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Antimicrobial potential of two medicinal plants (*Psammogeton biternetum* and *Haloxylon griffithii*) of District Kalat Balochistan, Pakistan

Muhammad Bilal[1], Saeed-Ur-Rehman[1], Saadullah Khan Leghari[1*], Kanval Shaukat[1], Abdul Kabir Khan Achakzai[1], Muhammad Anwar Khan Panezai[3], Aziz-Ud-Din[2], Sana Sadaf[3]

[1]*Department of Botany, University of Balochistan Quetta, Pakistan*

[2]*Department of Microbiology, Federal Urdu University Karachi Pakistan*

[3]*Department of Biochemistry, University of Balochistan Quetta Pakistan*

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Abstract

The main objective of this study was to explore the medicinal potential of two important plant species including; *Haloxylon griffithii* and *Psammogeton biternetum*. The Leaf, stem, root and seed (methanolic and ethanolic) extracts of these plant were examined against some pathogenic microbes (fungal and bacterial strains) through agar well diffusion techniques. For anti-bacterial screening 4 gram positive (*Enterococcus faecalis*, *Staphylococcus aureus, Staphylococcus epidermidis and Bacillus subtilis*) and 14 gram negative (*Acinetobacter baumannii*, *Escherichia coli, Salmonellla typhi, Proteus sp, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Salmonella paratyphi A, Salmonella enterica, Shigella boydii, Shigella sonnei, Klebsiella oxytoca, Klebsiella pneumoniae and Serratia marcescens*) bacterial species were selected. For antifungal activities 5 fungal species (*Candida albicans, Aspergillus niger, Penicillin, Aspergillus flavus and Mucor*) were chosen for examination. Results revealed that *Haloxylon griffithii* leaves was effective against all test species except *Bacillus subtilis*. *Psammogeton biternetum* was not active against *Bacillus subtilis, Acinetobacter baumannii, Proteus mirabilis, Proteus vulgaris, Salmonella paratyphi A, Salmonella enterica, Shigella boydii,* and *Shigella sonnei. Psammogeton biternetum* exhibited highest antimicrobial activity for *Pseudomonas aeruginosa* with 37mm ZI (zone of inhibition). *Haloxylon griffithii* was highly effective against *Proteus* with 31mm ZI. Furthermore, ethanolic extracts were found to be more effective than methanolic extracts. The extracts were least active against fungal strains. Results revealed that *Candida albicans, Aspergillus flavus* and *Mucor* are completely resistant against the selected medicinal plants.

* **Corresponding Author:** Saadullah Khan Leghari ✉ saadbotany@yahoo.com

Introduction

Use of medicinal herbs as ancient medication is one of the regularly used practices in subcontinent. Plants have always been a source of cure to different diseases due to presence of many phytochemical compounds. Drugs that are obtained from herbal sources have a remarkable influence in preventing and curing lethal diseases. People in most developed countries use traditional medicine in health care (Fransworth, 1993). Locally, various plants are used as medicines at the preliminary levels in different regions of the world. Majority of the people are dependent on herbal plants for health care (Bhalodia *et al.*, 2011).

Research by WHO on regular biological drugs like gentamycine showed such medicines are not good enough against the microbes any longer. There are many drugs that were effective once but do not show activity against harmful bacterial strains (Pushpa *et al.*, 2011). A few medications may likewise have symptoms that cause different abnormalities in the body as aside effect. These circumstances desperately compelled researchers for seeking drugs which are affordable, protected and biologically degradable and with least side effects (Kumar *et al.,* 2010).

Traditional medication plays a pivotal role in the medicine of developed nations. Herbal plants have been used for a certain period as solutions for human diseases because these are medicinally important (Suneetha *et al.*, 2013). *Cassia alata* (L.) is an important medicinal plant native to Asian countries, especially India and Pakistan. Distinctive parts of the plant were observed to show medicinal properties such as antimicrobial and analgesic. The treatment of ring worm carried out with the help of leaves of this plant. It is traditionally very useful against skin diseases in man and cattle (Deepak *et al.*, 2014).

Population explosion increased the need of natural resources to meet the need of food, clothes medicine etc. The use of ethnomedicinal knowledge has gained great concerns over the past few years because of its effective and low or no side effects in comparison to allopathic medicine. Besides the plants are used to make various lifesaving drugs. Rahman *et al.,* (2011) reported the use of medicinal plants through studying their medical application via latest methods. Long range of medicinal plant parts are used as raw material for medicines. However, numbers of plants have been explored for antibacterial activity but still some local plants of some areas remain under the shell and remain unexplored for the diverse antimicrobial potential that the local community is blissfully utilizing (Martins *et al.,* 2001). The growing problem of bacterial resistance to the available antibiotics has felt the need to search for new antibacterial agents.

Kalat is one of the big cities of Balochistan. It is located 28° 57' & 29° 20' north side and 66° 35' & 67° 31' east. It covers an area of 6621 sq. km with an elevation of 1525 to 1982 meters above sea level. Kalat is among the coldest cities of Balochistan. The average rainfall is recorded 15.23 mm in winter season and snowfall is also recorded in December and January.

The annual minimum temperature was $-4.34°C$ for the years 1993-1998, which was recorded at the meteorological center Kalat, whereas maximum temperature was $31.71°C$ July (Tareen *et al.*, 2010). Kalat is blessed with diverse medicinal and ornamental plants due to its geographical location. The natives of Kalat basically depend on the use of herbal plants for treatment of various diseases.

Psammogeton consists of 6 species, majority is found in sandy dunes of deserts in different parts of Turkmenistan, Pakistan, Afghanistan and Iran (Nasrabadi *et al.,* 2009). *P. biternatum* Edgew commonly known as “izbotk” in Kalat and “sparki” in other regions. It is an annual herb that ranges from 15 to 22cm in length. It has small flowers that are white or pink in color. It flowers from March to April.

It is an annual plant that is located in several regions of District Kalat. *Psammogeton biternetum* is locally used for the cure of malaria, whooping cough, typhoid

and chest diseases by the people of region (Ikram *et al.,* 2015). *P. biternatum* Edgew seeds are very beneficial for postpartum infections (after delivery) if ground and mixed in water and taken between meals (Bibii *et al.*, 2017).

Haloxylon griffithii belongs to family Chenopodiaceae, it is also known by the name of cat family. It consists of hundred genera and approximately twelve hundred species (Ali & Qaiser., 2001). Some of its types are weed and grow in barren soils. Thirteen species of Haloxylon are located predominantly in the dry regions of the South-West Asia and Arabian deserts and North-African.

The species of this genus are shrubs or sometimes small trees (Ali *et al.,* 2001). Five species of haloxylon are found in Pakistan out of six species found in central Asia (Mhaskar *et al*., 2000). *H. griffithii* is widely distributed in the forests of Balochistan. It is commonly found in the regions of Harboi, Kalat, Hanna Urak, Spinni road and Wali Tangi, Quetta. Hence, the present study was carried out to find out the antibacterial activity of *Haloxylon griffithii* and *Psammogeton biternetum* against some pathogenic microbes.

Material and methods

Procurement of plant samples

The plant samples were collected from the Kalat city and out skirts. These were recognized and authenticated by Dr. R. B. Tareen, Department of Botany, University of Balochistan, and Quetta.

Plant extract preparation

The collected plant parts were chopped into small pieces and shade dried at 32-35°C. About 50g of each plant specimen was powdered with the help of an electric blender. Each sample was extracted with methanol, and ethanol.

After 24 hours, the extracts were filtered via fine filtration with the help of Whatman filter paper. Then the filtrate was concentrated in rotary flask evaporator, until dried (Doughari, 2006).

Test species

Various microorganisms were used for the study that include fungi and number of bacteria. The test organisms were obtained from CASVAB (Center for Advance studies in Vaccinology and Biotechnology), Civil Hospital Quetta, Federal Urdu University Karachi and Microbiology Lab, Department of Microbiology, University of Balochistan, Quetta. All the bacterial strains were grown in nutrient broth and incubated at 37°C for at least two days (Doughari, 2006).

Agar well diffusion methods

Agar well diffusion method was used to explore antimicrobial potential of *Psammogeton biternetum* and *Haloxylon griifthii*. It is a common method used to test the antimicrobial activity of plants extracts.

In this process the surface of agar plate was inoculated by dispersal of volume of the microbial inoculums on the total surface of agar. Then, a hole was punched with a diameter of 6 to 8 mm with a sterilized cork borer and an extract solution of volume (20–100 µL) at chosen concentration was poured into the well. Afterwards, agar plates were incubated under appropriate conditions depending upon the organism under investigation. The anti-microbial agent was diffused in the agar medium and inhibited the growth of the bacteria (Chaman *et al.,* 2013).

Results

The data clearly shows that *Haloxylon griffithii* leaves are comparatively more effective against all test plant species. *Psammogeton biternetum* was not effective against *Bacillus subtilis, Acinetobacter baumannii, Proteus mirabilis, P. vulgaris, Salmonella paratyphi A, S. enterica, Shigella boydii,* and *S. sonnei*. Both plants were found to be ineffective against *Bacillus subtilis*. *Psammogeton biternetum* shows the highest antimicrobial activity against *Pseudomonas aeruginosa* with 37mm ZI (zone of inhibition). *Haloxylon grifthii* was highly effective against *Proteus* with 31mm ZI. However, ethanolic extracts were found to be more effective than methanolic extracts (Table 1).

Table 1. Antibacterial activity of *Psammogeton biternetum* and *Haloxylon griffithii* leaf in methanolic and ethanolic extracts.

	Organisms	Inhibition Zone (mm)			
		Psammogeton biternetum		*Haloxylon griffiti*	
		Ethanol	Methanol	Ethanol	Methanol
	Gram Positive				
1.	*Enterococcus faecalis*	15	4	17	15
2.	*Staphylococcus aureus*	27	12	27	20
3.	*Staphylococcus epidermidis*	15	6	19	11
4.	*Bacillus subtilis*	-	-	-	-
	Gram Negative				
1.	*Acinetobacter baumannii*	-	-	17	14
2.	*Escherichia coli*	27	12	21	16
3.	*Salmonellla typhi*	25	11	21	18
4.	*Proteus sp*	31	25	31	22
5.	*Proteus mirabilis*	-	-	11	10
6.	*Proteus vulgaris*	-	-	17	12
7.	*Pseudomonas aeruginosa*	37	8	19	13
8.	*Salmonella paratyphi A*	-	-	27	19
9.	*Salmonella enterica*	-	-	19	15
10.	*Shigella boydii*	-	-	25	19
11.	*Shigella sonnei*	-	-	17	16
12.	*Klebsiella oxytoca*	15	5	-	-
13.	*Klebsiella pneumoniae*	9	4	16	15
14.	*Serratia marcescens*	13	8	27	21

Considering, the stem extracts of both plants it was observed that the stem extracts were less active against various strains of bacteria. The maximum action was detected in methanolic extracts of *Haloxylon biternetum* against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The extracts showed zero activity against *Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis, Acinetobacter baumannii, Salmonella paratyphi A, S. enterica, Shigella boydii, S. sonnei* and *Proteus mirabilis* (Table 2).

Table 2. Antibacterial activity of *Psammogeton biternetum* and *Haloxylon griffithii* stem in methanolic and ethanolic extracts.

	ORGANISMS	Inhibition Zone (mm)			
		Psammogeton biternetum		*Haloxylon grifthii*	
		Ethanol	Methanol	Ethanol	Methanol
	Gram Positive				
1.	*Enterococcus faecalis*	13	11	-	-
2.	*Staphylococcus aureus*	-	-	12	19
3.	*Staphylococcus epidermidis*	-	-	-	-
4.	*Bacillus subtilis*	-	-	-	-
	Gram Negative				
1.	*Acinetobacter baumannii*	-	-	-	-
2.	*Escherichia coli*	10	8	-	-
3.	*Salmonellla typhi*	5	4	18	15
4.	*Proteus sp*	11	10	17	17
5.	*Proteus mirabilis*	-	-	-	-
6.	*Proteus vulgaris*	4	2	-	-
7.	*Pseudomonas aeruginosa*	8	6	11	19
8.	*Salmonella paratyphy A*	-	-	-	-
9.	*Salmonella enterica*	-	-	-	-
10.	*Shigella boydii*	-	-	-	-
11.	*Shigella sonnei*	-	-		-
12.	*Klebsiella oxytoca*	8	4	-	-
13.	*Klebsiella pneumoniae*	4	-	7	5
14.	*serratia marcescens*	10	4	-	-

The stem extracts of *Psammogeton biternetum* were found to be more effective against test strains as compared to *Haloxylon griffithii* (Table 2).

The seed extracts of *Psammogeton biternetum* showed great deal of activity against most of the test species. Ethanolic extracts were comparatively more effective than the methanolic extracts. Minimum antimicrobial activity was observed against *Acinetobacter baumannii* and highest activity was observed against *Pseudomonas aeruginosa Shigella sonnei, Klebsiella oxytoca, Serratia marcescens* and *Salmonella paratyphi* A showed complete resistance against the extracts of *P. biternetum* seeds.

Table 3. Antibacterial activity of *Psammogeton biternetum* seeds in methanolic and ethanolic extracts.

	Organisms	Inhibition Zone (mm)	
		Ethanol	Methanol
	Gram positive		
1.	*Enterococcus faecalis*	18	11
2.	*Staphylococcus aureus*	12	17
3.	*Staphylococcus epidermidis*	18	15
4.	*Bacillus subtilis*	8	-
	Gram Negative		
1.	*Acinetobacter baumannii*	4	-
2.	*Escherichia coli*	7	12
3.	*Salmonellla typhi*	18	17
4.	*Proteus sp*	26	27
5.	*Proteus mirabilis*	18	17
6.	*Proteus vulgaris*	15	11
7.	*Pseudomonas aeruginosa*	25	27
8.	*Salmonella paratyphi A*	-	-
9.	*Salmonella enterica*	19	27
10.	*Shigella boydii*	17	17
11.	*Shigella sonnei*	-	-
12.	*Klebsiella oxytoca*	-	-
13.	*Klebsiella pneumoniae*	5	11
14.	*Serratia marcescens*	-	-

Concluding, it was observed that the selected plants (*P. biternetum* and *H. griffithii*) are potentially effective against the selected bacterial strains. The seeds of izbotk and leaves of bundi are most effective with maximum zones of inhibition (Table 3). The roots of *H. griffithii* showed zero activity in both ethanolic and methanolic extracts against all test species (Table 4). Both plants were highly effective against *Escherichia coli, Salmonellla typhi* and *Pseudomonas aeruginosa*.

Table 4. Antibacterial activity of *Haloxylon griffithii* root in methanolic and ethanolic extracts.

	Organisms	Inhibition Zone (mm)	
		Ethanol	Methanol
	Gram positive		
1.	*Enterococcus faecalis*	-	-
2.	*Staphylococcus aureus*	-	-
3.	*Staphylococcus epidermidis*	-	-
4.	*Bacillus subtilis*	-	-
	Gram Negative		
1.	*Acinetobacter baumannii*	-	-
2.	*Escherichia coli*	-	-
3.	*Salmonellla typhi*	-	-
4.	*Proteus sp*	-	-
5.	*Proteus mirabilis*	-	-
6.	*Proteus vulgaris*	-	-
7.	*Pseudomonas aeruginosa*	-	-
8.	*Salmonella paratyphi A*	-	-
9.	*Salmonella enterica*	-	-
10.	*Shigella boydii*	-	-
11.	*Shigella sonnei*	-	-
12.	*Klebsiella oxytoca*	-	-
13.	*Klebsiella pneumoniae*	-	-
14.	*Serratia marcescens*	-	-

The results show that the selected plants are least active against selected fungal species. The study carried out experiment on fungal strains i.e. *C. albicans, A. niger, penicillin sp, A. flavus* and *Mucor*. *P. biternetum* showed least activity against tested fungal strains. The seeds extract of *P. biternetum* are only active against *A. niger* and *Penicillin sp* with 3mm and 2mm IZ (Table 5). *H. griffithii* showed activity against *A. niger* and *Penicillin sp*. Maximum activity was shown in the ethanolic extracts of leaf of *H. griffithii* (10mm IZ) (Table 6).

Table 5. Antifungal activity of *Psammogeton biternetum* (stem, leaf and seed) in methanolic and ethanolic extracts.

	Fungal strains	Zone of inhibition(mm)					
		Stem		Leaf		Seed	
		Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol
1.	*Candida albicans*	-	-	-	-	-	-
2.	*Aspergillus niger*	-	-	-	-	3	-
3.	*Penicillin*	-	-	-	-	2	-
4.	*Aspergillus flavus*	-	-	-	-	-	-
5.	*Mucor*	-	-	-	-	-	-

Discussion

From the present research, it was evident that *P. biternetum* and *H. griffithii* possess a promising antibacterial action against the investigated organisms. The results obtained from Agar diffusion assay method supports that gram-negative bacteria are sensitive to the extracts than gram-positive bacteria. Similar observations were observed Cimanga *et al.,* (2002) that *P. aeruginosa* was found to be most sensitive against tested concentrations. Similar results were also reported by Salama *et al.,* (2010). Ethanolic extracts had high antimicrobial potential than methanolic extracts, the difference in antibacterial and antifungal actions for different plant parts was also stated Ajaib *et al.*, (2014).

The results of present research clearly show that the antibacterial and antifungal activity fluctuate with the plants and plant part used. Thus, the study establishes the value of plants to be used in ayurveda, which could be of substantial interest to the growth of new medicinal drugs.

Table 6. Antifungal activity of *Haloxylon griffithii* (stem, leaf and root) in methanolic and ethanolic extracts.

	Fungal strains	Zone of inhibition(mm)					
		Stem		Leaf		Root	
		Ethanol	Methanol	Ethanol	Methanol	Ethanol	Methanol
1.	*Candida albicans*	-	-	-	-	-	-
2.	*Aspergillus niger*	2	-	10	-	-	-
3.	*Penicillin*	3	-	2	-	6	-
4.	*Aspergillus flavus*	-	-	-	-	-	-
5.	*Mucor*	-	-	-	-	-	-

Furthermore, the selected plants show high inhibition zone against *Pseudomonas aeruginosa, Candida albicans and Aspergillus niger* ZI of 13, 14 and 18mm respectively. Chaman *et al. (*2013) reported similar results. The disappearance of bacterial colonies may be due to the presence of chemicals like alkaloids. Ramya *et al.*, (2008) stated several positive outcomes of ethanolic leaf extracts of Sadbahar against many bacteria (gram positive and gram negative) i.e. *E. coli, P. aeruginosa, S. marcescens, S. typhii, S. aureus, S. pyrogens, B. cereus and B. subtilis*. Methanolic extracts of both plants (*P.*

biternetum and *H. griffithii*) were least active against bacterial and fungal strains. However, Ethanolic extracts have shown good antimicrobial activity. From the previous research it has been found that extracts prepared in specific solvents enhances the antimicrobial potential of plant. Thongson *et al.,* (2004) findings strongly support these findings.

The appearance and spread of multidrug-resistant (MDR) bacterial pathogens have significantly threatened the present antibacterial treatment (Boucher *et al.*, 2009). MDR bacterial infections are responsible for increasing mortality, staying longer in hospitals, and increased cost of treatment and attention (Giamarellou, 2010). The addition of traditionally used drugs including phytomedicine, if they prove harmless and operative, into national health care system is recommended by World Health Organization (Marasini *et al.*, 2015). Although many medicinal plants (>500 plants) have been reported to be used by people since ancient times for primary health care. However, there has been a paucity in data regarding their in vitro or in vivo efficacy (Kunwar and Bussmann, 2008).

Various reported literature like Cheruiyot *et al.,* (2009) and Ramzi *et al.,* (2005) were in accordance to our findings, claiming the antimicrobial activities of methanolic plant extracts of *Psidium guajava* and *Boswellia elongate* respectively against test species were least effective. Ajaib *et al.,* (2013) found alike results during the investigation of antioxidant and antimicrobial activities of *Rivina humilis* L.*, Echinochloa colona* (Linn.) *and Sporobolus coromandelianus* (Retz.) Kunth. Thus, it can be concluded that test plant species has great antibacterial potential against diverse bacterial strains that could help the pharmaceutical industries to efficiently use these plants that were remained unexplored yet.

Conclusion

The present study concludes that the selected plants (*P. biternetum* and *H. griffithii*) extracts have prominent effects against propagation of investigated bacterial species but did not show efficient results against fungal strains. Hence, the results exhibit the possibility of using these extracts for the ailment of harmful infections. The study further concludes that the seeds and leaves of *P. biternetum* are better active for microbial activities. Moreover, ethanolic extracts were better than the methanolic extracts and proved to be highly effective against vast range of microbial strains. So, it can be concluded that both plants possess medicinal properties against wide range of bacterial and fungal strains and can be used as better substitute to expensive drugs available in market.

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