered in Baluchistan as a cure for Jaundice. Due to above mentioned fact that this plant is very useful and the fact that little information is available on its biological activity, there is a need to find out more about the potential of this plant as antimicrobial agent.

The present study is therefore, designed to assess the potency of the plant extract on selected microorganisms.

## Material and methods

The plant was collected from Rawalakot Azad Kashmir. This was authenticated by Department of Botany University of Azad Jammu & Kashmir Muzaffarabad. Root were separated and dried under shade at room temperature. The dried root were ground into powder and stored in glass bottle.

The dried powdered 50gms of *Berberis lycium* were soaked in to 250ml in each solvent for 7-10 days. Each mixture was shaken after 24 hours. After ten days each extract was filtered with Whatman filter paper in separate bottles and were preserved until required.

Pure cultures of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Salmonella typhi* and a fungus *Candida albicans* were obtained from Microbiology Section, Drug control and Traditional Medicine Division, NIH Islamabad.

The culture media for bacteria was prepared by dissolving 20mgs of dehydrated nutrient agar in 1000ml of distilled water, boiled and stirred. The prepared media was autoclaved for 15 minutes at 121°C and 15psi. The PH of media was maintained at  $7.0 \pm 0.2$  at 25°C.

The culture media for fungus were prepared for dissolving 65gms of dehydrated sabourand's dextrose agar medium in 1000ml of distilled water by with constant stirring autoclaved for 15 minutes at 121°C and 15psi. The PH of medium was adjusted  $5.6 \pm 0.2$  at 25°C.

### Antimicrobial Activity:

The antimicrobial activities of the three extracts were determined by using the paper-disc method, describing by Casal [7] and Chung *et al.* [8]. Zones of inhibition for each extract on each organism were measured and recorded in millimeter.

### Preparation of Inoculum:

A 24 hours old culture of each bacterium and 72 hours old culture of fungus was used as inoculum for the test.

The culture was prepared on slants of their respective media. Each test tube was labeled with the name of bacterium and fungus.

These test tubes were incubated for 24 hours at 37°C and 72 hours at 25°C respectively.

### Preparation of Petri Dishes:

The sterilized Petri dishes were also labeled

with the bacterial and fungal names. 1ml of dilution of each bacterium and fungus was poured into previously labeled Petri dishes. The sterile nutrient agar at a temperature not more than 45°C was poured in the Petri dishes containing bacterial suspension and sabourand's dextrose agar medium in molten state was poured in Petri dishes containing fungal suspension. All the Petri dishes were rotated gently to mix the inoculum and the media were allowed to become solidified at room temperature.

Uniform filter paper discs were made. These discs were soaked in the solution of aqueous, petroleum ether and ethanolic crude extracts of the root of *Berberis lyceum* and were placed in the Petri dishes at their labeled position. Another set of Petri dishes were prepared in the same way in which commercially available antibiotic disc i.e. Tetracycline 50ug were placed on the top of the medium.

#### Incubation of Petri Dishes:

The Petri dishes containing the bacterial culture were incubated at 37°C for 24 hours while plates with fungal suspension were incubated at 25°C for 72 hours. After the incubation time, all the Petri dishes were examined for the presence of zones of inhibition as an indication of antimicrobial activity. The zones were measure in millimeter and were tabulated in table.

#### Statistical Analysis:

At least three replicates were measured for all assays, and all assays were performed twice. The mean values, standard deviations and statistical differences were evaluated through analysis of variance [24].

**Table 1:** Antimicrobial activity of *Berberis lycium* root extracts. (Zones of inhibition in mm)

S. No.	Extracts	<i>S. aureus</i>	<i>S. typhi</i>	<i>E.coli</i>	<i>S. epidermidis</i>	<i>B. subtilis</i>	<i>C. albicans</i>
1	Aqueous	20.00±0.33	22.00±0.38	23.00±0.30	24.00±0.25	20.00±0.28	27.00±0.31
2	Ethanolic	25.00±0.74	16.00±0.12	23.00±0.50	15.00±0.12	21.00±0.23	27.00±0.65
3	Petroleum Ether	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
4	Tetracycline 50ug	26.00±0.61	28.00±0.47	27.00±0.31	26.00±0.50	26.00±0.45	25.00±0.32

#### Ethanolic Extract:

The crude ethanolic extract of *Berberis lycium* root show high activity against *Candida albicans*, *S. aureus* and *E. coli* (27.00±0.65mm, 25.00±0.74mm and 23.00±0.50mm) and low activity against *S. epidermidis*, *Salmonella typhi* and *B. subtilis* (15.00±0.12mm, 16.00±0.12mm and 21.00±0.23mm). Kokoska *et al.* [16] studied antimicrobial activity of crude ethanolic extracts of 16 Siberian medicinal plants that tested against 5 species of microorganisms. Out of 16 plants

## Results and discussion

In the present study the antimicrobial activity of *Berberis lycium* roots extracts were observed against Gram +, Gram- bacteria and a fungal strain. The medicinal plant (*Berberis lycium*) was selected on the basis of their local medicinal uses and collected from Dreak Rawalakot distt. Poonch Azad Kashmir.

In this investigation the zones of inhibition produced by crude extracts were recorded against the microorganisms used. For the extraction of plant material a broad solvent extraction was carried out. *Berberis* spp. contain Berberine and other chemical compounds [14]. The compounds may active against test organisms.

The antimicrobial activities of ethanolic, aqueous and petroleum ether crude extracts of root of *Berberis lycium* were summarized in table.

#### Aqueous Extract:

The maximum zone of inhibition were observed against *Candida albicans*, *Styphlococcus epidermidis* and *E. coli* (27.00±0.31mm, 24.00±0.25mm, and 23.00±0.30mm) while minimum zone of inhibition against *Salmonella typhi*, *B. subtilis* and *Staphylococcus aureus* (22.00±0.38mm, 20.00±0.28mm and 20.00±0.33mm). Anjum and Khan [2] study the antimicrobial activity of crude extracts of *Cuscuta reflexa* against Gram +, Gram-and fungal strains. On the whole all the crude extracts were active against tested microorganisms. Chloroform and petroleum ether extracts were found to be more effective against the microorganisms used.

Comparison of the present research work with the work of Anjum and Kahn showed that the polar extracts were more active than the non-polar.

tested, 12 showed antimicrobial activity against one or more species of microorganisms. Present work was also be compared with the work of Kokoska under same laboratory condition which were very closed and identical in magnitude with the results discuss above.

#### Petroleum Ether Extract:

There was no significant activity shown by the petroleum ether extract of root of *Berberis lycium*.

### Conclusion:

*Berberis lycium* root possess antimicrobial activity. This can explain the rationale for the use of the plant in treating infections in traditional medicine. The plant could be a veritable and cheaper for conventional drugs since the plant is easily obtainable and the extract can easily be made via a simple process of maceration or infusion. On considering the present work, it is necessary to isolate the active principle from the plant extracts and to carryout pharmaceutical studies.

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