doi.org/10.36721/PJPS.2020.33.1.REG.027-032.1

Antimicrobial activity of different solvent extracted samples from the leaves and fruits of *Capsicum annuum*

Jehan Bakht[1]*, Nimra Noor[1], Arshad Iqbal[2] and Mohammad Shafi[3]
[1]Institute of Biotechnology and Genetic Engineering, The University of Agriculture, Peshawar, KPK, Pakistan
[2]Department of Botany, Islamia College, Peshawar, KPK, Pakistan
[3]Department of Agronomy, The University of Agriculture, Peshawar, KPK, Pakistan

Abstract: The current research describes the antimicrobial potential of methanol, n-hexane, n-butanol, ethyl acetate and aqueous extracted samples from the leaves and fruits tissues of *Capsicum annuum*. Different solvent extracted samples were screened against six pathogenic microorganisms including five bacterial and one fungal specie by disc diffusion susceptibility assay using 1, 2 and 3 mg disc^{-1} concentrations. When analyzed statistically the data showed that different solvent extracted samples from both leaves and fruits of *Capsicum annuum* revealed varying degrees of antibacterial and antifungal activities. n-butanol and ethyl acetate extracted fractions from both leaves and fruits showed significant inhibition of growth against all the tested microorganisms at 1, 2 and 3 mg disc^{-1} concentrations. *Escherichia coli* were completely resistant to aqueous extracts obtained from the leaves at all the three concentrations. *Klebsiella pneumonia* was resistant to n-hexane extracted fraction from leaves at 1mg disc^{-1} concentration, however, was susceptible at 2 and 3 mg disc^{-1} concentrations. The growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were effectively inhibited by all the solvent extracted fractions from the fruits while aqueous fraction was not able to inhibit the growth of *Bacillus subtilis*. The growth of *Candida albicans* was effectively inhibited by ethyl acetate extracted fraction from leaves at 3 mg disc^{-1} concentration.

Keywords: Antibacterial activity, antifungal activity, disc diffusion assay, *Capsicum annuum*

INTRODUCTION

Plants have been recognized for their medicinal potential since ancient times. Infectious diseases are the leading cause of death worldwide. The microbes are getting resistant against known antibiotics which have become a global concern. This antibiotic resistance leads to emergence of multi drug resistant pathogens which is a great threat to the efficacy of many existing antibiotics (Parekh and Chanda, 2007). Some serious side effects are also associated with the available antibiotic including allergic reaction caused by antibiotic like penicillin and killing the normal flora of the body thereby impairing normal functions of the body. Different approaches and techniques have been followed in order to develop innovative drugs that are safe and reliable. Researchers are doing great effort to discover novel and innovative antimicrobials agents that can cure diseases having no or fewer side effects. At present about 60% of the world's population uses medicinal plants to treat different health problems (Ahmad *et al.,* 2012). A huge amount of higher plants are still to be explored as a source for new drugs. Among the approximately 250,000-500,000 plant species, only a small proportion has been screened phyto-chemically Being source of many powerful drugs, such medicinal plants and their derived products are known to have antibacterial, and antifungal activities etc (Bakht *et al*., 2018; Bilal *et al.,* 2018; Ayaz *et al.,* 2017; 2018; Wajid *et al.,* 2017). Historically pharmacological screening of compounds of natural or synthetic origin has been the source of innumerable therapeutic agents. New biologically active molecules discovered as a result of random screening has been most prolific in the area of antibiotics.

Capsicum annuum belongs to family *Solanaceae* commonly known as Nightshade family comprises of many species of agricultural and medicinal importance all over the world. Its common names include Red pepper, Green pepper in English and Mirchi in Hindi. Traditionally, medicinal properties like stomachic, anti-inflammatory, analgesic are known to be possessed by *Capsicum annuum*. Numerous species of this family are planted for their fruits, such as the pepper, the aubergine and the tomato. The genus *Capsicum* comprises more than 200 varieties and the fruits vary widely in size, shape, flavor and sensory heat. Specific substances known as “capsaicinoids” are responsible for fruit pungency which is the characteristic of the genus *Capsicum.* “Capsaicinoids” is a group of compounds that includes more than 20 alkaloids (vanillylamines). Phenolics, principally flavonoids such as luteolin and quercetin are also characteristic of the genus *Capsicum*. Primary source of antioxidants for humans are dietary polyphenols like phenolic acids and flavonoids and are obtained from plants including spices, fruits, herbs and vegetables. A protective role of carotenoids and flavonoids against stroke, coronary heart disease, and some forms of cancer

**Corresponding author:* e-mail: jehanbakht@yahoo.co.uk

has been reported (Nascimento *et al.,* 2013; Saha *et al.,* 2013).

Keeping in view the medicinal importance of *Capsicum annuum,* the present research work was carried out to determine the antimicrobial activity of different solvent extracted samples from the leaves and fruits of *Capsicum annuum* against different bacterial species (gram positive and gram negative) and fungi through disc diffusion assay.

MATERIALS AND METHODS

Plant material collection

The plant material (leaves, fruits) of *Capsicum annuum* (chili) was collected from the Farms of The University of Agriculture, Peshawar, Pakistan. The plant specimen was identified at the Department of Botany University of Peshawar, Pakistan. In order to remove dirt and dust, plant material was thoroughly washed with distilled water, shade dried at room temperature for two weeks followed by grinding to fine powder using an electric grinder.

Crude extract preparation, fractionation and antibacterial activity

The methods of Bakht *et al*. (2014) was carried out for crude extract, their fractionation and antimicrobial activity of different samples from *Capsicum annuum* against different microbes (table 1).

STATISTICAL ANALYSIS

The experiment was repeated in triplicate and MSTAT (Version 5.4) computer software was used for the analysis of the data. Least Significant Difference (LSD) test was employed upon obtaining significant differences among means of different concentration at $p<0.05$ (Steel *et al*., 1997).

RESULTS

Antimicrobial activity of leaves extracted samples

Escherichia coli showed highest susceptibility to ethyl acetate extracted fractions measuring inhibitory activity of 53.3% at concentration of 3 mg disc^{-1} followed by 48.3% and 37% ZI at 2 and 1 mg discs^{-1} respectively of the same extract when compared with controls (fig. 1). The data also indicated 47% inhibitory zone by n-butanol extracted fraction at concentration of 3 mg discs^{-1}. Moderate activity was shown by n-hexane and methanolic extracts i.e., 40% and 35% ZI at 3 mg discs^{-1} respectively. No activity was shown by aqueous extracted fraction against *Escherichia coli.* N-butanol extracted fractions showed highest growth inhibition of 56.4% against *Bacillus subtilis* at the highest concentration of 3mg discs^{-1} followed by 52% and 43.5% ZI at 2 and 1 mg discs^{-1} respectively compared to other samples and controls (fig. 2). Good activity of 53.2% was shown by ethyl acetate extracted fractions at 3 mg discs^{-1}. Lowest activity was recorded for aqueous extracted samples (22.5% ZI) at 1 mg discs^{-1}.

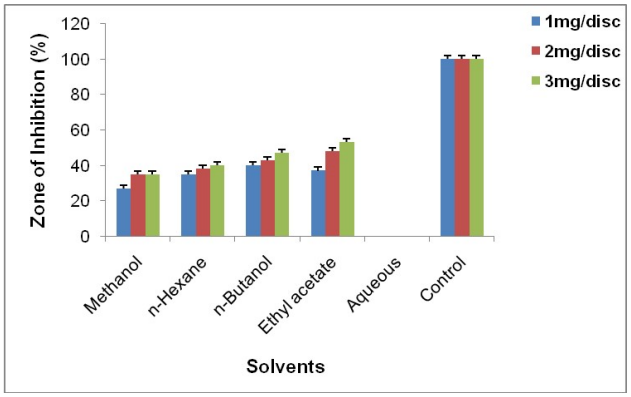

Fig. 1: Antibacterial activity of different solvant exracted samples from the leaves of *Capsicum annuum* against *Escherichia coli* (Bar shows LSD values at P<0.05).

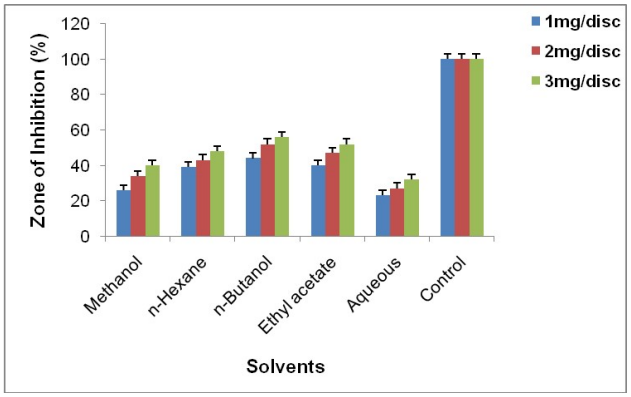

Fig. 2: Antibacterial activity of different solvant extracted samples from the leaves of *Capsicum annuum* against *Bacillus subtilis* (Bar shows LSD values at P<0.05).

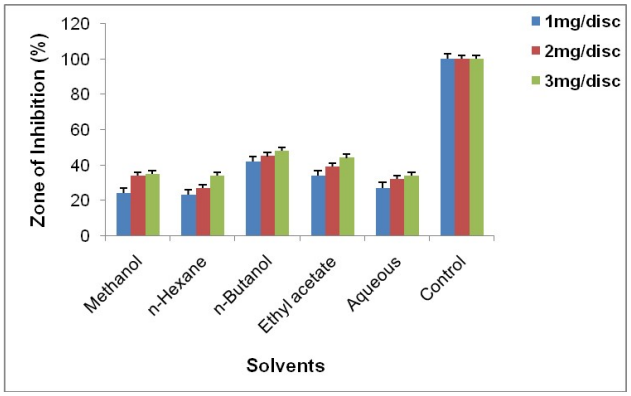

Fig. 3: Antibacterial activity of different solvant extracted samples from the leaves of *Capsicum annuum* against *Staphylococcus aureus* (Bar shows LSD values at P<0.05).

Highest activity of 48.3% against *Staphylococcus aureus* was shown by n-butanol extracted fractions at a concentration of 3mg disc^{-1} followed by 45.1% and 42% ZI at 2 mg and 1mg disc^{-1} respectively of the same sample

(fig. 3). Inhibitory zone of 43.5% was shown by ethyl acetate extracted fraction at concentration of 3mg disc^{-1}. Lowest activity was measured by n-hexane (22.5% ZI) at 1mg disc^{-1}. Highest activity of 51.4% was measured by n-butanol extracted fraction at concentration of 3 mg disc^{-1} followed by ethyl acetate extracted fraction having 49% inhibitory zone at the same concentration against *Pseudomonas aeruginosa* (fig. 4). N-hexane and methanol extracted samples showed activities of 37% and 31% at 3 mg disc^{-1}. On the other hand, aqueous extracts showed lowest activity of 26.4% at 3 mg disc^{-1} concentration (fig. 4). *K. pneumonia* showed highest susceptibility to ethyl acetate extracted fraction having maximum ZI of 53% followed by 47% and 35% inhibitory zones at 2 and 1 mg discs^{-1} respectively. Inhibitory zones of 44% and 38% were shown by methanol and n-butanol extracted fractions at 3 mg discs^{-1} concentration respectively (fig. 5). *Candida albicans* was highly susceptible to ethyl acetate extracted fractions with 53% zone of inhibition at concentration of 3 mg disc^{-1} followed by 48.4% ZI by n-butanol extracted fraction at the same concentration (fig. 6). The lowest inhibition activities of 21.2% each were measured by aqueous and n-hexane extracted fractions at 1 mg disc^{-1} concentration.

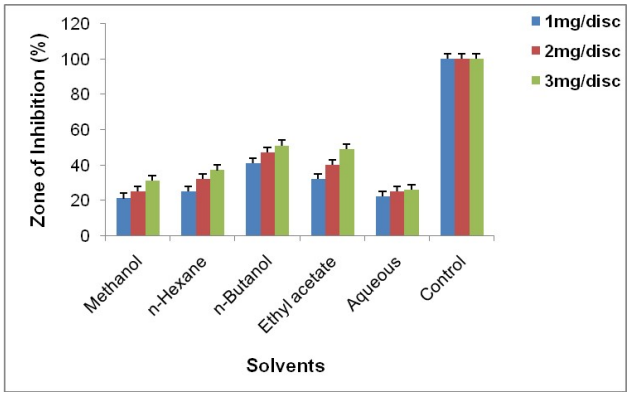

Fig. 4: Antibacterial activity of different solvant extracted samples from the leaves of *Capsicum annuum* against *Pseudomonas aeruginosa* (Bar shows LSD values at P<0.05).

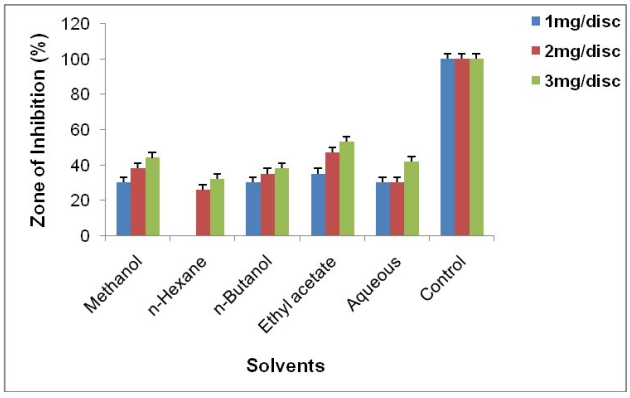

Fig. 5: Antibacterial activity of different solvant extracted samples from the leaves of *Capsicum annuum* against *Klebsiella pneumonia* (Bar shows LSD values at P<0.05).

Antimicrobial activity of fruit extracted samples

N-hexane and ethyl acetate showed highest inhibition zone (50%) against *E. coli* at 3 mg disc^{-1} followed by methanolic extract (47% ZI) at the same concentration (fig. 7). Moderate activities of 42% and 40% were shown by n-butanol and aqueous extracted samples respectively at 3 mg disc^{-1} concentration. N-butanol extracted fractions showed 66.1% inhibition zone against *Bacillus subtilis* at concentrations of 3 mg disc^{-1}. No activity was shown by aqueous extracted fractions at any of the three concentrations. N-hexane and ethyl acetate extracted samples showed 52% ZI followed by crude methanolic extract recording 50% inhibition zone at 3 mg disc^{-1} concentration (fig. 8). *S. aureus* showed highest susceptibility to n-hexane extracted fraction at 3 mg disc^{-1} yielding 48.3% ZI (fig. 9). Inhibitory zone of 45.1% was shown by ethyl acetate extracted fractions at 2 and 3 mg disc^{-1} concentrations respectively followed by 43.5% and 42% ZI by crude methanol and n-butanol extracted samples at concentration of 3 mg disc^{-1} respectively compared with controls. The aqueous extract recorded moderate activity against *S. aureus* having 35.4% ZI at highest concentration.

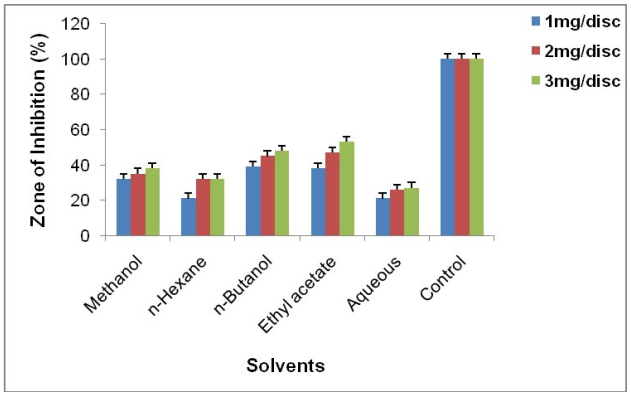

Fig. 6: Antibacterial activity of different solvant extracted samples from the leaves of *Capsicum annuum* against *Candida albicans* (Bar shows LSD values at P<0.05).

Highest activity of 50% was shown by n-butanol extracted fraction at concentration of 3 mg disc^{-1} (fig. 10). Inhibition activity of 48.5% was shown by ethyl acetate extracted fraction and 40% and 45.5% ZI by methanolic and n-hexane fractions respectively at the highest concentration. Lowest activity was shown by aqueous extracts at 1 mg disc^{-1} concentration when compared with controls and other extracts. Highest growth inhibition (40% ZI) was measured by ethyl acetate extracted fraction against *Klebsiella pneumonia* at concentration of 3 mg disc^{-1} followed by 35% and 32% ZI at 2 and 1mg disc^{-1} respectively (fig. 11). Crude methanolic and n-butanol extracts showed moderate activities at the highest concentration having 35% and 38% zones of inhibition respectively. Lowest activity was shown by aqueous extracts (21.2% ZI) at 1 mg disc^{-1} concentration. N-hexane extracted fractions showed 32% zone of inhibition

at both 2 and 3 mg disc^{-1} concentrations compared with controls. Maximum growth inhibition (47% ZI each) was shown by n-hexane, n-butanol and ethyl acetate extracted fractions at concentration of 3 mg disc^{-1}. These extracts also effectively inhibited *Candida albicans* at 1 and 2 mg disc^{-1} concentrations. Crude methanolic extracts also reduced the growth of the tested microbe recoding 45% zone of inhibition at 3 mg disc^{-1}. Activity of 33.3% was shown by aqueous extracted samples at the highest concentration compared with other extracts and controls.

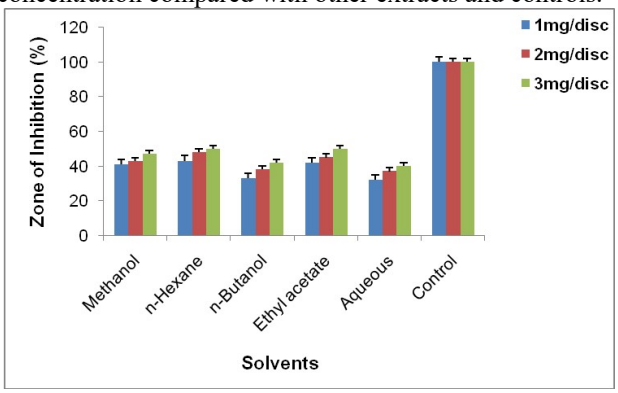

Fig. 7: Antibacterial activity of different solvant extracted samples from the fruits of *Capsicum annuum* against *Escherichia coli* (Bar shows LSD values at P<0.05).

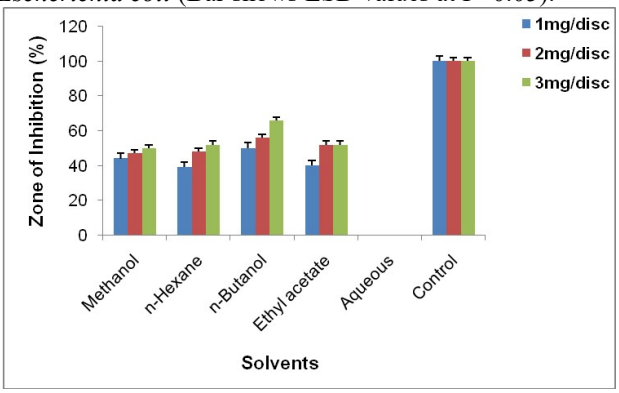

Fig. 8: Antibacterial activity of different solvant exracted samples from the fruits of *Capsicum annuum* against *Bacillus subtilis* (Bar shows LSD values at P<0.05).

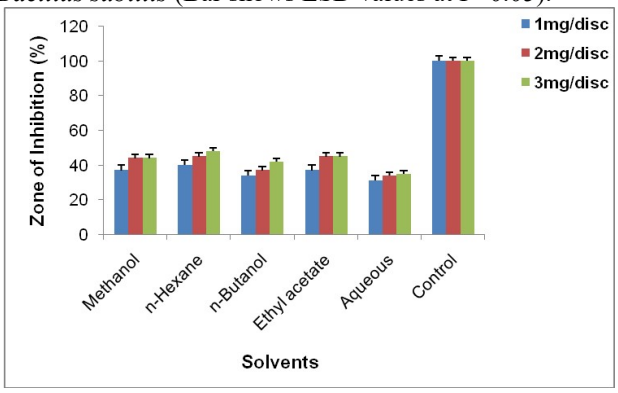

Fig. 9: Antibacterial activity of different solvant extracted samples from the fruits of *Capsicum annuum* against *Staphylococcus aureus* (Bar shows LSD values at P<0.05).

DISCUSSION

Analysis of the data indicated that *Escherichia coli* was highly susceptible to ethyl acetate and n-butanol extracted samples causing maximum reduction in its growth at the highest concentration when compared with other samples and controls. Moderate activity was shown by n-hexane and methanolic extracts at the tested concentrations when compared with other samples and controls. These results are in agreement with Keskin and Toroglu (2011) who investigated the antimicrobial activities of different solvent extracted samples from *Capsicum annuum* and reported that ethyl acetate and methanolic extracts had shown antibacterial activity against *Escherichia coli.* N-butanol and ethyl acetate extracted fraction was very effective to control the growth of *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* at the highest concentration compared to controls and other samples (Chichewicz and Thorpe, 1996). Methanolic and aqueous extracts revealed moderate activities while lowest activity was noted for n-hexane at 1 mg disc^{-1}.

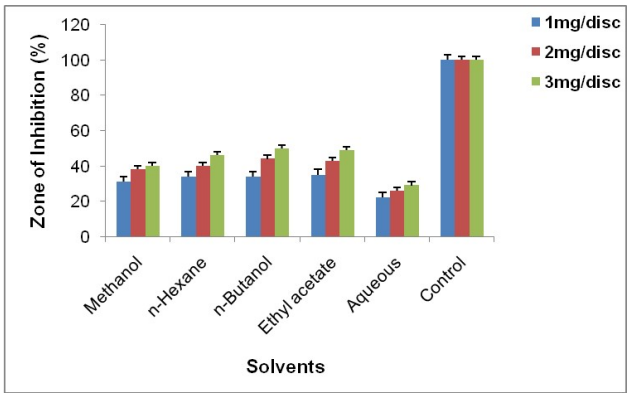

Fig. 10: Antibacterial activity of different solvant extracted samples from the fruits of *Capsicum annuum* against *Pseudomonas aeruginosa* (Bar shows LSD values at P<0.05).

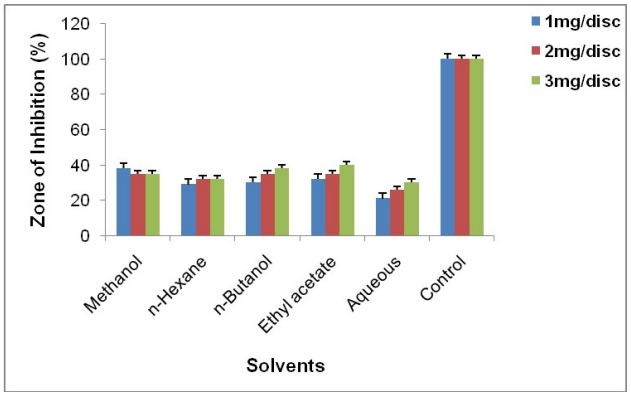

Fig. 11: Antibacterial activity of different solvant extracted samples from the fruits of *Capsicum annuum* against *Klebsiella pneumonia* (Bar shows LSD values at P<0.05).

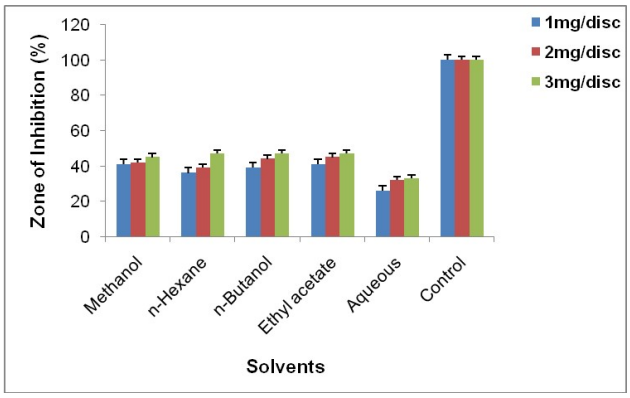

Fig. 12: Antibacterial activity of different solvant extracted samples from the fruits of *Capsicum annuum* against *Candida albicans* (Bar shows LSD values at P<0.05).

These findings are in agreement with Nevry *et al.* (2012) who reported that both methanolic and aqueous extracts of fruit were found to be effective against *Staphylococcus aureus*. N-hexane and methanol extracted samples showed moderate activities at 3 mg disc^{-1} when compared with other samples and control. These results are in agreement with those reported by Sahraei *et al.* (2014) who concluded possible antibacterial effects of *Capsicum annuum* against *P. aeruginosa*. The results also indicated that aqueous extracts showed lowest activity 3 mg disc^{-1} concentration. The antibacterial activity of crude methanolic, n-hexane, n-butanol, ethyl acetate and aqueous extracts from the leaves of *Capsicum annuum* against *Klebsiella pneumonia* implied that *K. pneumonia* showed highest susceptibility to ethyl acetate extracted fraction at all the tested concentration compared with other samples and controls. The data also suggested that good inhibition activities were shown by methanol and n-butanol extracted fractions at the highest concentrations of 3mg discs^{-1} concentration. These results are in agreement with those reported by Sahraei *et al.* (2014). *K. pneumonia* was resistant to n-hexane extracted samples at 1 mg disc^{-1} concentration. It is also clear form the results that moderate activity was shown by aqueous extracts when compared with other extracts and controls. *Candida albicans* was highly susceptible to ethyl acetate extracted fractions at the highest concentration of 3 mg disc^{-1} concentration followed by n-butanol extracted fraction at the same concentration. Lowest inhibition activities were recorded by aqueous and n-hexane extracted fractions at 1 mg disc^{-1} concentration. Moderate antifungal activity was shown by crude methanolic extracts at all the three tested concentrations when compared with other samples and controls. These results corresponds to those reported by Eeturk (2006) who concluded that *C. albicans* was inhibited by methanolic extracts of *Capsicum annuum*.

The antibacterial activity of crude methanolic, n-hexane, n-butanol, ethyl acetate and aqueous extracts from the fruit (green chilli) of *Capsicum annuum* against *Escherichia coli* indicated that almost all the extracted samples were effective to control the growth of *E. coli* at all the three concentrations. N-hexane and ethyl acetate showed highest inhibition zone at 3 mg disc^{-1} when compared with other samples and controls. The data also indicated that moderate activities were recorded for n-butanol and aqueous extracted samples at 3 mg disc^{-1} concentration compared to controls. N-butanol extracted fractions showed good activity against *Bacillus subtilis* at the highest concentrations of 3 mg disc^{-1}. Crude methanolic extract, n-hexane and ethyl acetate extracted samples also inhibited the growth of the tested bacterium at 3 mg disc^{-1} concentration. Similar results were stated by Yasurin (2015) who revealed that extracts were found to exhibit varying degrees of inhibition against *B. subtilis. S. aureus* was highly susceptible to n-hexane extracted fraction at 3 mg disc^{-1}. It is clear from the ethyl acetate extracted fractions showed activity at both 2 and 3 mg disc^{-1} concentrations followed by crude methanol and n-butanol extracted samples at concentration of 3 mg disc^{-1} respectively compared with controls. The results also revealed that aqueous extracted fractions measured moderate activity against *S. aureus* at highest concentration. These findings are in agreement with Nevry *et al.* (2012) who reported that both methanolic and aqueous extracts of fruit were found to be effective against *Staphylococcus aureus*. The data also indicated that different solvent extracted samples effectively controlled the growth of *P. aeruginosa* at all the three tested concentrations. Highest activity was shown by n-butanol extracted fraction at concentration of 3 mg disc^{-1}. The data further indicated that lowest activity was shown by aqueous extracts at 1 mg disc^{-1} concentration when compared with controls and other extracts.

Analysis of the data revealed highest growth reduction by ethyl acetate extracted fraction at the highest concentration when compared with other extracts and controls. The data also suggested that crude methanolic and n-butanol extracts showed moderate activities at the highest concentration. These results are in agreement with those reported by Eeturk (2006) who concluded that different solvent extracted samples from *Capsicum annuum* effectively reduced the growth of *Pseudomonas aeruginosa* among both Gram-positive and Gram-negative bacteria. It can be seen from the results that lowest activity was shown by aqueous extracts at 1 mg disc^{-1} concentration. Maximum growth inhibition was recorded by n-hexane, n-butanol and ethyl acetate extracted fractions at highest concentration of 3 mg disc^{-1}. The data further revealed that these extracts also effectively inhibited *Candida albicans* at 1 and 2 mg disc^{-1} concentrations. Crude methanolic extracts also reduced the growth of the tested microbe at 3 mg disc^{-1}. Moderate activity was shown by aqueous extracted samples ZI at the highest concentration when compared with other extracts and controls.

Table 1: Microbial strains used during the experiment

Microbial Species	Gram strain type	Details of the Microbial strains used
K. pneumoniae	Negative	Clinical isolate obtained from The Department of Microbiology, Quaid-I-Azam University Islamabad Pakistan
P. aeruginosa	Negative	ATCC # 9721
S. aureus	Positive	ATCC # 6538
B. subtilis	Positive	Clinical isolate obtained from The Department of Microbiology, Quaid-I-Azam University Islamabad Pakistan
E. coli	Negative	ATCC # 25922
C. albicans		ATCC # 10231. Plant Pathology Department, The University of Agriculture Peshawar KPK Pakistan

CONCLUSION

n-butanol and ethyl acetate extracted fractions showed good inhibitory activities against all test microorganisms in case of leaves and fruits (green chili). Maximum antibacterial activity was shown by n-butanol extracted fractions from fruits (green chili) against *Bacillus subtilis* at maximum concentration. *Klebsiella pneumonia* was highly resistant to methanol, n-butanol and aqueous extracts obtained from red chili. Ethyl acetate extracted fractions derived from leaves showed highest antifungal activities. In comparison to leaves and red chili extracts, green chili extracts showed good inhibition activities against all test microorganisms.

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