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ANTIMICROBIAL ACTIVITY OF *BERBERIS LYCEUM* ROYLE AGAINST DIFFERENT MICROOGANISMS

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ABSTRACT

Plants are biosynthetic laboratories for many useful compounds those are playing important role in human health. Ingredients of some medicinal plants are very much effective against bacterial growth and can be used to minimize their activities. A study was conducted to assess the possible affects of *berberis lyceum Royle* against the 18 bacteria, 4 fungi and yeast strains. The root extracts were prepared in methanol and aqueous media. Where as antimicrobial activities were assessed by using Disc diffusion method and Micro dilution assays. It was observed that methanol and aqueous root extracts of *berberis lyceum* were highly effective against different bacteria and fungi. The methanol extracts (135-260 µg/l) have inhibited growth of microorganisms more effectively as compared to aqueous extract (120-230 µg/l). The results obtained in present study indicates that root of *berberis lyceum* contained some phyto chemicals with antimicrobial activity and could be useful for pharmaceutical industries for development of new drugs for human and animal health.

Keywords: Antimicrobial activity, medicinal plants, drugs, health.

INTRODUCTION

Herbal medicines are used for their therapeutic or medicinal value. About 30% of the pharmaceutical products available in the market are manufactured from plants (Barbour *et al*., 2004). Medicinal plants are distributed worldwide, but they are most abundant in tropical countries. Pakistan is one of the few countries which have unique biodiversity, comprising of different climatic zones with a wide range of plant species (Shinwari and Malik, 2000). Approximately 6000 plant species with medicinal properties are found in Pakistan. In China traditional medicines are largely based on around 5000 plants those are used for treating 40% of urban patients and 90% of rural patients. In 1991 more than 700,000 tones of plant materials were used for preparation of medicines in China out of which 80% were collected from the wild. In India, where traditional health care system are very strong 400,000 registered traditional medical practitioner are in practice, compared to 332,000 registered doctors (Ahmad *et al*., 1998; Karman *et al*., 2003).

In nature there are large number of different types of antimicrobial compounds (Phytoalexins) that play an important role in the natural defence of all living organisms. Furthermore the development of antibiotic resistance is multi factorial, including the specific nature relationship of bacteria to antibiotics, how the antibacterial is used, host characteristics and environmental factors. To overcome the problem of antibiotic resistance, medicinal plants have been extensively studied as alternative treatments for diseases (Mullika *et al*., 2005).

Berberis lyceum Royle belonging to the family berberidacae is an important medicinal shrub. The ethnobotanical studies conducted in different areas of Pakistan indicates that roots, leaves and fruits of *berberis lyceum* has been used for treatment of wound healing, cough, affections of eyes, skin disease, jaundice diabetes, rheumatism and against mouth micro flora in the form of tooth paste (Shinwari and Malik, 2000).

In order to maintain health status of human and animals, the control of microbial growth is necessary. An antimicrobial agent is a chemical kills or inhibits the growth of microorganisms. Such a substance may be either a synthetic chemical or a natural product (Vorauthikumchai *et al*., 2004; Kumar *et al*., 2005). In recent years it was observed that multiple drugs resistance in human pathogenic microorganism has developed due to indiscriminate use of commercial antimicrobial drugs for treatment of various infections (Abdeolu and Oladimeji, 2005; Magiatis *et al*., 2001). Therefore scientist throughout the world are now trying to search out the new antimicrobial substance from various sources including medicinal plants (Barbour *et al*., 2004; Brantner *et al*., 1994).

Berberis lyceum Royle was not only fully analyzed for its phytochemicals but its antibacterial, antifungal and antiviral activities were also not investigated in the past.
The aim of present study was to evaluate the

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Table 1. Antimicrobial activity of Methanol extracts of *Berberis lyceum* (100 µg/disk tested against bacterial strains by using disk diffusion method. Zone of inhibition in diameter (mm) around test disk.

Microorganisms (Bacteria)	Number of strains[a]	*Berberis lyceum* extracts	Negative control (NC)	Standard antibiotic disk[b]
Bacillus amyloliquefaciens	1	14 mm/1	-	28 mm (SCF)
Bacillus cereus	2	8-22 mm/2	-	32 mm (OFX)
Bacillus licheniformis	1	30 mm/1	-	(SCF)
Bacillus magaterium	3	20-28 mm/3	-	10 mm (SCF)
Bacillus pumilus	2	22 -24 mm/ 2	-	25 mm (OFX)
Bacillus substilis	3	20-26 mm/ 3	-	30 mm (OFX)
Burkholdoria gladioli	3	12-18 mm/3	-	22 mm (NET)
Eschericha coli	1	16 mm/1	-	12 mm (OFX)
Klebsiella pneumonia	1	-		13 mm (OFX)
Micrococcus lutus	1	-	-	12 mm (OFX)
Proteus vulgaris	1	-	-	14 mm (OFX)
Plesiomonas shigelloides	1	-	-	12 mm (SCF)
Pseudomons putida	1	16 mm/l	-	25 mm (SCF)
Pseudomonos syringae	3	22-28 mm/3		25 mm (OFX)
Staphylococcus aureus	4	14-28 mm/4	-	26 mm (OFX)
Staphylococcus epidermis	3	12-22 mm/ 3	-	23 mm (SCF)
Streptococcus pneumonia	2	14 mm/2	-	12 mm (OFX)
Streptococcus pyogenes	2	-	-	14 mm (OFX)
Total bacteria species 18	Strains 35	12-30 mm / 29	-	

SCF; Sulbactum and Cefoperazona; OFX, Ofloxan; NET, Netilmicin (Positive control); NC, Negative Control (Methanol); NT; Not tested.

antimicrobial potential of *berberis lyceum* against various microorganisms

MATERIALS AND METHODS

In the present study root extracts of *berberis lyceum* were tested against bacteria and fungi. The study was conducted in Biochemistry Lab., University of Arid Agriculture, Rawalpindi during February-April, 2006.

Collection of samples

The root samples of *berberis lyceum* were collected from hilly area of Kotli sattian, dist., Rawalpindi about 65 km from Islamabad. The taxonomic study of plant was carried with the help of taxonomist. The plant was registered (voucher number 117) in Herbarium of Pakistan Science Foundation.

Preparation of samples

Root of *Berberis lyceum* were subjected to shadow drying followed by oven drying at 80^{o}C for over night and then converted into powdered form. Total 100 g of samples was added 300 ml of methanol and extracted in soxhlet apparatus for 4 hours at temperature less then the boiling point of solvent. The extract was further concentrated by rotary evaporator and residue was stored for further process. Where as in case of aqueous media same amount of sample was dissolved in water and boiled, filtered and saved for further process.

Microorganisms

A total 18 microbial culture belonging to 35 bacterial species and 4 fungi and yeast were used in this study. The identified microorganisms were obtained from Microbiology lab. Quaid-i-Azam University, Islamabad. National Institute of Health and Biotechnology Centre of National Agriculture Research Council, Islamabad.

Antimicrobial activity

The root extracts of *berberis lyceum* prepared above were again dissolved in similar methanol and water (30mg/ml) and sterilized by filtration though 0.45 um Millipore filters. The antimicrobial activity test was carried out by disk diffusion (Barnabas and Nagarajan, 1988) by using 100 µl of suspension containing 10^4 spore/ml of fungi spread on nutrient agar (NA), Saboured dextrose agar (SDA) and potato dextrose agar (PDA) media respectively. The disks (6 mm) containing 10 µl of extracts (300 µg/disk) with the concentration of 30 mg/ml were impregnated in the inoculated agar. Negative control

Table 2. Antimicrobial activity in aqueous extracts of *berberis lyceum* (100 µg/disk tested against bacterial strains by using disk diffusion method. Zone of inhibition in diameter (mm) around test disk

Microorganisms (Bacteria)	Number of strains[a]	*Berberis lyceum* extracts	Negative control (NC)	Standard antibiotic disk [b]
Bacillus amyloliquefaciens	1	12 mm/1	-	26 mm (SCF)
Bacillus cereus	2	10-20 mm/2	-	30 mm (OFX)
Bacillus licheniformis	1	26 mm/1	-	-(SCF)
Bacillus magaterium	3	18-24 mm/3	-	10 mm (SCF)
Bacillus pumilus	2	15 -20 mm/ 2	-	22 mm (OFX)
Bacillus substilis	3	20-22 mm/ 3	-	28 mm (OFX)
Burkholdoria gladioli	3	10-16 mm/3	-	22 mm (NET)
Eschericha coli	1	14 mm/1	-	12 mm (OFX)
Klebsiella pneumonia	1	-		13 mm (OFX)
Micrococcus lutus	1	-	-	12 mm (OFX)
Proteus vulgaris	1	-	-	14 mm (OFX)
Plesiomonas shigelloides	1	-	-	12 mm (SCF)
Pseudomons putida	1	14 mm/l	-	25 mm (SCF)
Pseudomonos syringae	3	18-24 mm/3		24 mm (OFX)
Staphylococcus aureus	4	10-22 mm/4	-	26 mm (OFX)
Staphylococcus epidermis	3	12-20 mm/ 3	-	23 mm (SCF)
Streptococcus pneumonia	2	10-22 mm/2	-	12 mm (OFX)
Streptococcus pyogenes	2	-	-	14 mm (OFX)
Total bacteria species 18	Strains 35	10-26 mm/29	-	

SCF; Sulbactum and Cefoperazona; OFX, Ofloxan; NET, Netilmicin (Positive control) NC Negative control aqueous); NT; Not tested

Table 3. Antimicrobial activity of Methanol extracts of *berberis lyceum* extract (100 µg/disk tested against yeast and fungi isolate by using disk diffusion method . Zone of inhibition in diameter (mm) around test disk

Microorganisms	Number of strains[a]	*Berberis lyceum*	Negative control (NC)	Standard (Antibiotic disk[b])
Yeast				
Candida albicans	6	12-25mm/6	-	14 mm (SCF)
Fungi				
Alternaria Alternate	2	-	-	NT
Aspergillus flavus	2	-	-	NT
Fasarium Oxysporum	2	-	-	NT
Penicillium spp	2	-	-	NT
Total isolates	14	12-25 mm/ 6isolates	-	

SCF; Sulbactum and Cefoperazona; OFX, Ofloxan; NET, Netilmicin (Positive control) NC, Negative control Methanol); NT; Not tested

was prepared by using similar solvents of plant extracts. Whereas Ofloxacin (10 µl/disk), Sulbactum (30 µg) added Cefoperazone (75 µg), (105 µg/disk) and or netilnicin (30ug/disk) were used as positive control to determine the sensitivity of each strain/isolate for each microbial species tested. The inoculated plates were incubated at 37^{o}C for 24 hours in the case of clinical bacteria strains, 48 hours for yeast and 72 hours for fungi isolate. Where as plant extract associated microorganism were inoculated at 27^{o}C (Mori *et al*., 1997; Fukai *et al*., 2004). Antimicrobial activity was assessed by measuring inhibition zones in reference to test organisms and each process was repeated to get accurate results (Barnabas and Nagarajan, 1988; Davis, 1994).

Micro dilution assays

The minimum inhibitory concentration (MIC) values were determined for microorganism those were sensitivity to *berberis lyceum* extract in disk diffusion assay. The inocula of microorganisms was prepared from 12 hours breath cultures and suspension was adjusted to 0.5

Table 4. Antimicrobial activity of aqueous extracts of *berberis lyceum* extract (100 µg/disk tested against yeast and fungi isolate by using disk diffusion method Zone of inhibition in diameter (mm) around test disk

Microorganisms	Number of strains [a]	*Berberis lyceum*	Negative control (NC)	Standard (Antibiotic disk[b])
Yeast				
Candida albicans	6	10-22mm/6	-	14 mm (SCF)
Fungi				
Alternaria Alternate	2	-	-	NT
Aspergillus flavus	2	-	-	NT
Fasarium Oxysporum	2	-	-	NT
Penicillium spp	2	-	-	NT
Total isolates	14	10-22 mm/6 isolates	-	

SCF; Sulbactum and Cefoperazona; OFX, Ofloxan; NET, Netilmicin (Positive control) NC Negative control (aqueous), NT; Not tested.

Table 5. The MIC (µg/ml) values of *berberis lyceum* extracts (methanol) tested against microorganism in micro dilution assays.

Microorganisms	*Berberis lyceum* extracts	Standard (Maxipime)
Bacillus amyloliquefaciens	200	135
Bacillus cereus	200	260
Bacillus licheniformis	100	-
Bacillus magaterium	100	-
Bacillus pumilus	200	35.5
Bacillus substilis	200	135
Escherichiccoli coli	200	22.5
Pseudomonas syingae	100	32.5
Pseudomonas putida	200	135
Candida albicans	40.5	-

McFarland culture, turbidity. The concentration of 100 µg/ml of plant extract was prepared in 10 % dimethylsulfoxide (DMSO) and serial dilution was made ranging from 10 µg/ml in 10 sterile test tubes (containing nutrient broth), therefore on the basis of micro dilution assays activity of plant extract against bacterial strains were determined (Clark, 1996).

The plates were prepared by dissolving 95 µl of nutrient broth and 5 µl of the inoculums, 100 µl of plant extract and 100 µl from serial dilution was taken in each plates. For negative control each plate was containing 195 µl of nutrient broth without the compound and 5 µl of the inoculums. Maxipime (Bristol-Myers Squibb) at concentration ranging from 8.5 – 500 µg/ml was prepared in nutrient broth and selected as positive control. The plates were covered with sterile plate sealer. The contents of each plate were mixed on a shaker at 2500 rpm for 25 second and incubated at suitable temperature for 24 hours. Microbial growth was determined by measuring absorbance by micro titer (US) at 600 nm, which was further confirmed by apply 5 µl of samples from each plates on nutrient agar media. The plant extracts in this study was tested twice for each organism. The minimum inhibitory concentration was defined as lowest concentration of the compound to inhibit the growth of microorganisms (Karman *et al*., 2003).

RESULTS AND DISCUSSION

The root extracts of *Berberis lyceum* prepared in two different solvents and tested against microorganisms. The results pertaining antimicrobial activity of root extracts are summarized in the following sections (Tables: 1-6).
In the present study the methanol and aqueous root extracts of *berberis lyceum* were applied to control the growth of different microorganisms and 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 (Ahmad *et al*., 1998). In the present study methanol and aqueous root extracts of *berberis lyceum* was applied against 35 strains of 18 bacteria species as well as 6 *Candida albicans* isolates (Tables 1-6). It was observed that methanol extract has inhibited the growth of these organism significantly as compared to aqueous extracts as reported by Karman *et al.,* 2003. The antimicrobial activity of extracts was assessed and quantified by

Table 6. The MIC (µg/ml) values of *Berberis lyceum* extracts (aqueous) tested against microorganism in micro dilution assays.

Microorganisms	*Berberis lyceum* extracts	Standard (Maxipime)
Bacillus amyloliquefaciens	200	120
Bacillus cereus	200	230
Bacillus licheniformis	100	-
Bacillus magaterium	100	-
Bacillus pumilus	200	30.5
Bacillus substilis	200	130
Escherichiccoli coli	200	20.5
Pseudomonas syingae	100	30.5
Pseudomonas putida	200	120
Candida albicans	35.5	-

presence or absence of inhibition zone, zone diameter and MIC values. The sensitivity of plant extracts found in this study was 12-30 mm and 135-260 µg/ml for methanol extract where as 10-26 mm and 120-230 µg/ml for aqueous extracts. Where as for isolates 12-25 mm and 40.5 µg/ml (methanol extract) and 10-22 mm and 35.5 µg/ml when aqueous extract was applied against growth of microorganism (Tables1-6). The results obtained during this study revealed that root of *Berberis lyceum* contained some active phytochemicals those have ability to control the growth of some microorganism (Barnabas and Nagarajan, 1988; Brantner *et al*., 1994). Therefore this is first study that will highlight the antimicrobial activity of *Berberis lyceum* Royle. The microorganism those was effected by plants extract could have some difference in their cell walls or inheritance antimicrobial resistance genes as plasmids can easily be transferred among bacterial strains. Therefore on the basis of results obtained in present study the root extracts of this plant can be helpful for development of new and useful drugs for different infections of human and animals.

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