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In Vitro Evaluation of Antibacterial Activity of *Berberis lycium* against Clinical Isolates

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ABSTRACT

***Berberis Lycium* is medicinally important plant. Most species of genus *Berberis* are used in traditional Chinese and Ayurvedic medicine. The objective of the present study is to assess the antibacterial activity of methanolic and aqueous root extracts of *Berberis lycium*. In vitro antimicrobial activities were investigated by using the agar well diffusion method and disc diffusion method. The zones of inhibition produced by extracts were recorded against selected test isolates (*Bacillus, Micrococcus, S.aureus, Salmonella, Proteus, Klebsiella, E.coli, Shigella, Acinitobacter* and *Pseudomonas*). Methanolic extract was active against all bacteria except Shigeella, whereas aqueous root extract was active against *Bacillus, Micrococcus, S.aureus, Salmonella, Proteus, Klebsiella* and *E.coli*. Results showed that the methanolic extract has significantly inhibited the growth of selected microorganisms as compared to aqueous extract. These plant can be further subjected for the isolation of active compounds and the development of new drugs that are effective for the treatment of different diseases.**

Keywords: *Antimicrobial activity, agar well diffusion, Berberis lycium, medicinal plants*

INTRODUCTION

Humanity has always been dependent upon the plants for their essential needs of life. Medicinal plants have perceived restorative medical uses. At present, as per world wellbeing association, around 80% of human populace depends on folk medicines to cure infections (Mansoor *et al.*, 2013). Restorative plants have been a crucial part for the survival of life on the planet. These plants have been utilized for the remedy of various diseases and disorders. Once a tribe got this learning, it was exchanged to next eras inside or outside the tribes as people cure. In this manner the medicinal plants delivered the folk medicine for hundreds of years and now this people data is being approved by the scientific research (Gurib-Fakim, 2006).

The American Society for Microbiology has proposed the advancement of novel antibiotics in view of the rise of multi drug resistant pathogens. In spite of the fact that the antibiotics assume a crucial part in the treatment of various sicknesses, they likewise have symptoms. There is an open swing towards natural cure in various countries (Bukhari *et al.*, 2011; Kakar *et al.*, 2012; Sarwat *et al.*, 2012). Today, a great diversity of plants, generally utilized as medications, are being screened for their antimicrobial activity and chemical compounds (Kakar *et al.*, 2012). Wild plants have dependably been the matter of center and have dependably been utilized for their vast potential. In developing countries medicinal plants give a proper alternative option to essential health care system, high safety margins and lesser expenses (Haq *et al.*, 2011).

Pakistan has a rich resources of medically essential plants. Local communities are using medical plant solutions for hundreds of years (Gilani *et al.*, 2010). These plants are utilized to treat any sort of sickness from cerebral pain to cut and wounds (Bhardwaj and Gakhar 2005). Local individuals utilized these plants as a part of the type of powder, chemicals and compounds (Hussain *et al.*, 2011; Saeed *et al.*, 2012)]. many plants are being examined

by various scientits and investigated for their antimicrobial properties (Hussain *et al.*, 2011).

Berberis lycium is indigenous to Nepal yet it is dispersed in the distinctive parts of the world. The plant is rich in the Himalayan locale of India and Pakistan. The entire plant particularly root is generally utilized for the treatment of various human infections in Pakistan (Khan, 2001). The different compound constituent of the plant are *berberine, berbamine*, *chinabine*, *karakoramine*, *palmatine balauchistanamine*, *gilgitine*, *helumine*, *punjabine*, *sindamine*, *chinabine* acidic corrosive, maleic corrosive, ascorbic corrosive. The plant contains real alkaloid *berberine* which is an isoquinoline alkaloid and umbellitine. Normally found in root or root bark of the *Berberis lycium*, and different *Berberis* species (Sharma, 2003). It has antimicrobial property, anti-diabetic properties, antioxidant properties, anti-hyper lipidemic property, hepatoprotective property, antimutagenic property, pesticidal property and wound mending property.

Berberic lycium is extremely successful against numerous microorganisms' particularly microbial growths. Its different parts and concentrates are utilized against various microscopic organisms, for example, *Micrococcus luteum*, *Bacillus subtilis*, *Bacillus cereus*, *Enterobactor aerogenus*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Streptococcus pneumonia*. The alcoholic and fluid concentrate of *B. lycium* has been accounted for to have more diversed and wide range movement against various bacterial strains when contrasted with parasitic strains (Jabeen, Saleem *et al.*, 2015). The antimicrobial movement is fundamentally because of the alkaloid *berberine* which is dynamic constituent of the plant and compelling against an extensive variety of creature including Bacteria, Fungi, protozoa *Trypanomonas* and *Plasmodia*. The antimicrobial action relies on pH and the innoculum size of selected test microorganisms. The component of activity of profoundly sweet-smelling about planar quaternary structure of Berberine is credited to its capacity to intercalate with DNA. In blend with the intercalation, protein synthesis(which is the real method of activity of *berberine*) could be responsible for observed cytotoxic effects because both targets are vital to a living cell (Čerňáková and Košťálová, 2002).

MATERIALS AND METHODS

Collection of Sample: The root samples of *Berberislycium* were collected from hilly area of Kotli paien, dist. Hazara Division. Fresh root materials were washed by sterile distilled water, air dried and then ground into fine powder with an electric blender. The powder was stored in airtight bottles until required for further analysis.

Extract Preparation: The dried powdered 50gms of *Berberis lycium* were soaked into 250 ml aqueous and methanolic medium for 7-10 days. Every mixture was shaked daily, after ten days each extract was filtered with Whatman filter paper in separate bottles and were refrigerated until required. Commercially available antibiotic Tetracycline powder was use as positive control. Antibiotic solution were prepared by dissolving in sterile distilled water (Hussain, Khan *et al.* 2011).

In Vitro Antibacterial Activity: The antimicrobial activities of the aqueous and methanolic root concentrates of *Berberis* Lycium were examined by utilizing the agar well diffusion technique. Muller Hinton agar medium was set up by utilizing 36g/L agar saturate in distilled water. Muller Hinton agar medium was filled each petri plate and permitted to cool to 45°C to solidify. 24 hours old bacterial cultures were utilized as inoculum for the test. Suspension of each bacterial species was set up in clean ordinary saline by altering turbidity to

a likeness of 0.5 McFarland standard, at which the quantity of cells was thought to be 1.5 x 108 cfu/ml. Lawn of each bacterial suspension were made on Muller Hinton agar plates. Wells of 6 mm distance apart were made in the agar with a sterile borer. Roughly 50µl of every root concentrate and antibiotics arrangement (Tetracycline) were stacked into the wells and left for couple of minutes for the dissemination of concentrates. The plates were incubated at 37°C for 24 hours. The zones of inhibition were measured for every concentrate utilizing a millimeter ruler and the outcomes were recorded (Sarwat *et al.*, 2012).

RESULTS

In the study, methanolic extract gave zone of inhibitions against *Pseudomonas* (20mm), *Acinetobacter* (19mm), S. aureus (22mm), *Salmonella* (26mm), *Klebsiella* (13mm), *Bacillus*(20mm), *E. coli*(18mm), *Proteus* (17) and *Micrococcus* (19mm) while *Shigella* remain resistant. Ethanolic extract gave zone of inhibitions against, S. aureus (12mm), *Salmonella* (10mm), *Klebsiella* (9mm), *Bacillus*(18mm), *E. coli* (8mm), *Proteus* (10)

Table I. Antibacterial activity of Methanolic and Aqueous root extract of *B. lycium*

	Zone Of Inhibition (mm)		
Microorganism	**Methanolic Extract**	**Aqueous Extract**	**Tetracycline**
Pseudomonas	20mm	0 mm	0 mm
Acinetobacter	19mm	0 mm	22mm
S.aureus	22mm	12mm	29mm
Salmonella	26mm	10mm	26mm
Klebsiella	13mm	9mm	-
Bacillus	20mm	18mm	26mm
E.coli	18mm	8mm	0 mm
Shigella	0 mm	0 mm	0 mm
Proteus	17mm	10mm	0 mm
Micrococcus	19mm	15mm	0 mm

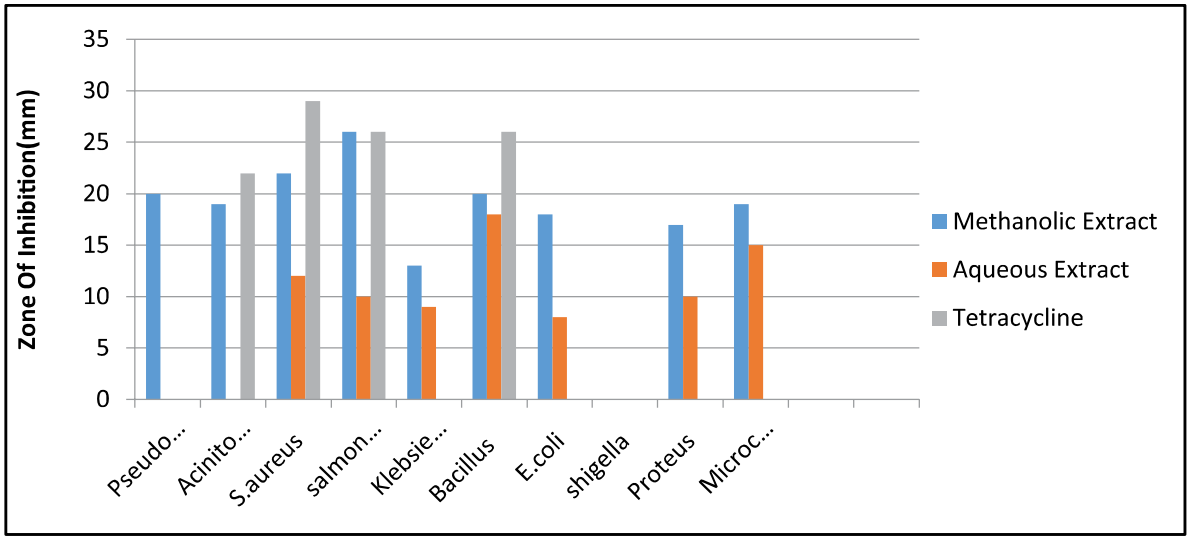
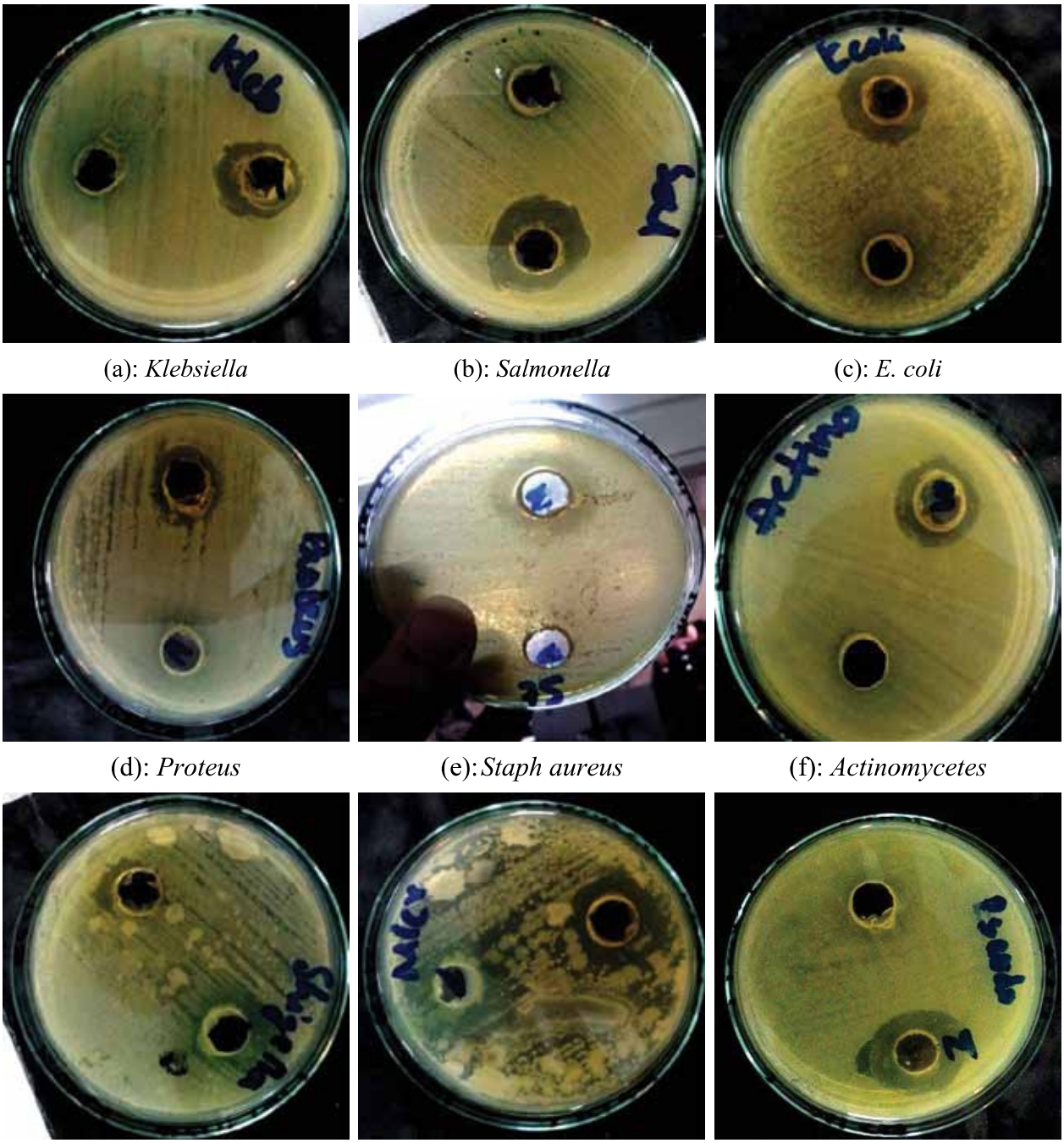


Figure 1. Graphical study of zone of inhibitions against different microorganisms



(a): *Klebsiella* (b): *Salmonella* (c): *E. coli*

(d): *Proteus* (e): *Staph aureus* (f): *Actinomycetes*

(g): *Shigella* (h): *Micrococcus* (i): *Pseudomonas*

Figure 2 (a-i). Antibacterial pattern of berberis lyciun root extracts against different microorganisms

and *Micrococcus* (15mm) while *Shigella*, *Pseudomonas* and *Acinetobacter* remain resistant. Tetracycline gave zones against *Acinetobacter* (22mm) while *S.aureus* (29mm), *Salmonella*(26mm) and *Bacillus*(26) other seems to be resistant.

DISCUSSION

The objective of the present study is to check the activity of root extracts of *Berberislycium*. The medicinal plant was utilized on the basis of their therapeutic properties. Extraction was carried out in aqueous and methanolic

medium. *Berberis* contain Berberine and other chemical compounds that are responsible for affectivity (Hussain*et al.*,2011) So, the plant is screened for their antimicrobial activity against selected test organisms.

Each extract showed a different degree of inhibition against different microorganisms. In this analysis, the zones of inhibition produced by extracts were recorded against the test microorganisms. It was found that methanol extracts has provided better results as compared to aqueous extracts. Therefore methanol is considered as useful solvents for assessment of antimicrobial activities (Ahmed*et al.*, 1998). It was also reported by (Karaman *et al.*, 2003)that methanol extract has inhibited the growth of different microorganism organism significantly as compared to aqueous. Because of the fact that alkaloids are highly soluble in polar solvents (Irshad *et al.*, 2013).

The methanolic extract of *Berberis lycium* root showed activity against all test isolates except *Shigella*. Sensitivity of plant extracts found in this study was maximum against *Salmonella* (26mm), followed by *S.aureus*(22mm), *Pseudomonas*and*Bacillus*(20mm),*Acinitobacter* and *Micrococcus* (19mm), *E.coli* (18mm), *Proteus* (17mm) and *Klebsiella* (13mm). There is no effect of methanolic extract observed on *S.aureus Klebsiella* and *E.coli* reported by (Sarwat *et al.*,2012). Inhibitory effect of extract against *E.coli* (12mm), *Pseudomonas* (11 mm), and *S.aureus* (10 mm) was also reported by (Irshad *et al.*,2013; Stermitz *et al.*,2000).

The aqueous root extract showed higher activity against *Bacillus* (18mm) followed by *Micrococcus* (15mm), *S.aureus* (12mm), *Salmonella* and *Proteus* (10mm), *Klebsiella* (9mm) and *E.coli* (8mm) while there is no activity shown by the extract against *Shigella*, *Acinitobacter* and *Pseudomonas*. Against *S.aureus* 10mm zone of inhibition is reported by (Jabeen 2015). Aqueous root extract showed inhibitory zones against S. aureus (20mm), *Salmonella* (22mm), *E.coli* (23mm), *Bacillus* (20mm) reported by (Hussain *et al.*, 2011).

The study showed that plants are proof to be more therapeutic then chemical drugs and the use of herbal drugs may be more successful without any side effects. This could provide a way to overcome many diseases successfully.

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