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**RESEARCH ARTICLE**

# EVALUATION OF ANTI-BACTERIAL ACTIVITY OF HIGH VALUED MEDICINAL PLANT EXTRACTS AGAINST MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA AND PROTEUS VULGARIS

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**ABSTRACT**

Human pathogenic bacteria have employed high levels of multidrug resistance (MDR) with enhanced morbidity and mortality. MDR develop hindrance in disease control by intensifying the possibility of spreading of resistant pathogens, thus, declining efficacy of treatment. MDR in bacterial infections has impaired the current antimicrobial therapy and demanding the search for other alternatives. Search for natural product extracting from high valued medicinal plants are the alternative source for discovery of new drugs useful against MDR bacteria. Henceforward, this study is designed to investigate the *in vitro* antibacterial activity of selected medicinal plants extracts against *Pseudomonas aeruginosa* and *Proteus Vulgaris* in comparison with commercial antibiotic discs using disc diffusion method. Different fractions of eight high valued medicinal plants were screened. The fractionated extract of *Chenopodium album, Quercusincana, Zizipus jujube leaves, Zizipus jujube Flower, Grevillea robusta, Corydalis govaniana, Solanum nigrum* showed antibacterial activity confirming through zone of inhibition ranging from 0-18.3 mm against *Pseudomonas aeruginosa* and *Proteus Vulgaris.* While *Trifoliumrepens*, *Lamoniumcabulicium*failed to control bacterial growth on disc diffusion. We suggest that the protocol used in this study is useful for the investigation of more plants used for antibacterial activity and plant having active natural products serve as a source for antibacterial compounds.



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## INTRODUCTION

The development and spread of antibiotic resistance in pathogenic bacteria is a continuous threat both to humans as well as animals (Bush *et al*., 2011). The molecular mechanisms of bacterial resistant to antibiotics are very complex. They are also significantly more expensive to diagnose. Naturally antibiotic resistance is far much less common than the acquired one resulted from conjugation of plasmid and transmitted horizontally (Davies and Davies, 2010). Thus resulted in the simultaneous development of resistance to several antibiotic classes creating very dangerous multidrug-resistant (MDR) bacterial strains. Some of MDR Bacteria are also known as ''superbugs'' (Alanis, 2005). The spread of MDR bacteria in community remains as critical healthcare problem (Brusselaers *et al*., 2011).

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Despite to advances in antibiotic therapy, infectious complications remain an important cause of mortality and morbidity among hospitalized patients thus leading to considerable clinical and economic burden (Salgado *et al*., 2005). Pseudomonas aeruginosa and Proteus vulgar is are MDR bacteria. These bacteria are found in soil, water, human skin, throat, and stool of a healthy human naturally. Common diseases caused by Pseudomonas aeruginosa in human urinary tract infections are ventilator-associated pneumonia, surgical site infection, respiratory, ocular, ear, skin and soft tissue infections.

Proteus Vulgaris are opportunistic pathogen; commonly it can cause urinary and septic infections. It also cause severe kidney infection which is because of its attachment to host urothelial cells (Fuchs *et al*., 1996; Hsu *et al*., 1994; Thornsberry and Yee, 1996). Due to the increase of resistance to antibiotics, there is an urgent need to develop new and innovative anti-microbial agents. Different plants sources have long been

investigated as potential sources of new antibacterial agents. Because of their low toxicity, there is a long tradition of using dietary plants in the treatment of infectious disease (Djeussi *et al*., 2013). The present study is designed to determine the antibacterial activity of eight selected high valued medicinal plants (Chenopodiumalbum, Quercusincana, Zizipus jujube, Zizipus jujube, Trifoliumrepens, Grevillearobusta, Lamoniumcabulicium, Corydalis govaniana, Solanumnigrum) against MDR Pseudomonas aeruginosa and Proteus vulgaris.

## MATERIALS AND METHODS

### Collection of Plant Materials

A total of eight medicinal plants were used in the present study: *Trifoliumrepens, Quercusincana, Zizipus jujube, Chenopodium album, Lamoniumcabulicium, Solanum nigrum, Grevillea robusta,* and *Corydalis govaniana.* These plants were collected from different areas of district Swat and Lower Dir, Khyber Pakhtunkhwa, Pakistan as shown in Table 1.

### Growth medium

Different bacterial growth media including Nutrient agar (Merck, Darmstadt, Germany) and McConky Agar (Oxoid Ltd, Hampshire, UK) were used for the culturing and sub-culturing of the selected bacterial strains.

### *In-vitro* antibiotic sensitivity test

The antibiotic sensitivity and pattern of resistant of each plant extract was determined against MDR bacteria including *Pseudomonas aeruginosa* and *Proteus vulgaris.* In addition, for comparative analysis, the commercial antibiotic discs (Ciprofloxacin, Cefepime, Moxifloxicin, Gentamicin, and Ceftriaxone) used as standard. Discs prepared from plant extracts and commercial antibiotic discs were applied on the surface of the agar plates with the help of sterile forceps separated at an equal distance from each other. All plates were incubated for 24 hours at 37°C.

**Table 1. List of Plant Material used and their area of collection**

| S. No | Plant name | Plant part used | Area of sample collection |
|---|---|---|---|
| 1 | *Trifoliumrepens* | Seeds | |
| 2 | *Gravilearobista* | Leaves | |
| 3 | *Zizipusjujuba* | Leaves, flowers | Lower Dir, Khyber Pakhtunkhwa, Pakistan |
| 4 | *Chinipodium album* | Leaves | |
| 5 | *Lamoniumcabulicium* | Leaves | |
| 6 | *Solinumnigrum* | Seeds | |
| 7 | *Quercusincana* | Leaves | Swat Khyber Pakhtunkhwa, pakistan |
| 8 | *Corydalis govanniana* | Flower | |

### Plant extracts preparation

The plants materials were shad dried and then chopped into small pieces performed previously (Bhalodia and Shukla, 2011). The crude extracts were separated through filtration rotary evaporator(Hossain *et al*., 2014). The isolated plant extracts were stored in an airtight bottle at 4°C until further use.

### Different concentration discs preparation

A total of 1g/10 ml of crude extract was dissolved in distilled water for preparation of different concentration of discs from these isolated plant extracts. After preparing different concentration of each extract the disc were subjected with each concentration. About 10ul of each concentration was poured on individual disc. Different discs were prepared having different concentration of (10ug, 50ug, 100ug, 750ug and 1gm). The 5 mm discs were prepared from the filter paper using a punching paid. All the prepared discs were sterilized(Thomas and Veda, 2008). Each sterile disc was individually treated with each extract of plant in various concentrations (10ug, 50ug, 100ug, 250ug, 500ug, 750µg, 1g). Over flow of the compounds was avoided on the outer surface of the discs and were placed for drying and then stored at 4°C.

### Tested microorganisms

The MDR bacteria used in this study were *Pseudomonas aeruginosa* and *Proteus vulgaris.* Both bacteria were collected from government hospitals of Khyber Pakhtunkhwa Pakistan.

The effectiveness of the drug and fraction of crude extract was determined by measuring the diameter of the zones of inhibition using digital verniercaliper that resulted from the diffusion of the active ingredient in the medium surrounding the plant extracts discs as well as commercial antibiotics. After incubation, the zones of inhibition were observed around the discs on nutrient agar plates.

## RESULTS

The chloroform, methanolic, ethyl acetate extracts from the screened plant material (*Chenopodium album, Quercusincana, Zizipus jujube* leaves, *Zizipus jujube* Flower, *Trifoliumrepens, Grevillearobusta*, *Lamoniumcabulicium, Corydalis govaniana, Solanumnigrum*) showed variable zones of inhibition (ranging from 0-17.3, 0-18.3, 0-16) respectively against *Pseudomonas aeruginosa*. Only the chloroform extracts of *Chenopodium album* showed antibacterial activity (17.3mm). The methanolic extracts of *Chenopodium album* (7.3mm), *Quercusincana* (7mm), *Zizipus jujube* leaves (18.3mm), *Zizipus jujube* flower (8mm), *Grevillea robusta* (6.3mm), *Corydalis govaniana* (7mm) and *Solanumnigrum* (13.3mm). Similarly only the ethylacetate extracts of *Chenopodium album* showed antibacterial activity (16mm) as shown in Figure 1. Only the chloroform extracts of *Chenopodium album* showed antibacterial activity (17.3mm). The tested commercial antibiotics (Ciprofloxacine, Cefepime, Moxifloxicin, Gentamicin, and Ceftriaxone) showed different antibacterial activities (22.1mm, 34.8mm, 27.3mm, 8mm, and 20.4mm) against *Pseudomonas aeruginosa* respectively as shown in Table 2.

**Table 2. Antibacterial efficacy of organic extracts fractions (Ethyl acetate, methanolic and chloroform) of different plant parts against multi-drug resistance (MDR *Pseudomonas aeruginosa***

| Type of MDR bacteria | | List of commercial antibiotics | | | | | Zone of inhibition of *Chenopodium album* (mm) | | | Zone of inhibition of *Quercusincana* (mm) | | | Zone of inhibition of *Zizipusjujube* leaves (mm) | | | Zone of inhibition of *Zizipus jujube* Flower (mm) | | | Zone of inhibition of *Trifoliumrepens* (mm) | | | Zone of inhibition of *Gravilliarubista* (mm) | | | Zone of inhibition of *Lamoniumcabulicium* (mm) | | | Zone of inhibition of *Corydalis govaniana* (mm) | | | Zone of inhibition of *Solinumnigrum* (mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ciprofloxacin | Cefepime | Moxifloxicin | Gentamicin | Ceftriaxone | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate |
| *Pseudomonas aeruginosa* | | 22 | 35 | 27 | 9 | 20 | 19 | 8 | 20 | 0 | 8 | 0 | 0 | 19 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 14 | 0 |
| | | 23 | 35.3 | 26.2 | 8 | 19.5 | 16 | 7 | 15 | 0 | 7 | 0 | 0 | 18 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 12 | 0 |
| | | 21.4 | 34.3 | 28.7 | 7 | 21.9 | 17 | 7 | 13 | 0 | 6 | 0 | 0 | 18 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 14 | 0 |
| | Mean Values | 22.1 | 34.8 | 27.3 | 8 | 20.4 | 17.3 | 7.3 | 16 | 0 | 7 | 0 | 0 | 18.3 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 6.3 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 13.3 | 0 |

**Table 3. Antibacterial efficacy of organic extracts fractions (Ethyl acetate, methanolic and chloroform) of different plant parts against multi-drug resistance (MDR *Proteus vulgaris)***

| Type of MDR bacteria | | List of commercial antibiotics | | | | | Zone of inhibition of *Chenopodium album* | | | Zone of inhibition of *Quercusincana* | | | Zone of inhibition of *Zizipus jujube* leaves | | | Zone of inhibition of *Zizipus jujube* flower | | | Zone of inhibition of *Trifoliumrepens* | | | Zone of inhibition of *Gravilliarubista* | | | Zone of inhibition of *Lamoniumcabulicium* | | | Zone of inhibition of *Corydalis govanniana* | | | Zone of inhibition of *Solinumnigrum* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Ciprofloxacine | Cefepime | Moxifloxicin | Gentamicin | Ceftrixone | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate | Chloroform | Methanolic | Ethyl acetate |
| *Proteus vulgaris* | | 28 | 22 | 32 | 22 | 18 | 0 | 10 | 0 | 0 | 8 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 7 | 6 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 15 | 0 |
| | | 27.8 | 21.2 | 32.9 | 21.2 | 19 | 0 | 10 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 7 | 6 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 13 | 0 |
| | | 29.5 | 23.2 | 31.9 | 23.3 | 20 | 0 | 10 | 0 | 0 | 6 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 7 | 6 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 14 | 0 |
| | Mean Value | 28.4 | 22.4 | 32.2 | 22.2 | 19 | 0 | 10 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 7 | 6 | 0 | 0 | 0 | 0 | 0 | 7.3 | 0 | 0 | 14 | 0 |

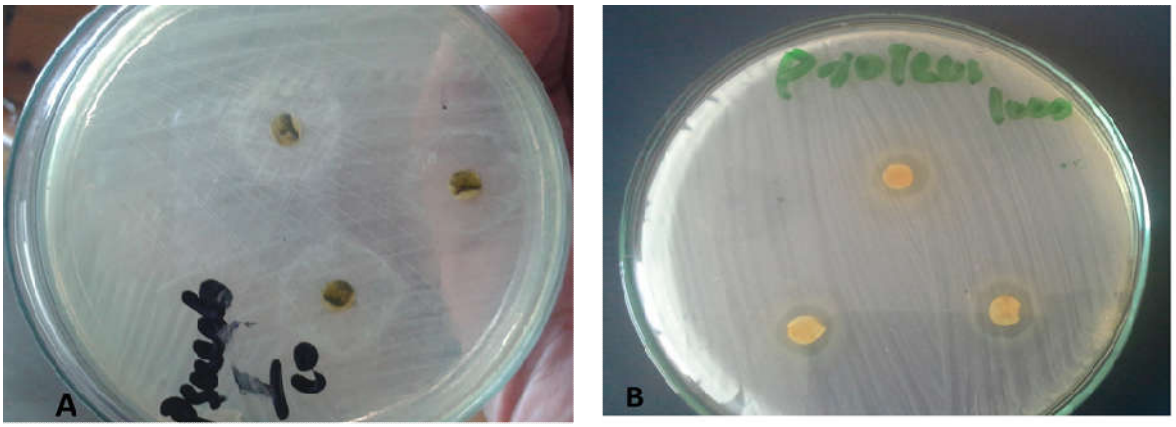


**Figure 1. An example of antibacterial activity of *Zizipus jujube* leaves against *Pseudomonas aeruginosa* (plate A) and plate B shows the antibacterial activity of *Solanum nigrum* against *Proteus vulgaris***

The chloroform, methanolic, ethyl acetate extracts from the screened plant material (*Chenopodium album, Quercusincana, Zizipus jujube* leaves, *Zizipus jujube* Flower, *Trifoliumrepens, Grevillearobusta*, *Lamoniumcabulicium, Corydalis govaniana, Solanumnigrum*) showed variable zones of inhibition (ranging from 0-7, 0-14, 0) respectively against *Proteus vulgaris*as shown in Figure 1. The methanolicextracts of *Chenopodium album* (10mm), *Quercusincana* (7mm), *Zizipus jujube* leaves (7mm), *Zizipus jujube* flower (7mm), *Grevillea robusta* (6mm), *Corydalis govaniana* (7.3mm) and *Solanumnigrum*(14mm). Similarly only the ethyl acetateextracts of*Chenopodium album* showed antibacterial activity (16mm). The tested commercial antibiotics (Ciprofloxacine, Cefepime, Moxifloxicin, Gentamicin, Ceftrixone) showed different antibacterial activities (28.4mm, 22.4mm, 32.2mm, 22.2mm, 19mm) against Pseudomonas aeruginosa respectively as shown in Table 2.

## DISCUSSION

Each fraction of the extract tested in present study exhibitedantibacterial activity on both studied bacteria. However, differences were observed between antibacterial activities of plant's different extracts depending on the organic solvent used for preparation. These differences could be due to the differences in the chemical composition of these extracts as the secondary metabolites of plants have many effects including antibacterial and antiviral properties (Cowan, 1999; Noumedem *et al*., 2013). Our data showed antibacterial activity of all three types of fractionsof *Chenopodium album*leaves against *Pseudomonas aeruginosa* which were in accordance with previous observation (Elif Korcan *et al*., 2013). Singh *et al* has also showed that aqueous extract of *Chenopodium album* revealed strongest antibacterial activity on *Staphylococcus aureus* and methanol leaf extract indicated strongest antibacterial activity on *Pseudomonas aeruginosa*. (Pandey and Gupta, 2014).

However, Amjad and Alizad mentioned that the flowers and leaves methanolic and ethanolic extracts of *Chenopodium album* don't have any activity against the tested bacterial strain *Pseudomonas aeruginosa* (Amjad and Alizad, 2012). Jan *et al,* 2012 investigated the antibacterial activity of thirty-three plant species belonging to 26 families. The results indicated that all medicinal plants showed anti-bacterial activity which is in accordance with our studied plant species (Jan *et al*., 2012). Our study showed that methanolic leaves fractionof *Zizipus jujube* have antibacterial activity against MDR *Pseudomonas aeruginosa* which is in accordance with the results of Bashir Ahmad *et al* 2011(Ahmad *et al*., 2013). It was previously shown that methanolic extract of exhibit moderate activity. We investigated that methanolic extract showed best activity on *Pseudomonas aeruginosa*. Moreover Majid *et al* reported that *Zizipus jujube* has no activity against *Pseudomonas aeruginosa* (Majid, 2014). Borchardt *et al* reported that *Trifoliumrepens* have no antibacterial activity against *Pseudomonas aeruginosa* on any extract which is according to our results (Borchardt *et al*., 2008). According to the Sharif Ullah *et al*, *Grevillea robusta* had antimicrobial activity in chloroform extract and no activity in methanolic extract which is in contrast to our findings (Ullah *et al*., 2015).

In our results methanolic fraction show activity while the other two extracts (chlorofomic and ethyl acetate) fractions did not show any activity. Ethyl alcohol extract *Solanumnigrum* showed activity against *Pseudomonas aeruginosa* (Nithya *et al*., 2006). While, Parameswari. K showed that it hadno zone of inhibition against *Pseudomonas aeruginosa* (Parameswari *et al*., 2012). Using these plant extracts no previous study was conducted on *Corydalis govaniana* and *Lamoniumcabulicium.*
In *Chenopodium album* moderate antibacterial activities were recorded by *Proteus vulgaris* by K.P.Singh *et al*., 2011. Our study also showedmoderate result within a range of 10 mm zone of inhibition. T.M. Sridhar 2011 mentioned in his work that *Solanumnigrum* has activity in all the three extracts Ethanol, chloroform and ethyl acetate 10mm 21mm and 10.5mm respectively. While in our study only the methanolic extract show significant results with a zone of 14.4mm. While the other plants *Quercusincana, Grevillearobusta*, *Zizipus jujube, Trifoliumrepens, Lamoniumcabulicium, and Corydalis govaniana* have not been previously studied against *Proteus vulgaris.*

### Conclusion

Multidrug resistance (MDR) bacteria is an alarming issue in health care with enhanced morbidity and mortality. Present investigation revealed the pivotal role medical plants as an alternative to the commercial antibiotics. Further studies are

required to explore the bioactive constituents of the studied plants extracts.

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