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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2022 19(3): 1490-1497. **OPEN ACCESS**

Phytochemical analyses and antimicrobial activities of different solvent extracted samples of *cactus grandiflorus l*. (vanilla cactus)

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Plants derived bioactive compounds are gaining popularity for combatting infections caused by variety of microorganisms because of their efficiency and minimal side effects. In the current study, plant extract from *cactus grandifloras L* was used to analyze the composition of bioactive compounds in five different solvents (Aqueous, Methanolic, Butanol, n-Hexane, and Ethyl Acetate). Phytochemical analyses revealed higher concentration of bioactive compounds in Ethyl Acetate and Methanolic extracts as compared to other extracts. Moreover, the antimicrobial potential of the plant extract was also analyzed against various bacterial pathogenic strains (*Escherichia coli, Bacillus subtilis, Xanthomonas campestris, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumonia*) and fungal strain i.e *Candida albicans*. All the pathogens showed susceptibility to all the solvent extracted of *C. grandifloras* L. Overall aqueous (water) extract exhibited least antimicrobial activity as compared to other extracts. An increase in antimicrobial and antifungal activity was observed by increasing concentration from 1-3 mg/disc for bacterial pathogens and 0.5-1.5 mg/disc for fungal pathogen. Overall, 3 mg/disc and 1.5 mg/disc showed maximum antimicrobial activities. For *E. coli* maximum Zone of Inhibition (ZI) i.e 48.2% was shown by Butanol. In case of *B. Subtilis,* n-Hexane showed maximum activity i.e 61.12% ZI. For *X. campestris* and *S. aureus* maximum ZI i.e 45.15 and 38.7% was shown by Ethyl Acetate and Methanol respectively. Ethyl Acetate and Methanol were proven to be the best solvent extracts against *P. aeruginosa and K. pneumonia* both showing maximum ZI of 39.37 and 39.21% respectively. Best antifungal activity against *C. albicans* was shown by n-Hexane i.e 94% ZI at 1.5 mg/disc

Keywords: *Cactus grandiflorus L*., Antimicrobial activity, Phytochemicals, *Staphylococcus aureus*

INTRODUCTION

Recent advancement in medical field particularly drug discovery shows great progress with regard to resistance against microbial infections. However, microorganisms, through their evolutionary and genetic mechanisms, have developed counter resistance against the antibiotics used. These evolutionary adaptations of the pathogens offer challenges to control these infectious pathogens. The bacterial strains such as *Pseudomonas aeruginosa* (Multidrug-resistant), *Staphylococcus aureus* (Methicillin-resistant), and *Enterococcus* (Vancomycin-resistant) have acquired multi drugs resistance. To counter these infectious entities, different systematic chemical drugs such as synthetically made antibiotics, have been used. In addition, plants extract either in pure form or as crude extracts have been utilized for medicinal purposes (Parekh and Chanda, 2007). These extracts have different bioactive compounds that helps counter pathogenic agents and thus can be used for future drug development

A large number of medicinal plants with bioactive compounds inhibiting pathogens growth have been reported reference. These medicinal plants produce secondary metabolites to counter biotic and abiotic stresses (reference). The roots, stems, leaves, and barks of the plants are rich in bioactive compounds that counter the growth of pathogenic microbes (bakht et al. 2014). The concentrations of these bioactive compounds may different in different part i.e. antimicrobial activity of different plant extracts obtained have been reported against *S. aureus* and P. *aeruginosa* (Santos Filho et al. 1990). These secondary metabolites include terpenoids, alkaloids and phenolic compounds etc. which are reported to possesses various important phytotherapy activities i.e., anti-inflammatory, anti-cancer, anti-microbial, and anti-allergic (Mahato SB, Sen S 1997).

Cactus grandifloras L. belongs to cactus family *Seleniferous grandifloras* L., is a green climber herb. This plant is native to West Indies and Jamaica and is

cultivated worldwide as an ornamental plant. It is commonly called as night blooming cereus, Queen of the night, Sweet-scented cactus, Vanilla cactus and Torch thistle (Haque et al.2015). Its green stem and flowers are referred as the major source of bioactive compounds (Burt WM. H.,2003). It has been used for centuries to cure fever and difficulty in breathing in Jamaica (Burt WM. H 2003) and (Jones A O 1890). The current study was aimed at studying the phytochemical composition of the cactus grandifloras L and its potential as antimicrobial agent.

MATERIALS AND METHODS

This study was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan during 2017. The plant samples were collected from plant herbarium Mardan. After collection and identification, plants were washed for removal of adhered soil particles and left for drying in shaded area at room temperature for 7 days.

Crude Extract Preparation

After drying, the plant was grinded into a fine powder using electric grinder. The powder was transferred to a flask following the addition of methanol. The flask containing the solution was left on shaker for a week. After shaking filtration was performed with Grade 1 (Whatman) filter paper. Desiccation was performed under minimized pressure via rotary evaporator. The whole process was repeated thrice to obtain maximum extract.

Crude Extacts Fractionation

Crude extract was divided into two portions. One portion was stored for antimicrobial activity of crude extract while the other one was subjected to fractionation with different solvents. The crude extract was dissolved in distill water and poured in separating funnel with the addition of n-hexane followed by vigorous shaking. Shaking results in two distinct layers, the upper layer of n-hexane and lower layer of water (n-hexane lighter than water). Compounds soluble in upper layer were collected. The process was repeated three times to extract maximum levels of derived compounds in n-hexane. All fractions of n-hexane were combined and poured into round bottom flask in rotary evaporator and n-hexane was isolated from the fraction leaving behind semisolid n-hexane fraction. This fraction was dried in China dish by water bath at about 46°C and stored for future use. The whole process was repeated for butanol and ethyl acetate fractions. At last lower aqueous phase was dried via rotary and water bath.

Culture Media

Nutrient broth was used for shaking incubation and standardization of different microorganisms while nutrient agar media was used for culturing and growth of microbes. Media was prepared as described by Bakht *et al*. (2011).

Microorganisms Used

Antimicrobial activity of 5 solvents extracted samples were evaluated against different bacterial and fungal strains (Table 1). All the microbial stored cultures were freshened by inoculation of sterile loops on nutrient agar media plates in a laminar flow hood and incubated at 37°C for 24 h. Day after, the streaked cultures were refreshed on media plates and incubated at 37°C for 24 h. The second streaked cultures were then inoculated into the nutrient broth in flasks and subjected to shaking incubation for 18 h at 37°C (200 rpm).

Table 1: List of bacterial strains used in the present study

Microbial species/strains	Strain Type (Gram)	Microbial strain Sources
Escherichia coli	Negative	ATCC # 25922
Bacillus subtilis	Positive	Dept. of Microbiology, Quaid-I-Azam University Islamabad, Pakistan
Xanthomonas campestris	Negative	ATCC # 33913
Staphylococcus aureus	Negative	ATCC # 9721
Pseudomonas aeruginosa		
Klebsiella pneumonia	Negative	ATCC # 10231. Dept. of Plant Pathology, Uni. of Agric. Peshawar, Pakistan
Candida albicans	Fungal strain	ATCC # 10231. Dept. of Plant Pathology, Uni. of Agric. Peshawar, Pakistan

Antibacterial Bioassay

For antimicrobial bioassay, Nutrient agar media plates were inoculated with microbial cultures for 18-24 h (a standardized inoculum 1-2 10^7 CFU ml-1 0.5 McFarland Standard). Whatman No. 1 filter paper disc of in size 6mm were placed on petri plates by sterile forceps. The plant extract was applied in the volume of 6, 12 and 18 µl in concentration of 1mg, 2mg and 3mg respectively. After inoculation the plates were incubated at 37°C for 18-24 h. The zone of inhibitions (ZI) was recorded in mm around the disc.

Positive and Negative Control

Antibiotics i.e. Ciprofloxacin (6 µl $disc^{-1}$) and DMSO (6 µl $disc^{-1}$) were used as positive and negative control on disc. For fungi, fluconazole (50 µg µl 6mm $disc^{-1}$) were used.

Phytochemical analyses

The aqueous crude extract was used for screening of bioactive compounds like secondary metabolites (saponins tannins flavonoids alkaloids and terpenoids).

Test for saponins

About 0.5 g of crude extract was dissolved in 5 mL distilled water and filtered. Then 0.01 g of NaHCO3 was added and left for shaking. The presence of persistent foam indicates saponins.

Test for tannins

About 0.5 g of extract was taken in 1 mL distilled water, then 1-2 drops of 5% ferric chloride was added to solution and observed blue or greenish black color which indicates the presence of tannins.

Test for flavonoids

Dilute NaOH was added to about 0.2 g of crude extract and few drops of diluted HCl was added. As a result, colorless solution formed showed the presence of flavonoids.

Test for alkaloids

The extract 0.05 g in weight was mixed with 10 mL of diluted HCl. Then filter it, few drops of Mayer's reagent were added to the solution. White or creamy precipitates indicate alkaloids.

Test for terpenoids

In 10 mL of methanol, 0.8 g of extract was dissolved and then filter. 1 mL of chloroform and 2 mL of H_2SO_4 was added. The formation of reddish-brown color shows terpenoids.

Data analysis

The data is presented in triplicate. Standard deviation and means were calculated using Microsoft excel (2010).

RESULTS

Plants encounter biotic and abiotic stress via the production of several important phytochemicals including saponins, tannins, alkaloids, flavonoids and terpenoids. The presence of these chemical is believed to have roles in the combat against infectious pathogens. Phytochemicals analysis in the various fractions of *Cactus grandifloras* L. given in Tables 2. Phytochemicals concentrations of were assessed on the basis of color change by the addition of chemical required for detection. The color intensity showed the concentration of the phytochemical. Low, moderate and high concentrations were denoted by symbols as +, ++, and +++, respectively. According to the data in Table 2 n-butanol and ethyl acetate fractions from the leaf's samples showed high concentrations of these compounds. These fractions were also found with high inhibition activities against all bacterial strains. Therefore, this data may reveal that the high activities of these fractions might be due to the presence of comparatively higher phytochemicals concentrations. However, the concentrations of these phytochemicals increased in the same fractions from the stem samples (Table 2). These results confirm that the stem samples of *C. grandifloras* L. are more effective than those of the leaves samples to counteract the growth of bacterial strains. Also, the aqueous fraction contained no or very low concentrations of the phytochemicals.

Table 2: List of Phyto-chemical constituents of *Cactus grandifloras* L.

Solvents	Saponins	Tannins	Alkaloids	Flavonoids	Terpenoids
Water	+	_	_	+	_
Methanol	++	+++	+++	++	++
Butanol	+	+++	+	+	++
N-Hexane	++	+	++	+	++
Ethyl-Acetate	++	++	++	++	+++

Legend: += *Low concentration,* ++= *Moderate concentration,* +++= *High Concentration,* - = *Absent*

Antimicrobial Assay

Plant extract of *cactus grandifloras L* of aqueous, Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *Escherichia coli* is shown in figure 1. Data revealed that *E. coli* showed susceptibility to all the extracts. Highest ZI was shown by Butanol i.e 29.11, 40.29 and 48.82% followed by Methanol with ZI of 27.94, 37.64 and 45.29 at all three (1, 2 and 3 mg/disc) concentrations, while least ZI was shown by aqueous (water) at all three (1, 2 and 3 mg/disc) concentrations, respectively. The effect of different solvent extracted sample have direct relation with dosage concentration and zone of inhibition increase dosages increase the zone of inhibition and vice versa.

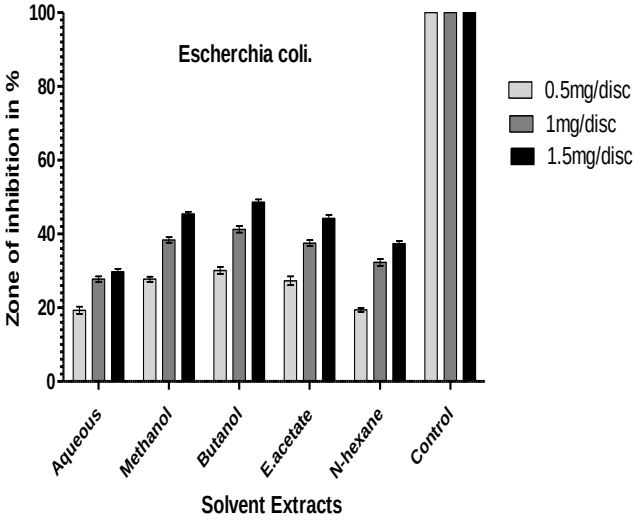

Figure 1: Antibacterial activity of aqueous, Ethyl Acetate, methanol, and butanol extracts of *cactus grandifloras L* against *Escherichia coli.*

Plant extract of *cactus grandifloras L* of aqueous, Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *Escherichia coli* is shown in figure 2. Data revealed that Incase *B. subtilis,* n-Hexane has proven to be the best extract showing maximum ZI i.e 24.41, 37.70 and 61.12% at the all three (1, 2 and 3 mg/disc) concentrations. Following n-Hexane, Methanolic showed 23.23, 35.00 and 40.00% ZI at all three (1, 2 and 3 mg/disc) concentrations, respectively. While least ZI was shown by aqueous (water) at all three (1, 2 and 3 mg/disc) concentrations, respectively. The effect of different solvent extracted sample have direct relation with dosage concentration and zone of inhibition increase dosages increase the zone of inhibition and vice versa.

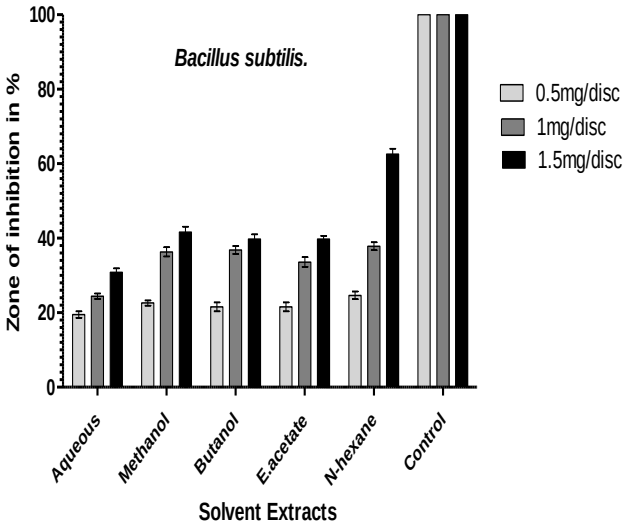

Figure 2:Antibacterial activities of aqueous, Ethyl Acetate, methanol, and butanol extracts of *cactus grandifloras L* against *B. subtilis*

Plant extract of *cactus grandifloras L* of aqueous, Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *X. campestris* is shown in figure 3. Data revealed that Incase *X. campestris* of Ethyl Acetate showed highest ZI of 27.63 37.00 and 45.15% followed Methanol with ZI of 23.90, 36.72 and 44.80% at all the three (1, 2 and 3 mg/disc) concentrations, respectively. Aqueous (water) showed minimum ZI at all three (1, 2 and 3 mg/disc) concentrations, respectively The effect of different solvent extracted sample have direct relation with dosage concentration and zone of inhibition increase dosages increase the zone of inhibition and vice versa.

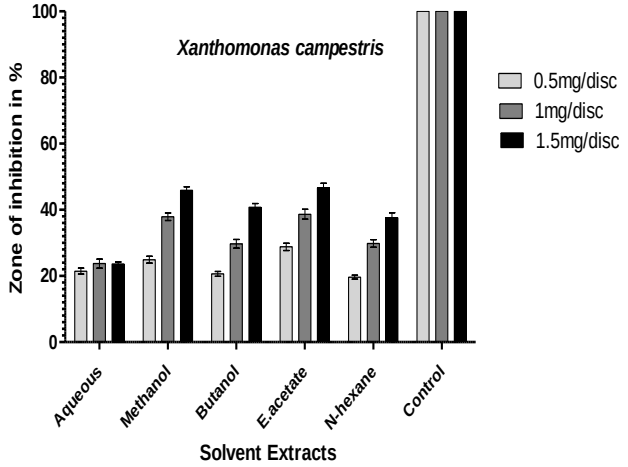

Figure 3: Antibacterial activities of aqueous, E. Acetate, methanol, and butanol extracts of *cactus grandifloras L* against X. *campestris*

Plant extract of *cactus grandifloras L* of aqueous, Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *S. aureus* is shown in figure 4. Data revealed for *S. aureus*, maximum ZI 21.30, 34.88 and 38.27% was shown by methanol followed by n-Hexane with ZI of 21.30, 30.55 and 37.97% at all the three (1, 2 and 3 mg/disc) concentrations, respectively. Aqueous (water) showed minimum ZI at all three (1, 2 and 3 mg/disc) concentrations, respectively. The effect of different solvent extracted sample have direct relation with dosage concentration and zone of inhibition increase dosages increase the zone of inhibition and vice versa.

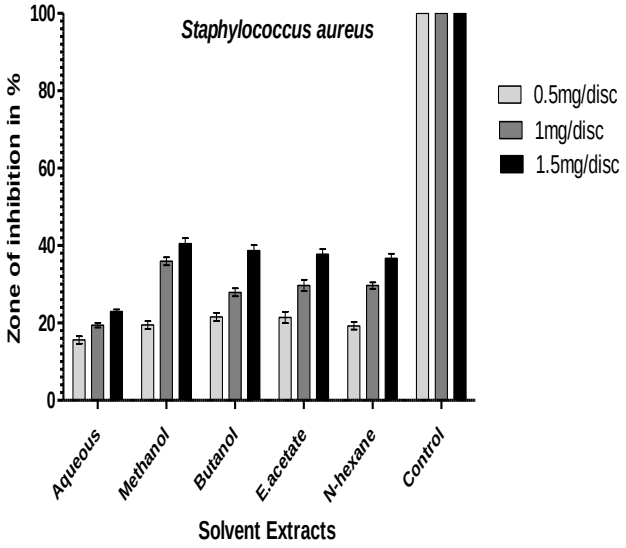

Figure 4:Antibacterial activities of aqueous, E. Acetate, methanol, and butanol extracts of *cactus grandifloras L* against *S. aureus*

Plant extract of *cactus grandifloras L* of aqueous, Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *P. aeruginosa* is shown in figure 5. Data revealed that *P. aeruginosa* showed susceptibility to all the extracts. Highest ZI for *P. aeruginosa* was shown by Ethyl Acetate i.e 27.94 and 39.37% followed by Methanol with ZI of 24.45% and 38.74 at 1 and 3 mg/disc concentrations, respectively. While at 2 mg/disc, to Methanol exhibits good antimicrobial activity i.e 35.57% as compared to Ethyl Acetate showing 33.02% ZI. Methanolic extracts showed maximum antimicrobial effect i.e 27.78, 31.57 and 39.21% respectively. Aqueous (water) showed minimum ZI at all three (1, 2 and 3 mg/disc) concentrations, respectively. The effect of different solvent extracted sample have direct relation with dosage concentration and zone of inhibition increase dosages increase the zone of inhibition and vice versa.

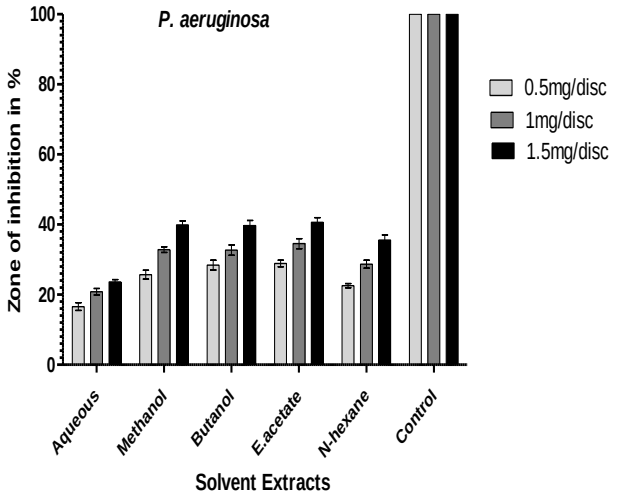

Figure 5: Antibacterial activities of aqueous, E. Acetate, methanol, and butanol extracts of *cactus grandifloras L* against *P. aeruginosa*

Plant extract of *cactus grandifloras L* of aqueous, Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *K. pneumonia* is shown in figure 6. Data revealed for *K. pneumonia*, maximum ZI 28.91, 34.88 and 38.27% was shown by methanol followed Ethyl Acetate with ZI of 23.63, 30.84 and 34.83% at all the three (1, 2 and 3 mg/disc) concentrations, respectively. Aqueous (water) showed minimum ZI at all three (1, 2 and 3 mg/disc) concentrations, respectively. The effect of different solvent extracted sample have direct relation with dosage concentration and zone of inhibition increase dosages increase the zone of inhibition and vice versa.

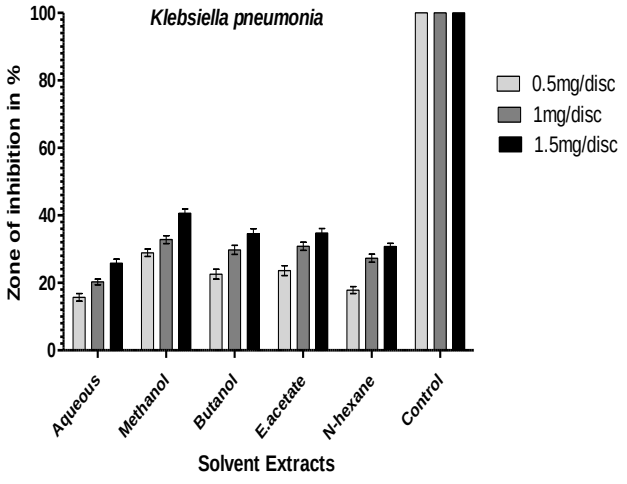

Figure 6: Antibacterial activities of aqueous, E. Acetate, methanol, and butanol extracts of *cactus grandifloras L* against *K. pneumonia*

Antifungal Assay

The antifungal effect of aqueous (water), Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *Candida albicans* is shown in Table 8. Data revealed that *C. albicans* showed susceptibility to all the extracts. Highest ZI for *Candida albicans* was shown by n-Hexane i.e 71, 78 and 95% at all the three (0.5, 1 and 1.5 mg/disc) concentrations, respectively followed by Butanol with maximum ZI of 64 and 71% at 1 and 3mg/disc concentrations, respectively. Ethyl Acetate at 1 mg/disc concentration exhibited good antifungal activity (63%) as compared to Butanol showing 60% antifungal activity. aqueous (water) showed minimum antifungal activity as compared to other extracts at all the three concentrations.

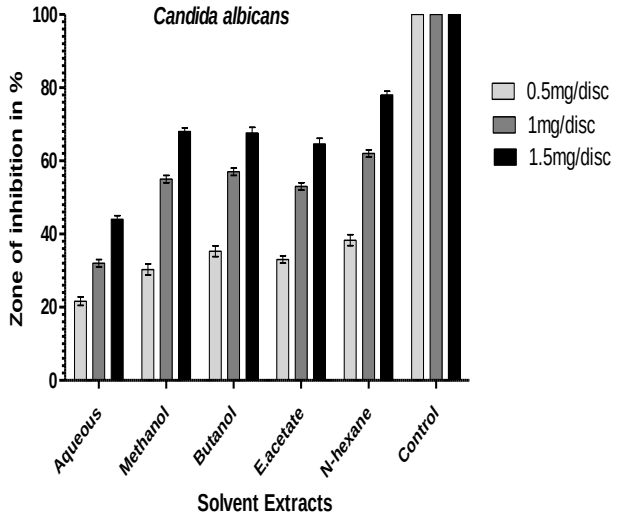

Figure 7: Antifungal activities of aqueous, E. Acetate, methanol, and butanol extracts of *cactus grandifloras L* against *Candida albicans*

DISCUSSION

The given research work proposed that the screening for different bioactive compound and their antimicrobial Potential of cactus grandi plant using different solvent fraction like butanol, methanol, ethyl acetate, n-hexane and aqueous. Phytochemical analyses revealed higher concentration of bioactive compounds in Ethyl Acetate and Methanolic extracts while less performance were shown by aqueous extract similar result were shown by Daoud et al. (2012) Data revealed that *E. coli* and *B. subtilis* are susceptibility to all the extracts at Highest ZI 48.82% as compared to Positive control (Ciprofloxacin) and negative control (DMSO) shoed in Table 2 and 3. Similar results were also presented by Daoud et al. (2012) and Borchardt et al. (2008). The antimicrobial effect of aqueous (water), Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *Xanthomonas campestris* and *Staphylococcus aureus* is shown in Table 4 and 5. The data showed that *X. campestris* and *S. aureus* were susceptible to all the extracts. Incase *X. campestris* of Ethyl Acetate showed highest ZI 45.15% followed Methanol 44.80% ZI. For *S. aureus*, maximum ZI 38.27% was shown by methanol followed by n-Hexane with ZI 37.97% at all the three concentrations, respectively. Aqueous (water) showed minimum ZI at all three concentrations, respectively similar results were also proposed Pranting et al. (2010), Akhbari et al. (2012), Gautam et al. (2012) and Muhammad et al. (2013). The antimicrobial effect of aqueous (water), Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *Pseudomonas aeruginosa* and *Klebsiella pneumonia* is shown in Table 6 and 7. Data revealed that *P. aeruginosa* and *K. pneumonia* showed susceptibility to all the extracts. Highest ZI 39.37% for *P. aeruginosa* was shown by Ethyl Acetate followed by Methanol with ZI of 24.45% and 38.74 at all three concentrations, respectively. While at 2 mg/disc, to Methanol exhibits good antimicrobial activity 35.57% as compared to Ethyl Acetate showing 33.02% ZI. Methanolic extracts showed maximum antimicrobial effect ZI against *K. pneumonia* at the all three (1, 2 and 3 mg/disc) concentrations. Following Methanolic, Ethyl Acetate showed maximum ZI for *K. pneumonia.* ZI at all three (1, 2 and 3 mg/disc) concentrations, respectively. While least ZI was shown by aqueous (water) at all three (1, 2 and 3 mg/disc) concentrations, respectively, same results were suggested by Arora et al. (2007), Ahmad et al. (2006), Khan et al. (2011) Vuuren et al. (2008), Pranting et al. (2010), Sun et al. (2011). The antifungal effect of aqueous (water), Methanolic, Butanol, n-Hexane, and Ethyl Acetate extracts of *Cactus grandifloras* L. against *Candida albicans* is shown in Table 8. Data revealed that *C. albicans* showed susceptibility to all the extracts. Highest ZI for *Candida albicans* was shown by n-Hexane at all the three (0.5, 1 and 1.5 mg/disc) concentrations, respectively followed by Butanol with maximum ZI of 64 and 71% at 1 and 3mg/disc concentrations, respectively. Ethyl Acetate at 1 mg/disc concentration exhibited good antifungal activity (63%) as compared to Butanol showing 60% antifungal activity. aqueous (water) showed minimum antifungal activity as compared to other extracts at all the three concentrations our results agree with Phongpaichit et al. (2005).

CONCLUSION

It is concluded that different extracts from the bark of Cactus grandifloras showed antibacterial and antifungal activities against different microbes. Antimicrobial compounds of Cactus grandifloras were highly soluble in methanol followed by butanol, ethyl acetate and n-hexane. Antimicrobial compounds were not significantly soluble in water. This plant may further be used for antibiotic production against resistant pathogenic microbes.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEDGEMENT

The authors are thankful to the Institute of Biotechnology and Genetic Engineering, the University of Agriculture Peshawar, Pakistan for providing support.

AUTHOR CONTRIBUTIONS

ABR conceptualization. MA and BA Methodology, performance of experiments, SS and LK manuscript writing. AR, ZH data curation and formal analysis. RM and MU Writing – review & editing. All authors read and approved the final All authors read and approved the final version.

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