

REPORT

Antibacterial activity of local herbs collected from Murree (Pakistan) against multi-drug resistant *Klebsiella pneumoniae*, *E. coli* and methiciline resistant *Staphylococcus aureus*

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Abstract: Exploring healing power in plants emerged in prehistory of human civilization. Sustaining good health has been achieved over the millions of years by use of plant products in various traditional sockets. A major contribution of medicinal plants to health care systems is their limitless possession of bioactive components that stimulate explicit physiological actions. Luckily Pakistan is blessed with huge reservoir of plants with medicinal potential and some of them; we focused in this study for their medicinal importance. In this study we checked the antibacterial activity inherent in *Ricinus communis*, *Solanum nigrum*, *Dodonaea viscosa* and *Berberis lyceum* extracts for multidrug resistance bacterial strains *Klebsiella pneumoniae*, *E. coli* and methiciline resistant *Staphylococcus aureus*. MRSA showed sensitivity for *Ricinus communis*. Multidrug resistant *Klebsiella pneumoniae* was sensitive with *Pine roxburgii* and *Ricinus communis* but weakly susceptible for *Solanum nigrum*. Multidrug resistant *E. coli* was resistant to all plant extracts. Treatment of severe infections caused by the bacterial strains used in this study with *Ricinus communis*, *Pine roxburgii* and *Solanum nigrum* can lower the undesired side effects of synthetic medicine and also reduce the economic burden.

Keywords: *Ricinus communis*, *Solanum nigrum*, *Dodonaea viscosa* and *Berberis lyceum*.

INTRODUCTION

The occurrence of bacterial infection in man is on peak around the world. The use of antibiotic drugs is either very much expensive or causes undesirable side effects while some are ineffective due to bacterial resistance (Alper, 1998). There has been an alarming increase in development of resistance in bacteria to antibiotic drugs. Ruifang Zhang *et al.* in 2006 reported the serious rise in bacterial resistance in China; 22% average growth in project time 1994-2000, Kuwait; 17% average growth from 1999 to 2003 and U.S.; 6% spanning 1999 to 2002 (Ruifang *et al.* 2006). The gradual rise in use of development of bacterial resistance in past years has led to increase in advocacy for the use of natural products in the prevention and cure of bacterial infections. This surge to discover remedies in natural resources has been more sought for nearly last fifty years.

Plants have an abundant reservoir of medicinally important chemicals known as phytochemicals. This previously unattended resource has been a very important avenue for pharmacy researchers since the potential resistance of bacteria to various classes of antibiotics. At present, according to world health organization, approximately 80% of human population relies on this

folk medicine to cure life threatening diseases.

Pakistan has a rich reservoir of medicinally important plants especially in its sub-tropical and northern areas. According to the surveys carried out by the Pakistan Forest Institute, 500 tons of medicinal plants are produced in Hazara and Malakand, 16 tons in Murree Hills, 38 tons in Azad Kashmir and about 24 tons in Northern Areas.

Murree hills. Murree is situated on the southern slopes of the Western Himalayan foothills as they ascend to the northeast towards Kashmir. Murree features a subtropical highland climate. This type of area has cold, snowy winters, relatively cool summer with drastically escalated rain, in relation with lower altitudes, and frequent fog. The extracts of various parts of these plants are being used by natives of Murree to cure pain, fever, wounds and as anti-inflammatory agents.

Extracts of leaves and seeds of plants which are used for years by natives of Murree have been reported in various studies around the world for their medicinal importance. The seeds of *R. communis* have several traditional applications (Devendra and Raghavan, 1978 and Gaydou *et al.*, 1982). These are used for the treatment of warts, cold tumours, indurations of the breast, corns and moles (Gibbs *et al.*, 2002, Wilcox and Bodeker 2004 and Huguet

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Termes, 2001). The crude extracts of the leaves of *Dodonaea viscosa* possess antibacterial activity against *Streptococcus pyogenes* and *Staphylococcus aureus* and strong activity against Cocksackie virus B3 and influenza A virus (Getie *et al.* 2003). The leaf, seed and root extract of *S. nigrum* has shown antibacterial activity (Sridhar *et al.* 2011). Model animal studies have suggested the wound healing and anti-diabetic properties of the *Berberis lyceum* root extract. In rat, aqueous and methanol extracts of *Berberis lyceum* has wound healing properties (Asif *et al.*, 2007); Gulfaraz M *et al.* in 2008 also reported the antidiabetic activity of root extract of the plant in alloxan-induced diabetic rats (Gulfraz *et al.*, 2008). In this study we checked the antibacterial activity of aqueous extracts of leaves of *Ricinus communis*, *Solanum nigrum*, *Dodonaea viscosa* and roots of *Berberis lyceum* against multidrug resistant strains of *E. coli* (Extended Spectrum Beta Lactamase, ESBL), MRSA and *Klebsiella pneumoniae*.

MATERIALS AND METHODS

Materials

The plants *Ricinus communis*, *Dodonaea viscosa*, *Solanum nigrum* and *Berberis lyceum* were collected in the month of March 2011 from Murree after proper identification. Pus, blood and urine specimen were taken from infected patients, who were showing resistance to

specific classes of antibiotics, for wound, chest and urinary tract infection respectively after informed consent.

Preparation of agar media

Dissolve bacto-trypton (10g), yeast extract (5g) and NaCl (10g) in 900 ml distilled water. Adjust pH 7.5 with 4N NaOH. Add granulated agar and adjust the final volume to 1L with distilled water. Autoclave to sterilize agar medium. The agar medium was cooled to 55°C and then poured into autoclaved Petri-plates in between two flames in laminar flow hood. The media plates were solidified for further use.

Sensitivity and resistance profiling of bacterial strains

The specimens; pus, blood and urine were cultured on agar medium at 37°C overnight. The bacterial strains were sub-cultured to get single colonies. Heavy growth of bacterial strains of *Staphylococcus aureus*, *Klebsiella spp.* and *E. coli* was isolated. Each bacterial strain was sub-cultured and profiled for its sensitivity or resistance against different classes of antibiotics in a culture and sensitivity assay using disc method. Methicillin, Penicillin, Cephalosporin, monobactam and carbapenems were tested for their activity against each bacterial strain. ESBL production by bacterial strains was also checked. This experiment was done for a standard reference. The list of all antibiotics used is given in table 1.

Table 1: Effect of different antibiotics on *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* (*Staph aureus*)

Names of Antibiotics	Bacterial strains			Names of Antibiotics	Bacterial strains		
	ESBL		<i>Staph aureus</i>		ESBL		<i>Staph aureus</i>
	<i>E.coli</i>	<i>Klebsiella Pneumoniae</i>			<i>E. coli</i>	<i>Klebsiella pneumoniae</i>	
Imipenem	S	S	R	Fosfomycin	S	-	-
Meropenem	-	S	R	Urxin	R	-	-
Ciproxin	-	S	R	Nitrofurantoin	R	-	-
Ofloxacin	R	S	R	Tazocin	S	R	R
Levofloxacin	R	S	R	Sulzone	R	R	R
Amoxycillin	R	R	R	Chloramphenicol	-	R	S
Augmentin	R	R	R	Linezolid	-	-	S
Cephadrine	R	R	R	Vancomycin	-	-	S
Cefaclor	R	R	R	Doxycyclin	-	-	S
Cefuroxime	R	R	R	Septran	R	-	S
Cefixime	R	R	R	Cloxacillin	-	-	R
Ceftriaxon	R	R	R	Penicillin-G	-	-	R
Cefotaxime	-	R	R	Erythrocin	-	-	R
Cefaperazone	-	R	R	Fusidic Acid	-	-	R
Ceftazidim	-	R	R	Rifampicin	-	-	R
Ciprofloxacin	R	-	-	Cefpirom	R	-	R
Gentamycin	S	R	R	Ertapenem	-	-	R
Amikacin	S	R	R	Amikacin	-	-	-
Aztreonam	R	R	R				

Sensitive (S), Resistant (R)

Table 2: Effect of various plant extracts on *E. coli*, MRSA and *Klebsiella pneumoniae* (Zone of inhibition is given in mm)

Name of the plant	Plant part used for aqueous extract	Effect on Multidrug resistant bacteria (zone of inhibition in mm)		
		<i>E. coli</i>	MRSA	<i>Klebsiella pneumoniae</i>
<i>Ricinus Communis</i>	Leaves	Null	Good (7mm)	Good (7.5mm)
<i>Berberis Lyceum</i>	Roots	Null	Null	Null
<i>Dodonea viscosa</i>	Leaves	Null	Null	Null
<i>Pinus roxburgii</i>	Female cone	Null	Null	Good (7mm)
<i>Solanum Nigrum</i>	Leaves	Null	Null	Weak (4mm)

Preparation of phytoextract

The leaves of *Ricinus communis*, *Solanum nigrum*, *Dodonaea viscosa* and roots of *Berberis lyceum* were cut from fresh plants. These parts were washed with fresh water and thoroughly rinsed with de-ionized water. 15 grams of the leaves and roots were crushed fine in an electric grinder. The paste was dissolved in 100ml water. This aqueous solution was placed at room temperature for 2 weeks. The samples were centrifuged at 3000 rpm for 10 minutes. Aqueous layer or supernatant was collected as phytoextract in clean centrifuge tubes. The phytoextracts were lyophilized to concentrate. The volume of the phytoextract was reduced to one third after lyophilization.

Application of phytoextracts to the bacterial strains

Single colony of *Staph. aureus*, *Klebsiella spp.* and *E. coli* were cultured on agar medium by lawn formation. Concentrated aqueous phytoextracts were applied to the agar Petri plates by disc method. The agar plates containing the specific bacterial lawn and phytoextract was incubated at 37°C overnight. The zone of inhibition was measured in millimeter (mm).

RESULTS

The response of *Staphylococcus aureus*, *Klebsiella spp.* and *E. coli* to various antibiotic drugs which were used as standard and shown in table 1. *E. coli* and *Klebsiella pneumoniae* were ESBL producing strains. *Staphylococcus aureus* was resistant to methicilline and hence it is identified as Methicilline resistant *Staphylococcus aureus* (MRSA). It was observed that all bacterial strains used were resistant to Penicillins, Cephalosporins, Carbapenems and Monobactam class of drugs. Moreover *E. coli* and MRSA were ESBL (ESBL) producing strains.

Antibacterial activity of phytoextracts

The concentrated aqueous extract of *Ricinus communis* leaves showed good antibacterial activity against multidrug resistant MRSA and *Klebsiella pneumoniae* (ESBL). In this case the zone of inhibition was measured 7 and 7.5 mm respectively. *Klebsiella pneumoniae* (ESBL) was fairly sensitive for aqueous extract of female cone of *Pinus roxburgii* with a 7mm zone of inhibition. But this extract did not show any activity against *E. coli*. The

aqueous extract of *Solanum nigrum* leaves came up with a weak sensitivity to *Klebsiella pneumoniae*, a 4mm zone of inhibition was observed. *E. coli* (ESBL) was resistant to the extracts of leaves of *Ricinus communis*, *Solanum nigrum*, *Dodonaea viscosa*, roots of *Berberis lyceum* and female cone of *Pinus roxburgii*. The results of antibacterial activity of different plant phytoextract are shown in table 2.

DISCUSSION

Plants synthesize abundant amount of aromatic substances including phenols or their oxygen substituted derivatives (Olukoya et al., 1992). These chemical weapons help the plant to tolerate microorganism, insect and herbivore intruders (Cowan, 1999). Other important plant chemicals include Terpenoids and Tannins which respectively have the ability to inhibit bacterial growth (Aureli, 1992) and set bacterial proteins ineffective by weak bonding like hydrogen bonding.

A smart work has been reported on antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory features of the plant extracts (Samy and Ignacimuthu, 2000, Palombo and Semple, 2001, Kumaraswamy et al. 2002, Stepanovic et al. 2003, Bylka et al. 2004, Behera and Misra, 2005, Govindarajan et al. 2006). These reports have been utilized for the identification of principle agent in conferring the antimicrobial ability of plant. However a glimpse amount of reports highlight the antibacterial properties of medicinal plant extracts against multidrug resistant strains of pathogenic bacteria. The present study shows interesting findings for the antibacterial activity of *Ricinus communis* leaf extracts against MRSA and *Klebsiella pneumoniae*; intolerance of *Klebsiella pneumoniae* with aqueous extract of *Pinus roxburgii* Female cone and a weak ability of *Solanum nigrum* leaf extract to prevent *Klebsiella pneumoniae* growth. However multidrug resistant MRSA and *Klebsiella pneumoniae* were resistant to *Berberis Lyceum* and *Dodonaea viscosa* extracts of leaves and Female cone respectively. Multidrug resistant *E. coli* showed resistance to all aqueous extracts of various parts of plants used in this study as shown in table 2.

The aqueous extract of leaves of *Ricinus communis* show good activity against multidrug resistant *K. pneumoniae* and MRSA (Jombo1 and Enenebeaku, 2008). The alcohol and water extracts of fermented seeds of *Ricinus communis* had been reported with potential antibacterial activity against several bacteria. Very few reports are present for the antibacterial potential of *Pinus roxburghii*; our study indicates that its female cone has good activity against multidrug resistant bacteria.

This study concludes that plants have a great reservoir to fight against pathogens of human beings. These plants extract needs a prompt validation and should be employed in reducing the infectious disease burden at lower costs.

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