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Tariq Mahmood
Department of Microbiology, Faculty of Health Sciences Hazara University, Pakistan

Muhammad Hussain
Department of Microbiology, Faculty of Health Sciences Hazara University, Pakistan

Syed Wajid Ali Shah
Department of Microbiology, Faculty of Health Sciences Hazara University, Pakistan

Najeebullah
Department of Microbiology, Faculty of Health Sciences Hazara University, Pakistan

Mujaddad Ur Rehman
Department of Microbiology, Abbottabad University of Science and Technology Pakistan

Azam hayat
Department of Microbiology, Abbottabad University of Science and Technology Pakistan

Muhammad Ayub Jadoon
Department of Microbiology, Abbottabad University of Science and Technology Pakistan

Sulaiman Bahadur
Department of Microbiology, Abbottabad University of Science and Technology Pakistan

Raheem Bahadar
Department of Pharmacy, COMSATS Institute of Information Technology Abbottabad Pakistan

Muhammad Usman
Department of Pharmacy, COMSATS Institute of Information Technology Abbottabad Pakistan

Hameed Ur Rehman
Department of Chemistry, Kohat University of Science and Technology, KPK, Pakistan

Wasim Ullah
Department of Chemistry, Kohat University of Science and Technology, KPK, Pakistan

Javid Khan
Department of Microbiology, Abbottabad University of Science and Technology Pakistan

Correspondence
Javid Khan
Department of Microbiology, Abbottabad University of Science and Technology Pakistan

Comparative anti-microbial study of different parts of *Opuntia littoralis* against specific human pathogens

Tariq Mahmood, Muhammad Hussain, Syed Wajid Ali Shah, Najeebullah, Mujaddad Ur Rehman, Azam hayat, Muhammad Ayub Jadoon, Sulaiman Bahadur, Raheem Bahadar, Muhammad Usman, Hameed Ur Rehman, Wasim Ullah and Javid Khan

Abstract
An emergency need was felt to discover and search for new antibiotics, because of emergence of multi drug resistant strains of bacteria and appearance of species with reduced susceptibility to already existing antibiotics. The presence of microorganisms has been exposed by human beings since immemorial time of human history and the thought about medicinal herbs and shrubs that they have the ability of healing potential has been proved in many research works even today. By using water, ethyl alcohol, acetone and ethyl acetate, four types of extracts were prepared from *Opuntia littoralis* extract. All the extracts were tested against ten human pathogens. Agar disc diffusion method was applied for antibacterial activity. It was observed that extracts of fruits showed high activity than the extracts of leaves. The highest activity was observed for *Ervinia carotovora* (20-28 mm), A. tumefaciens (16-23 mm) for fruit sample in acetone extract followed by A. tumefaciens (20-25 mm in each conc) of both sample in ethyl acetate extracts. The *Opuntia* species proved high antimicrobial activities. Comparatively, the zone of inhibition was high for fruit sample than stem.

Keywords: *Opuntia littoralis,* antimicrobial activity, disc diffusion method, controls

Introduction
Opuntia littoralis is also known as coastal prickly cactus. This plant is mostly found in sandy soil. *Opuntia littoralis* grow as irregular clusters or shrub like mounds the size of which may reach to 10 feet tall. The leaves of these plants are modified stems. These stems are jointed, fleshy and flattened. The stems are known as cladodes usually called as pads. The stems have special types of axillary or lateral buds which are called as areoles. The areoles are unevenly dispersed over the stems. The areoles have the ability to produce hairs and spines. The spines are of two types namely large spines which are fixed and small hair like spines which is also called as glochids. The glochids are radially detached. The flowers of the *Oppuntia littoralis* are radially symmetric and yellow in color. After flowering it gives a fruit which has a shape like a pear**.** The size of the fruit may reach to 2 to 3 inches long. The color of the fruit is red to purple when fully matured in late summer. Large numbers of bony and hard seeds are present in each fruit [1-3].

The people use *Opuntia littoralis* and other species of *opuntia* from immemorial time of human history [4]. Besides being consumed as food or beverages, most portions of the plants have been used as medicine and in modern times have also been commercially prepared as capsules, drinks, pills or powders. Various preparations are prepared from *Opuntia littoralis* and other species of *opuntia* which are used in diabetes by diabetic patients. These preparations have also anti-inflammatory and analgesic properties. Antiviral and anti oxidants activities has also shown by these preparations. These preparations have also the ability to control weight and sugar level of the blood. These preparations are used by the pregnant women which makes the delivery easy. These are also used in the treatment of asthma and other respiratory disorders [5-7]. These have also shown a useful activity in diarrhea, dysentery, dyspnea, gastritis, colitis and other disorders of alimentary canal. High cholesterol level can be controlled by these preparations. Measles, gonorrhea, syphilis, inflammation of the eyes, snake bite, sore throat, and inflammation of the vigina can also be treated by using the preparations of this plant. Hot preparations of *Opuntia littoralis* and other species of *opuntia* are kept on the

breasts of the women to increase the flow of the milk. These hot preparations can also be used in skin disorders such as erythema and as well as in the treatment of rheumatic disease. The pulp obtained from the leaves of the plant has been used in many countries as a bandage to cover their burns, wounds cuts and fractures and are believed to kill the pain and promote healing [8]. Decoctions obtained from the fruits of the plant can be used as purgatives [9]. The cladodes are helpful as infective agents and can also used in the treatment of whooping cough and ulcer [10, 11]. Preparations of the fruits are used in the disorders of urinary system especially in women [12, 13]. Although preparations of the fruits are consider non toxic but it can cause adverse effects like mild diarrhea, nausea, increased stool volume, increased stool frequency, abdominal fullness, and headache [14]. The purpose of the study was to evaluate comparatively the antimicrobial activity of the of different of parts *Opuntia littoralis.*

Materials and Methods

The present research work was conducted from January 2016 to June 2016 on Comparative antimicrobial study of different parts of *Opuntia littoralis* against specific microbes was performed in Pakistan Council of Scientific and Industrial research (PCSIR), laboratories complex, Peshawar, Pakistan.

Collection of Plant

The plant was collected from the local area of District Mardan, named Rustam KPK in the month of January to June 2016. The stem and fruit were collected only.

Processing of plant

Oppuntia littoralis leaves, stem, fruit samples was rinsed with tap water to remove soil, dust, mud and other undesired materials and then kept under shadow for drying. The plant samples were crushed and grounded to powder form. After grinding, 1200 gm of powder was taken from each sample and divided into four equal parts. Four types of extracts were made of each sample. They were water based, ethyl alcohol based, acetone based and ethyl acetate based. Each sample was soaked in conical flask by adding 500 ml corresponding solvents. To facilitate the extraction, all the flasks were warmed and shacked. All the flasks were covered with stoppers. For 24 hours, all the flasks were kept at room temperature. All the soaked samples was filtered by whattman filter paper. The process was repeated two times. Through vacuum rotary evaporator at specified conditions, different viscous crude extracts were obtained. These were weighed for further processing. For antimicrobial activity, every sample crude extract in 0.5 mg concentration was dissolved in 3 ml of dimethyl sulphoxide.

Disc Diffusion method

The antibacterial activity of the *Opuntia littoralis* was determined using agar disc diffusion method. In disk diffusion method first of all the agar surface was streaked with a soft sterile swab for, *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Agrobacterium tumificious, Bacillus atrophous, salmonella typhi, Erwinia carotovora Staphylococcus aureus, Klebsiella Pneumoniae* and *Candida albicans*. The prepared culture was swabbed uniformly throughout the agar surface. Then the inoculums were left to dry for at least five minutes after the streaking has been completed. Then a double sterile disk was placed on the top and a single sterile disc was placed on the lower side of the Petri dishes which were labeled precisely. After the completion of placing disks on the agar plates 6 micro litter of extract was poured on the single dick and 12 micro litters was placed on the double disk with the help of sterile micropipette. DMSO was used for negative control. Levofloxacin was used for Gram negative and Azithromycine for Gram positive bacteria as a positive control, while Clotrimazole and Fluconazole for Candida albican. The process was done thrice. After 24 hour zone of inhibitions were checked by measuring the zone of inhibition with the help of calibrated scale.

Results

Aqueous extract of stems of the *Opuntia littoralis*

Table 1 shows that aqueous extract of stems have limited activity against the prescribed microorganisms. The highest zone of inhibition is against *Klesiebella Pneumoniae* followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The zone of inhibition against *Klesiebella Pneumoniae* is 18mm and 21mm in and 12 µl concentration respectively.

Table 1: Zone of inhibition of the aqueous extract of stems of the *Opuntia littoralis*

Extracts	Microbial species	Zone of inhibition of the extracts in mili meter in various conc			
		6 µl	12 µl	Positive control	Negative control
Aqueous extract of leaves	*Escherichia coli*	NA	NA	31mm	NA
	Pseudomonas aeruginosa	10mm	8mm	31mm	NA
	Salmonella typhi	NA	NA	36mm	NA
	Klesiebella Pneumoniae	18mm	21mm	36mm	NA
	Erwinia carotovora	NA	NA	27mm	NA
	Staphylococcus aureus	10mm	20mm	32mm	NA
	Bacillus subtilis	NA	NA	34mm	NA
	Bacillus atrophous	NA	NA	31mm	NA
	A tumificious	NA	NA	32mm	NA
	Candida albicans	NA	NA	31mm	NA

Zone of inhibition against *Staphylococcus aureus* is 10mm and 20mm and against *Pseudomonas aeruginosa* are 8mm and 10 mm in 6 µl and 12 µl concentrations. According to the table aqueous extract of stems show no activity against *Escherichia coli, Bacillus subtilis, Bacillus atrophous, Salmonella typhi, Erwinia carotovora, a tumificious* and *Candida albicans*.

Aqueous extract of fruits of the *Opuntia littoralis*

Table 2 shows that aqueous extract of fruits has maximum zone of inhibition against *Erwinia carotovora* followed by *Staphylococcus aureus, Candida albicans* and *a tumificious*. The zone of inhibition against *Erwinia carotovora* is 18mm and 28mm, against *Staphylococcus aureus* is 21mm and 22mm, against Candida albicans is 18mm and 21mm, and against *a tumificious* is 14mm and 19mm while no activity against *Escherichia coli*.

Table 2: Zone of inhibition of the aqueous extract of fruits of the *Opuntia littoralis*

Extracts	Microbial species	Zone of inhibition of the extracts in mili meter in various conc.			
		6µl	12µl	Positive control	Negative control
Aqueous extract of fruits	*Escherichia coli*	NA	NA	31mm	NA
	Pseudomonas aeruginosa	10mm	8mm	31mm	NA
	Salmonella typhi	12mm	17mm	36mm	NA
	Klesiebella Pneumoniae	15mm	10mm	36mm	NA
	Erwinia carotovora	18mm	30mm	27mm	NA
	Staphylococcus aureus	21mm	22mm	32mm	NA
	Bacillus subtilis	12mm	10mm	34mm	NA
	Bacillus atrophous	12mm	10mm	31mm	NA
	A. tumificious	14mm	19mm	32mm	NA
	Candida albicans	18mm	21mm	31mm	NA

Ethanolic Extract of stems of the *Opuntia littoralis*

Table 3 shows that Ethanolic extract of stems have maximum zone of inhibition against *Escherichia coli* which is 16 mm and 19 mm followed by *Staphylococcus aureus* having zone of inhibition of 14mm and 17mm in 6 µl and 12 µl concentrations. According to the table, it shows no activity against *Salmonella typhi, Klesiebella Pneumoniae, Erwinia carotovora* and *Candida albicans*.

Table 3: Zone of inhibition of Ethanolic Extract of stems of the *Opuntia littoralis*

Extract	Microbial species	Zone of inhibition of extract in mm in various concentrations			
		6 µl	12 µl	Positive control	Negative control
Ethanolic Extract of Stem	*Escherichia coli*	16mm	19mm	31mm	NA
	Pseudomonas aeruginosa	13mm	15mm	31mm	NA
	Salmonella typhi	NA	NA	36mm	NA
	Klesiebella Pneumoniae	NA	NA	36mm	NA
	Erwinia carotovora	NA	NA	27mm	NA
	Staphylococcus aureus	14mm	17mm	32mm	NA
	Bacillus subtilis	10mm	12mm	34mm	NA
	Bacillus atrophous	10mm	12mm	31mm	NA
	A. tumificious	10mm	12mm	32mm	NA
	Candida albicans	NA	NA	31mm	NA

Ethanolic extract of fruits of the *Opuntia littoralis*

Table 4 shows that Ethanolic extract of fruits has maximum zone of inhibition against *Erwinia carotovora* followed by *Staphylococcus aureus* and *A. tumificious*. The zone of inhibition against *Erwinia carotovora* is 18mm and 29mm, against *Staphylococcus aureus* is 21mm and 23mm and against *a tumificious* is 14mm and 20mm. It has no activity against *Escherichia coli*.

Table 4: Zone of inhibition of Ethanolic Extract of fruits of the *Opuntia littoralis*

Extract	Microbial species	Zone of inhibition of extract in mm in various concentration			
		6 µl	12 µl	Positive control	Negative control
Ethanolic Extract of fruits	*Escherichia coli*	NA	NA	31mm	NA
	Pseudomonas aeruginosa	12mm	10mm	31mm	NA
	Salmonella typhi	14mm	18mm	36mm	NA
	Klesiebella Pneumoniae	12mm	10mm	36mm	NA
	Erwinia carotovora	18mm	30mm	27mm	NA
	Staphylococcus aureus	21mm	23mm	32mm	NA
	Bacillus subtilis	15mm	10mm	34mm	NA
	Bacillus atrophous	8mm	10mm	31mm	NA
	A. tumificious	14mm	20mm	32mm	NA
	Candida albican	12mm	10mm	31mm	NA

Ethyl acetate based extract of stems of the *Opuntia littoralis*

Table 5 shows that ethyl acetate based extract of stems show maximum zone of inhibition against *Escherichia coli* and *a tumificious* followed by *Erwinia carotovora* and *Staphylococcus aureus.* The zone of inhibition against *Escherichia coli* is 25mm and 20mm and *a tumificious* is 20mm and 25mm in 6 µl and 12 µl concentrations respectively while zone of inhibition against *Erwinia carotovora* is 18mm and 15mm and against *Staphylococcus aureus* is 16mm and 14mm in 6 µl and 12 µl concentrations. It has no activity against *Pseudomonas aeruginosa*, *Bacillus atrophous* and *Candida albicans*.

Table 5: Zone of inhibition of Ethyl acetate based Extract of stems of the *Opuntia littoralis*

Extract	Microbial species	Zone of inhibition of extract in mm in various concentrations			
		6µl	12µl	Positive control	Negative control
Ethyl acetate based Extract of Stem	*Escherichia coli*	25mm	20mm	31mm	NA
	Pseudomonas aeruginosa	NA	NA	31mm	NA
	Salmonella typhi	11mm	15mm	36mm	NA
	Klesiebella Pneumoniae	10mm	8mm	36mm	NA
	Erwinia carotovora	18mm	15mm	27mm	NA
	Staphylococcus aureus	16mm	14mm	32mm	NA
	Bacillus subtilis	8mm	10mm	34mm	NA
	Bacillus atrophous	NA	NA	31mm	NA
	A. tumificious	20mm	25mm	32mm	NA
	Candida albicans	NA	NA	31mm	NA

Ethyl acetate based Extract of fruits of the *Opuntia littoralis*

Table 6 shows that ethyl acetate based extract of fruits has maximum zone of inhibition against *a tumificious* followed by *Erwinia carotovora, Salmonella typhi* and *Klesiebella Pneumoniae*. Zone of inhibition against *a tumificious* is 20mm and 25mm, against *Erwinia carotovora* is 17mm and 25mm, against *Salmonella typhi* is and *Klesiebella Pneumoniae* is 12mm and 14mm in 6 µl and 12 µl concentrations respectively. It shows no activity against *Bacillus subtillus*.

Table 6: Zone of inhibition of ethyl acetate based extract of fruits of the *Opuntia littoralis*

Extract	Microbial species	Zone of inhibition of extract in mm in various concentration			
		6 µl	12 µl	Positive control	Negative control
Ethyl acetate based Extract of fruits	*Escherichia coli*	10mm	11mm	31mm	NA
	Pseudomonas aeruginosa	10mm	12mm	31mm	NA
	Salmonella typhi	12mm	14mm	36mm	NA
	Klesiebella Pneumoniae	12mm	14mm	36mm	NA
	Erwinia carotovora	17mm	25mm	27mm	NA
	Staphylococcus aureus	15mm	20mm	32mm	NA
	Bacillus subtilis	NA	NA	34mm	NA
	Bacillus atrophous	10mm	8mm	31mm	NA
	A tumificious	20mm	25mm	32mm	NA
	Candida albicans	10mm	12mm	31mm	NA

Acetone based extract of fruits of the *Opuntia littoralis*

Table 7 shows that acetone based extract of the fruit show activity against almost all microorganisms except *Bacillus subtilis*. The highest activity was observed against *Erwinia carotovora* followed by *A. tumificious* and *Staphylococcus aureus*. The zone of inhibition against *Erwinia carotovora* is 20mm and 28mm in 6 µl and 12 µl concentrations respectively while the zone of inhibition against *A. tumificious* is 16mm and 23mm in 6 µl and 12 µl concentrations respectively. The zone of inhibition against *Staphylococcus aureus* is 16mm and 19mm in 6 µl and12 µl concentrations respectively

Table 7: Zone of inhibition of acetone based extract of the fruit of the *Opuntia littoralis*

Extracts	Microbial species	Zone of inhibition of the extracts in mili meter in various concentration			
		6µl	12µl	Positive control	Negative control
Acetone based extract of fruits	*Escherichia coli*	13mm	13mm	31mm	NA
	Pseudomonas aeruginosa	15mm	18mm	31mm	NA
	Salmonella typhi	13mm	16mm	36mm	NA
	Klebisila Pneumoniae	1omm	12mm	36mm	NA
	Erwinia carotovora	20mm	28mm	27mm	NA
	Staphylococcus aureus	16mm	19mm	32mm	NA
	Bacillus subtilis	NA	NA	34mm	NA
	Bacillus atrophous	15mm	10mm	31mm	NA
	A. tumificious	16mm	23mm	32mm	NA
	Candida albicans	8mm	10mm	31mm	NA

Acetone based extract of stems of the *Opuntia littoralis*

Table 8 shows that acetone based extract of stems has maximum zone of inhibition against *Escherichia coli* followed by *Staphylococcus aureus* and *A. tumificious*. The zone of inhibition against *Escherichia coli* is 20mm and 14mm, against *Staphylococcus aureus* is14mm and 16mm and against *A. tumificious* is 15mm and 12mm in 6 µl and 12 µl concentrations respectively.. Table also shows that acetone based extract of stems has no activity against *Pseudomonas aeruginosa, Bacillus atrophous* and *Candida albicans*.

Table 8: Zone of inhibition of acetone based extract of stems of the *Opuntia littoralis*

Extracts	Microbial species	Zone of inhibition of the extracts in mili meter in various conc			
		6 µl	12 µl	Positive control	Negative control
Acetone based extract of leaves	*Escherichia coli*	20mm	14mm	31mm	NA
	Pseudomonas aeruginosa	NA	NA	31mm	NA
	Salmonella typhi	7mm	8mm	36mm	NA
	Klesiebella Pneumoniae	8mm	10mm	36mm	NA
	Erwinia carotovora	1omm	8mm	27mm	NA
	Staphylococcus aureus	14mm	16mm	32mm	NA
	Bacillus subtilis	10mm	8mm	34mm	NA
	Bacillus atrophous	NA	NA	31mm	NA
	A. tumificious	15mm	12mm	32mm	NA
	Candida albicans	NA	NA	31mm	NA

Discussion

The antimicrobial impact of the medicinal plants is well known, proved and properly documented since the very beginning of human's history [15]. The plant extract of *Oppuntia littoralis* was tested for their possible antimicrobial activities and an attention-grabbing antimicrobial profile has been observed against the test micro organisms includes *Escherichia coli, Salmonella typhi, Klesiebella Pneumoniae, Erwinia carotovora, Staphylococcus aureus, Bacillus subtilis, bacillus atrophous, Pseudomonas aeruginosa*, and *Candida albicans* which is a fungus.. All extracts showed the zones of inhibition. It was observed that the highest activity was against *Erwinia carotovora* in Ethanolic and aqueous extracts of fruit which was 30mm in 12 µl concentration. It was also observed that extracts of fruits showed high activity than the extracts of stems. It was also noted that ethyl acetate and acetone based extracts showed higher activity than the other extracts. Ethyl acetate and acetone based extracts showed activity against almost all the tested microorganisms. In ethyl acetate based extract of stems the highest activity was observed against *Escherichia coli (25 mm and 20 mm),A. tumificious(20 mm and 25 mm)* and the lowest activity was observed against *Klesiebella Pneumoniae (10 mm and 8 mm)* and *Bacillus subtillus* (8mm and 10mm) in 6 and 12ul respectively. The zone of inhibition against *A tumificious* was 20 mm and 25 mm and *Erwinia carotovora* was 17 mm and 25 mm using 6 and 12 µl concentration of the ethyl acetate extract respectively. Acetone extract of stem showed high zone of inhibition against *Escherichia coli* (20 mm and 14 mm) followed by *Staphylococcus aureus* (14 mm and 16 mm) using 6 µl and 12 µl extracts respectively while the minimum zone was against *Salmonella typhi* which was 7 mm and 8 mm in respected concentration and has no activity against *Bacillus atrophous, Pseudomonas aeruginosa* and *Candida albicans*. The fruit extract in acetone showed much more activity than stems extract in acetone. The highest activity was against *Erwinia carotovora* (20 mm and 28 mm) using 6 µl and 12 µl concentration of the extract respectively. The water based extract of stems showed no activity against most of the bacteria and *Candida albicans.* However the extract of fruit in water showed activity almost against all bacteria except *E coli*. The highest zone of inhibition was against *Erwinia carotovora* (18 mm and 28 mm) followed by *Staphylococcus aureus (21mm and 22 mm) in 6 and 12*µl concentration. The extract of stems in ethanol showed highest activity against *E coli* which was 16 mm and 19 mm followed by *Pseudomonas aeruginosa* which was 13 mm and 15 mm using 6 µl and 12 µl concentration respectively. Eethanolic extract of fruits showed activity against almost all bacteria except E coli. The maximum activity was against *Erwinia carotovora* (18 mm and 29 mm) using 6µl and 12µlconcentration respectively. The minimum activity was against *Bacillus atrophous* which was 8 mm and 10 mm.

Conclusion

This research proves that *Opuntia littoralis* have antimicrobial prospective by hindering the growth of harmful microbial strains. The bacterial strains showed inhibition against the different extracts with varying diameter of zones of inhibition including Gram $^{+ve}$ strains, Gram $^{-ve}$ strains and fungus (*Candida albicans)*.

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