Mycopath (2016) 14(1&2): 37-41 **Research Article**

Evaluation of antimicrobial activity of some important xerophytes

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Abstract

The present research was conducted to evaluate the antimicrobial activities of certain selected xeric plants extracts against certain species of bacteria and fungi by minimum inhibitory concentration assay. The selected xerophytes species of District Bannu for minimum inhibitory concentration (MIC) were *Calligonum polygonoides*, *Sueda fruticosa*, *Peganum harmala* and *Rosa brunonii*. The antimicrobial activities were carried out in laboratory by using of various strains of bacteria and fungi such as *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger* and *Aspergillus flavus.* All the extract of the selected plant were found effective against bacteria and fungi except for *S. fruticosa* in which weak antimicrobial activities were found. Similarly, it was also investigated that *C. polygonoides* and *R. brunonuii* have a high potential of antibacterial activities against the pathogens with less MIC (50, 75 µg mL^{-1}). In case of antifungal activities the MIC value (50 µg mL^{-1}) of *R. brunonuii* and *C.* poygonoides, while in *S. fruticosa* both the activities were recorded weak with high MIC value (150, 200 µg mL^{-1}). It was concluded that amongst the elected xeric plants, *C. polygonoides* and *R. brunonuii* have strong antibacterial and antifungal activities with lower values of MIC as compared to *S. fruticose*. Our report also suggested that the selected medicinal plants of district Bannu have potential properties to inhibit bacteria and fungi growth. This is the first report describing the antimicrobial activities of several Mexican medicinal plants used in this study.

Keywords: Antibacterial, Antifungal, *Calligonum*, *Rosa brunonii*, Xeric plants.

Introduction

Medicinal plants, plays pivotal role in traditional medicine and is a subject of interest in pharmacological studies. Medicinal plants are the potential and important sources for new therapeutic compounds as well as drug development (Matu and van Staden, 2003). McKay and Blumberg (2007) reported that 80% population in developing countries for health on primary basis depends on medicinal plants. On the other hand, antibiotics played a key role in eradication of microbial infections. However, their over and unwise use has been proved a potential threats to human health as well as environment. Meanwhile, antibiotics have become less and less effective because of resistant potential of microbes against the commonly used antibiotics (Mateen *et al.*, 2011). Microbial resistance to antibiotics and increased infection rates is the matter of intense concern which demands to explore alternative potential drugs from various sources such as medicinal plants (Shahid *et al.*, 2008; Cordell, 2000). However, among the plant flora of the world, only about 20% have been subjected yet to pharmacological or biological testing hence, plant activities specially antimicrobial and antifungal gained much attention recently (Mothana and Lindequist, 2005; Sen and Batra, 2012). Medicinal herbaceous plants are widely used as a potential sources of traditional medicine (Dubey *et al.*, 2004). Similarly, natural products obtained from higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Kirtikar and Basu, 1990; Matalawska *et al.*, 2002).

Pakistan is the land of several indigenous tribes with a rich herbal knowledge about medicinal plants. Pakistan has diverse climatic conditions and geographical regions that have caused a wide distribution of medicinal plant species (Ahvazi, 2008). The pharmacognostic studies of the plants provides basis for proper collection, identification and investigation of plant. Such studies could be useful in the preparation of herbal medicine in Pakistani pharmacopoeia (Khan *et al.*, 2011). In this respect large numbers of studies have been done in different part of the world to assess the antimicrobial potential of medicinal plants (Ahmed *et al.*, 2000). Plants have the potential of antimicrobial activities because of having a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc.

(Hassan and Khalid, 1992; Gupta *et al.*, 2003). For substantiate, standard and authenticate drugs, these characteristics are very necessary for characteristic of plants (Garg *et al.*, 2011). In order to investigate new sources of antimicrobial agents in xeric plant of District Bannu, *Calligonum polygonoides*, *Sueda fruticose*, *Peganum harmala* and *Rosa brunonii* have been selected. *Calligonum polygonoides* is a woody small much branched or small tree leafless glabrous with whitish to pale brownish bark, found in desert areas or extreme xeric environment. It commonly grows on drought sandy soils or on sand dunes; it is a common plant of sand dunes in Southern Baluchistan and Trans-Indus plains, also found in certain area of district Bannu. This plant is commonly known as phog, locally known as Balanza. The *Sueda fruticose* is a perennial shrub belongs to family (Chenopodiaceae). This is a leaf succulent obligate halophyte which produces large a numbers of seeds under salty environment locally known as Thoman. Similarly, *Peganum harmala* is a wild annual drought tolerant medicinal herb native to dry areas grow in sandy and loam type of soils while *Rosa brunonii* is a deciduous, smooth, tall-climbing perennial shrub, it grow wild in forests in well cultivated; it prefers fertile, permeable soil. The present study was based to screen out the antimicrobial and antifungal activities of the mentioned xeric medicinal plants of district Bannu, Pakistan against some pathogenic bacteria and fungi for the purpose to find out new sources of antibacterial and antifungal agents.

Material and Methods

Collection of plant materials and preparation of extracts

The fresh leaves of *C. polygonoides*, *S. fruticose*, *P. harmala* and *R. brunonii* collected from different areas of District Bannu in Khyber Pakhtunkhwa Pakistan during 2013-14. Plant specimens were labeled along with the record of their local uses. Collected leaves were washed with distilled water to clean them from dust and were than dried under shade. The dried leaves were than crushed to powder and were soaked in 70% methanol for 72 hours at room temperature. The methanol extract was collected by use of Whatman filter No.1. Methanol and water were evaporated while obtained the plant extracts. After extraction, stock solution of 10 µg mL^{-1} was prepared, further dilutions of 50-200 µg mL^{-1} from stock solution for MIC value were also prepared.

Antibacterial and antifungal activities

Both the activities were performed by using the disc diffusion method. For bacteria, aseptic dextrose potato liquid nutrients medium (growth medium) were used and poured in autoclaved, Petri dishes up to 4mm depth while sabouraud dextrose agar (SDA) media used for anti-fungal activity. The strains of bacteria (*B. subtilis* and *E. colli*) and fungl strains (*A. niger* and *A. flavus*) were swabbed on Petri dishes after solidification of the media with the help of aseptic aluminum borer; four wells were cut in the agar layer of each petri plate. All the extracts were screened for their antibacterial and antifungal activities against bacterial strain i.e. *B. subtilis*, *E. colli* and fungal strains i.e. *A. niger* and *A. flavus*. In each of the four wells, equal amount of DMSO, tetra cycline and the same amount of methanol extract from stock solution (10 µg mL^{-1}) were dissolved in DMSO and further diluted to 50, 75, 100, 125, 150, 175 and 200 µg mL^{-1} of the extract and mixed with terbinofine of 950, 925, 900, 875, 850, 825 and 800 µg for the determination of MIC value in separate well. The wells of tetracycline were used as a control while DMSO as a standard treatment. The plates were then incubated for 24 h at room temperature (37 °C). The zones of growth inhibition around the disks were measured in mm after 24 h of in incubation at 30 °C. The MIC value was measured for each plant.

Results and Discussion

In the present research the MIC value were analyzed for the methanolic extracts of xeric plants of Disrict Bannu which are *Calligonum polygonoides*, *Sueda fruticose*, *Peganum harmala* and *Rosa brunonii* against the fungal and bacterial strains (*Aspergillus niger* and *Aspergillus flavus*), (*Bacillus subtilis*, *Escherichia colli*). The overall results were summarized in Table 3 and 4 also in Fig.1 and 2. Present results indicated that the MIC values were found higher i.e.150 and 200 µg mL^{-1} in extract of *Sueda fruticose* which shows the extent of resistance by the two mentioned strain of bacteria and fungi. Similarly, this study also explain that maximum activities of the *Calligonium poygonoides* and moderate activities of *Rosa brunonuii* against the pathogens with MIC values of 50 and 75 µg mL^{-1}, respectively. The methanolic extract of this plant was especially effective as antibacterial tools in the control of this strain of bacteria which protect us from illness and other undesirable microorganism. The result also showed that the inhibitory value also change with change of concentrations while in case of

antifungal activities the MIC value of *Rosa brunonuii* and *Calligonium poygonoides* showed lower value (50 µg mL^{-1}) for both. In case of The *Peganum harmala* extract with the MIC value found moderate i.e. 75 µg mL^{-1} (Table 3 and 4). Overall the result shows that *Calligonium poygonoides* and *Rosa brunonuii* have excellent antibacterial and antifungal activities against the mentioned strains of bacteria and fungi while *Peganum harmala* have moderate MIC value while in *Sueda fruticosa* the activities are weak with high MIC value.

Diseases caused by bacteria and fungi represent a serious public health risk and causes death of people round the world. To prevent this, people extensively using antibiotics which have several side effects, resistance in agents, as well as environmental hazards. Alternative to these, natural products obtained from medicinal plants could be of high interest to mitigate the increasing problem rising due to use of antibiotics. In ancient times, plants have been considering the potential source of drugs. Different plant extracts from folk medicinal plants have been tested to identify the drugs. In this respect, large numbers of studies have been conducted, demonstrating the usefulness of natural products obtained from plants in controlling various infectious diseases caused by microorganisms (Qaralleh, 2010; Njume, 2011). Ahmad *et al.* (2005) reported that natural products obtained from plants in pure or extract form provide prospects for new drugs for discoveries. According to Garg *et al.* (2011), plants are medicinally important because of the presence different compounds like flavonoids, alkaloids, terpenoids and tannins.

The present research revealed the antibacterial and antifungal activities of *C. polygonoides*, *P. harmala*, *S. futircosa* and *R. brunonii* extract against microbes (*B. subtilis, E. colli, A. niger* and *A. flavus*). The results showed that the MIC values are different for different plant species as well as for different microorganisms. MIC values of *C. polygonoides*, *P. harmala*, *S. futircosa* and *R. brunonii* shown in Table 3 and 4. Our present results regarding the biological activities were found in accordance with the reports of (Al-Zahrani and Al- Robai, 2007). Application of leaf extract of *C. polygonoide* reduced or inhibited the growth of bacterial strain (*B. subtilis* and *E. colli*) and fungi strains (*A. niger* and *A. flavus*). The result showed that methanol extract of dried leaves of *C. polygonoide* had inhibitory effect on bacteria and Fungi strains while extract of *S. futircosa* plant have less effect on Bacterial and Fungal strains. These reports were found in concordance with the reports published by Mateen *et al.* (2011) which stated that some plants can pose inhibitory effect against bacterial strains. Similarly, Bauer and Staden (2006) studied the antibacterial and antifungal activities of many medicinal plants used venereal disease. Zain *et al.* (2012) studied the antimicrobial activities of Saud Arabian desert plants, in this study *A. maurorum*, *C. murale* and *T. aphylla* showed significant antimicrobial activities against Gram negative and Gram positive bacteria, all the investigated plants extracts showed antifungal activities against *A. fumigatus, A. flavus and P. chrysogenum*. Similarly, Sajid *et al.* (2011) studied the phytochemical screening and antimicrobial activity of *F. cretica* plant extracts against *S. aureus, Escheria coli, P. aeruginosa, B. subtilis, S. epidermidis.*

Present activities could be of particular interest in this regard to find out its unexplored efficiency and a potential source for chemically interesting as well as biologically important drug candidates. *C. polygonoides* is more fertile plant on the basis of chemical nature hence further research will be useful and more fruitful due to its less amount of MIC value. The investigation reported in this article can be considered as the first information on the antibacterial and antifungal properties of the selected plants of District Bannu which may also contribute to the knowledge of antimicrobial potential of selected xerophytes species of the study region.

Table 1. Important xeric medicinal plants selected for biological activities

S. No.	Botanical name	Family	Local name
1	*Calligonum polygonoides*	Polygonaceae	Balanza
2	*Peganum harmala*	Zygophyllaceae	Spelanii
3	*Rosa brunonii,*	Rosaceae	Jangli gulap
4	*Sueda fruticosa*	Chenopodiaceae	Thomoon

Table 2. Bacterial and fungal strains used in the present study.

S.No		Code number
	Bacterial Strains	
1	*Bacillus subtilis,*	ATCC No.2063
2	*Escherichia colli*	ATCC No.25922
	Fungal Strains	
1	*Aspergillus niger*	ATCC No.545
2	*Aspergillus flavon*	ATCC No.610

Table 3. MIC Value for Different Methanolic Extract against *Bacillus Subtilis* and *Escherichia Colli.*

Methanolic extracts	MIC (µg mL^{-1})	
	Bacillus subtilis	***Escherichia coli***
Calligonum polygonoides	50.0±3.5	50.02±4.6
Rosa brunonii	75.0±3.6	75.01±5.7
Peganum harmala	100.0±5.2	100.02±6.8S
Sueda fruticosa	150.0±5.2	150.01±7.0

Table 4. MIC values for different methanolic extracts against *Aspergillus niger* and *Aspergillus flavon.*

Methanolic extracts	MIC (µg mL^{-1})	
	A. niger	***A. flavon***
Calligonum polygonoides	50.0±5.5	50.02±6.8
Rosa brunonii	50.0±4.5	50.01±5.9
Peganum harmala	75.0±4.0	75.01±7.2
Sueda fruticosa	200.0±5.4	200.02±6.7

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