

***In Vitro* antimicrobial activity of *Allium cepa* (dry bulbs) against Gram positive and Gram negative bacteria and fungi**

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Abstract: The present research was carried out at the Institute of Biotechnology and Genetic Engineering, the University of Agriculture Peshawar KPK Pakistan. Analysis of the data revealed that all the extracts from dry bulbs showed different ranges of antimicrobial activities. Ethyl acetate fractions showed inhibitory activities against all tested eight microbes including bacteria and a fungus while chloroform fractions inhibited all the microbes except *Pseudomonas aeruginosa*. Butanol fractions showed second highest activity at both lower and higher concentrations. Ethanol and water fractions were found least effective or ineffective. Among Gram positive microbes, *Staphylococcus aureus* was the most susceptible bacteria and the most resistant Gram negative bacteria were *Pseudomonas aeruginosa* and *Salmonella typhi*.

Keywords: Antimicrobial activity, *Allium cepa*, bacteria, fungi

INTRODUCTION

Traditional medicine is the oldest way of curing diseases and infections and therefore, various plants have been used in different parts of the world (Caceres *et al.*, 1991; Nweze *et al.*, 2004; Vineela and Elizabeth, 2005). Medicinal plants are known to possess curative potentials because of certain biological active substances. These active chemicals include terpenes, flavonoids, bioflavonoids, benzophenones, xanthenes, tannins, saponins, cyanates, oxalate and anthraquinones (Iwu, 1993). Human beings have been using products from animals, plants and microbial sources both in the pure forms and as crude extracts (Parekh and Chanda, 2007c). The identification of the chemical and physical properties of these compounds has resulted in the synthesis and production of more potent and safer drugs. Naturally derived compounds may also have applications in food industry controlling bacteria in foods (Delaquis and Mazza, 1995; Bowles and Juneja, 1998). Medicinal plant extracts as source of controlling the growth of food borne pathogens and food spoilage bacteria are emerging as alternatives to conventional natural preservatives as they are generally safe to humans and environmentally friendly (Thangavelu *et al.*, 2004). A large number of naturally derived antimicrobial compounds are found in different medicinal plants and their essential oils. These compounds are known to be safe, possessing varying degree of antimicrobial activity, and could prevent growth of food borne pathogens and spoilage bacteria. Different studies have reported that medicinal plants produce a large number of secondary metabolites with antimicrobial effects on pathogens (Mari *et al.*, 2003; Obagwu and Korsten, 2003; Bakht *et al.*, 2011 a,b,c,d; 2012; 2013 a,b). The common onion, *Allium cepa*, is a biennial herb; grow

to about 1.2 m in height, with 4 to 6 hollow, cylindrical leaves. In addition to their nutritional effects, the antibacterial and antifungal activities of *A. cepa* have been and continue to be extensively investigated. Compounds derived from onion have shown anti-inflammatory and antihistamine effects *in vitro* and in animal models (WHO, 1999; Griffiths *et al.*, 2002). *In vitro* studies have shown that onion possesses antibacterial (including *H. pylori*), antiparasitic, and antifungal activity (USDA, 2007; Rose *et al.*, 2005; Elnima *et al.*, 1983; Zohri *et al.*, 1995). Keeping in view the role of onion in medicinal plants, the present study was conducted to investigate the antimicrobial activity of different solvent extracted samples of onion.

MATERIALS AND METHODS

Plant material

The present study was conducted at the Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, KPK Pakistan. Plant material (dry onion bulbs) was collected from the local market in Peshawar, KPK, Pakistan. Onion bulbs were chopped and dried in the shade. The dried chopped bulbs were subjected to grinding by tissue homogenizer (InfinitenTM Tissue Mixer Mill) to make fine powder. The following procedure was followed for crude extract in different solvents.

Crude extract preparation

About 1000 g of dried powder was stirred into extraction drums containing five liters of ethanol. These extraction drums were kept at room temperature for six days. During this period, the drums were shaken twice daily. The methanol-soluble compounds were filtered using No.1 Whatman filter paper (WhatmanTM). Twenty five hundred ml fresh ethanol was added to the solid residue and the

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whole process was repeated three times. The filtered ethanolic solution was dried in a rotary evaporator (Rotavapor^R-R 210/R215; BUCHIL Labortechnik AG) by removing ethanol from the solution below 45°C under vacuum pressure. The semisolid extract was removed and dried in a china dish in a water bath at about 45°C.

Fractionation of crude extracts

Dried crude extract was divided into two parts. One part (10g) was poured into glass vials to be tested as crude ethanol extract for antimicrobial activity while the second part (100g) was transferred to a glass beaker for fractionation with different solvents. The second portion was dissolved in water and poured into a separation funnel. Twenty ml distilled petroleum ether was added to the funnel. The separation funnel was shaken to separate the two phases. Compounds soluble in the upper petroleum ether phase were collected and the lower aqueous phase was re-extracted thrice with petroleum ether. All fractions of petroleum ether were combined and dried to a semisolid state with a rotary evaporator. The semisolid petroleum ether fraction was dried in a china dish at about 45°C and stored in glass vials until used. The same process of fractionation was carried out with chloroform, ethyl acetate and butanol respectively, resulting in chloroform, ethyl acetate and butanol fractions. The lower aqueous phase at the end of the process was dried via rotary evaporator and water bath and used as aqueous fraction. At the end of the process (fig. 1), six different extracts, i.e. crude ethanol extract (1.7g), petroleum ether fraction (2.6g), Chloroform fraction (0.5g), ethyl acetate fraction (1.5g), butanol fraction (2g) and aqueous fraction (0.6g) were prepared one by one for antimicrobial testing.

Culture media

Nutrient agar medium (HiMedia Laboratories Pvt., Ltd.) was used for the culturing and growth of all microorganisms used in the study. Nutrient broth was used for shaking incubation and standardization of these microorganisms (AOAC, 1995; Tassou *et al.*, 2000).

Preparation of media

The required quantities of nutrient agar and nutrient broth were prepared and poured into conical flasks. Some of the nutrient broth (approx. 20 ml/test tube) was also poured into test tubes. All the media flasks and test tubes were plugged with cotton wool and sterilized in an autoclave. After sterilization, nutrient agar medium was poured aseptically into sterilized petri plates. A sterile environment was maintained during pouring to avoid contamination. The medium was allowed to solidify in petri plates for about an hour and the petri plates were placed in an inverted position (to avoid evaporation of water from the medium within the plates) in an incubator at 37°C for 24 hrs. After 24 hrs, uncontaminated plates were used for culturing of bacteria and fungi. The nutrient

broth in flasks (approx. 20 ml/flask) was used for shaking incubation of microorganisms while nutrient broth in test tubes was used for standardization of microbial cultures for disc diffusion tests.

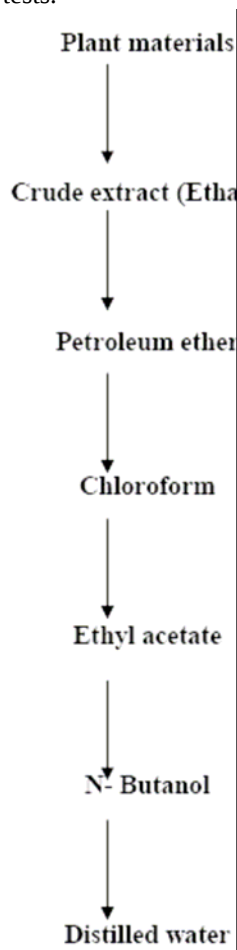


Fig. 1: Flow chart showing crude extract preparation and different fractions by various solvents.

Microorganisms used

Antimicrobial activity of different solvent extracted samples of *Allium cepa* was tested against different bacterial and fungal strains (table 1).

All microbial stock cultures were freshened by streaking using a sterile inoculation loop on nutrient agar medium plates in a laminar flow hood, then incubated at 37°C for 24 hrs. After 24 hrs, the streaked cultures were again sub-cultured on medium plates and incubated at 37°C for 24 hrs. The second streaked cultures were inoculated into nutrient broth in flasks and subjected to shaking incubation for 18 hrs at 37°C (200 rpm) in shaking water bath (GLSC-SBR-04-28).

Disc diffusion susceptibility method

In this method, nutrient agar media plates were seeded with 18-24 hrs cultures of microbial inoculums (a standardized inoculums $1-2 \times 10^7$ CFU ml⁻¹ 0.5

Table 1: Microbial strains tested for susceptibility to *Allium cepa* extracts.

Microbial species	Gram strain type	Details of the microbial strains used
<i>Bacillus subtilis</i>	Positive	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
<i>Candida albicans</i>	Fungus	Clinical isolate obtained from Hayatabad Medical Complex Peshawar KPK Pakistan
<i>Erwinia carotovora</i>	Negative	Plant Pathology Department KPK AUP Pakistan
<i>Escherichia coli</i>	Negative	ATCC # 25922
<i>Klebsiella pneumoniae</i>	Negative	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
<i>Pseudomonas aeruginosa</i>	Negative	ATCC # 9721
<i>Salmonella typhi</i>	Negative	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
<i>Staphylococcus aureus</i>	Positive	ATCC # 6538

McFarland Standard). Whatman No. 1 filter paper discs (6 mm in diameter) were placed with the help of a sterile forceps on the media and then plant extracts in concentrations of 1 and 2 mg disc⁻¹ in 6 and 12 µl volume were applied on the discs. Antibiotics (for Gram-positive bacteria = Azithromycin 50 µg per 6 µl; for Gram negative bacteria = Ciprofloxacin 30 µg per 6 µl and for *Candida albicans* = Clotrimazole 50 µg per 6 µl) as positive control and DMSO (6 µl disc⁻¹) as negative control were also applied on the discs. Inoculated plates were incubated at 37 °C for 18-24 hrs. The next day zones of inhibition were recorded in mm around the discs in each plate.

RESULTS

The present research investigates the antimicrobial (antibacterial and antifungal) potential of different solvent extracted samples from the dried bulbs of onion against eight different microbial species. Among them, seven were bacteria (Gram positive and Gram negative) and one was a fungus. Fig. 2 shows the antimicrobial potentials of six different solvent extracted samples of dried onion bulbs against *Bacillus subtilis*. The data indicated that petroleum ether, butanol and water extracted samples did not inhibit the growth of *B. subtilis* at low concentrations (1 mg disc⁻¹) when compared with their positive controls. All these extracts exhibited zero percent inhibition of *B. subtilis* grown on nutrient agar media at low concentration. However, when the concentration was increased to 2 mg disc⁻¹, these solvent extracted samples inhibited the growth of *B. subtilis* (22% by pet-ether; 25% by butanol and 28% by water). Ethyl acetate and chloroform on the other hand, were more effective against *B. subtilis* at both concentrations when compared with other solvent extracted samples (39% by ethyl acetate and 41% by chloroform at 2 mg disc⁻¹). The data further suggested that ethanol extracted samples did not inhibit the growth of *B. subtilis* at any concentration showing 0% ZI (Zone of Inhibition). Fig. 3 reveals the antifungal activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples against *Candida albicans*. Petroleum ether, butanol and ethanol extracted samples were not effective in controlling the

growth of *C. albicans* at low concentration (1 mg disc⁻¹) when compared with positive controls. However, at higher concentrations (2 mg disc⁻¹) these solvent extracted samples showed more inhibition against *C. albicans* (38% by petroleum ether; 35% by butanol; and 27% by ethanol). The data further suggested that ethyl acetate and chloroform extracted samples were more effective against *C. albicans* as compared to other samples (58% by ethyl acetate and 49% by chloroform at 2 mg disc⁻¹). Water extracted samples did not inhibit the growth of *C. albicans* at any of concentration recording 0% ZI.

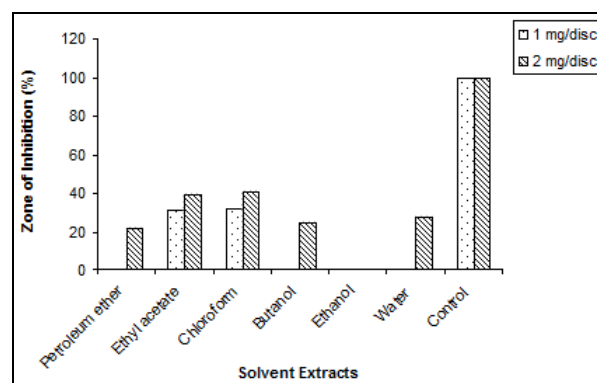


Fig. 2: Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* against *Bacillus subtilis* by disc diffusion assay

The antibacterial activities of six different solvents extracted samples from the dried bulbs of onion against *Erwinia carotovora* (Gram negative) is presented in fig. 4. Chloroform and butanol extracted samples had inhibitory effect against *E. carotovora* at both concentrations. Chloroform reduced the growth of *E. carotovora* by 72% and butanol by 37% at 2 mg disc⁻¹ concentration. The data further showed that ethyl acetate reduced the growth of *E. carotovora* by 49% at high concentration. Ethanol extracted samples showed zero percent inhibition at both concentrations. Data regarding the antimicrobial activity of dried bulbs of onion against *Escherichia coli* is shown in fig. 5. *E. coli* were susceptible to ethyl acetate, chloroform and water extracted samples at both

concentrations. *E. coli* was highly susceptible to ethyl acetate extracted samples (61% ZI) followed by chloroform (45% ZI) and water (31% ZI) at 2 mg disc⁻¹. The data also indicated that petroleum ether, butanol and ethanol extracted samples did not inhibit the growth of *E. coli* at any concentration and measured 0% ZI. Chloroform and butanol extracted samples revealed inhibitory effect against *Klebsiella pneumonia* at both concentrations when compared with positive controls. Petroleum ether, ethyl acetate and water extracted samples on the other hand, were effective only at 2 mg disc⁻¹ concentration. Petroleum ether measured 21%, ethyl acetate 31% and water extracted samples 34% zone of inhibition. Ethanol extracted samples did not inhibit the growth of *K. pneumonia* at any concentration showing 0% ZI (fig. 6).

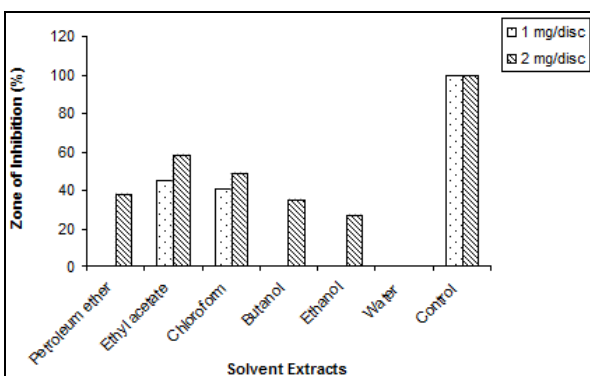


Fig. 3: Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water Extracted samples from *Allium cepa* against *Candida albicans* by disc diffusion assay.

Fig. 7 shows data concerning antimicrobial activity of dried bulbs of onion against *Pseudomonas aeruginosa* when applied in different concentrations. Petroleum ether, chloroform, butanol, ethanol and water extracted samples were not effective in controlling the growth of *P. aeruginosa* at any concentration showing 0% zone of inhibition when compared with positive controls (azithromycin). These results suggested that *P. aeruginosa* was highly resistant to all these fractions. Ethyl acetate, however, showed varying degree of inhibition i.e. 0% inhibition at 1 mg disc⁻¹ concentration and 53% inhibition when concentration was increased to 2 mg disc⁻¹. Fig. 8 shows data pertaining to petroleum ether, chloroform ethyl acetate, butanol, ethanol and water extracted samples against *Salmonella typhi*. The data indicated that *S. typhi* was resistant to petroleum ether, butanol, ethanol and water fractions of onion and showed zero percent zone of inhibition. Ethyl acetate on the other hand, reduced the growth of *S. typhi* at higher concentration (31% ZI at 2 mg disc⁻¹) only. Chloroform fraction was effective in reducing the growth of *S. typhi* at both concentration showing 27% ZI at 1 mg disc⁻¹ and 39% ZI at 2 mg disc⁻¹. Butanol, ethanol and water extracted

samples were ineffective to control the growth of *S. aureus* at any concentration. The data further suggested that petroleum ether, chloroform and ethyl acetate fractions inhibited the growth of *S. aureus* at lower and higher concentrations. Petroleum ether fraction reduced the growth of *S. aureus* by 61% and 77%, ethyl acetate by 51% and 60% and chloroform by 44% and 48% at 1 and 2 mg disc⁻¹ concentration respectively (fig. 9).

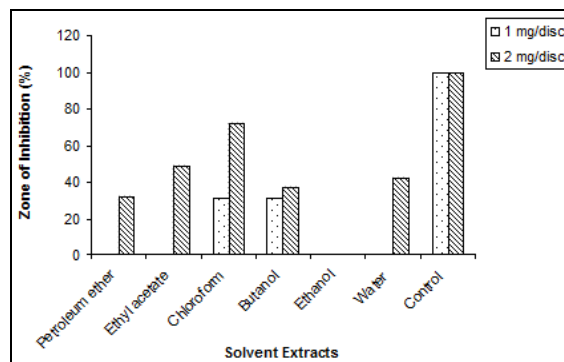


Fig. 4: Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* against *Erwinia carotovora* by disc diffusion assay

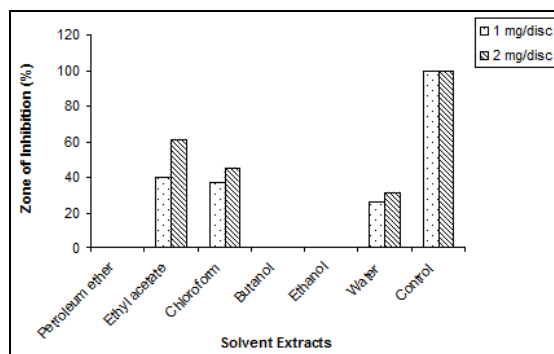


Fig. 5: Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* against *Escherichia coli* by disc diffusion

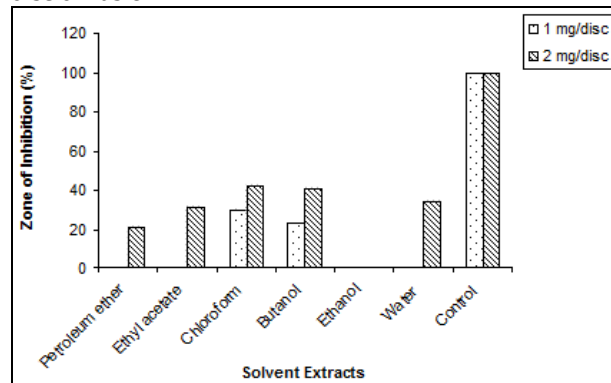


Fig. 6: Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* against *Klebsiella pneumoniae* by disc diffusion assay.

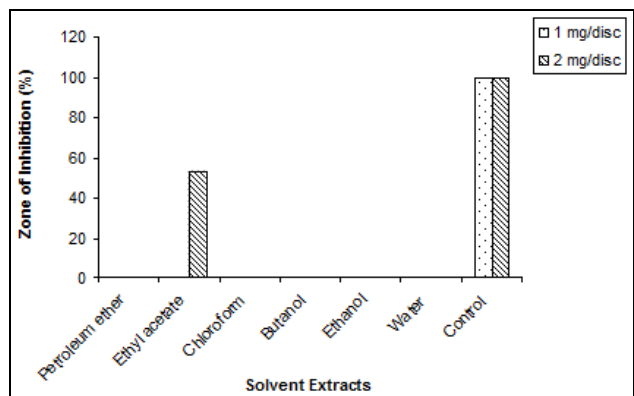


Fig. 7: Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* against *Pseudomonas aeruginosa* by disc diffusion

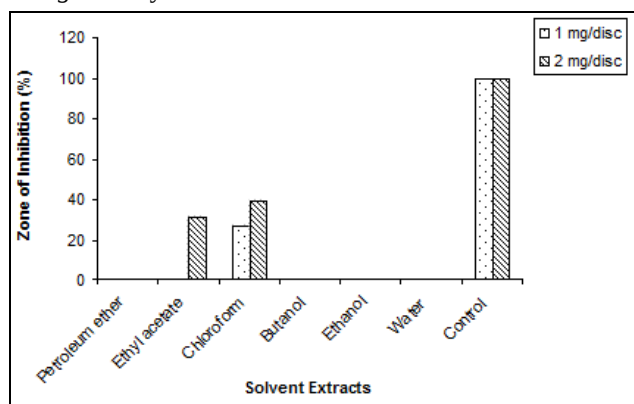


Fig. 8: Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* against *Salmonella typhi* by disc diffusion

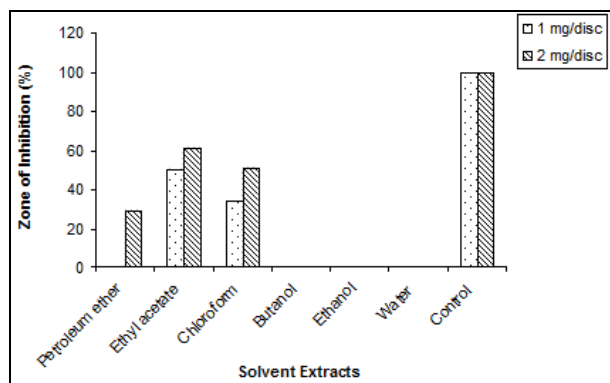


Fig. 9: Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* against *Staphylococcus aureus* by disc diffusion

DISCUSSION

The *in vitro* antibacterial and antifungal potentials of different solvent extracted samples from the dried bulbs

of onion was studied against eight different microbial species including Gram positive and Gram negative bacteria and one fungus. Petroleum ether, butanol and water extracted samples did not reduce the growth of *B. subtilis* at low concentrations compared with positive controls. However, at higher concentration (2 mg disc⁻¹) these solvent extracted samples were effective to inhibit the growth of *B. subtilis*. Ethyl acetate and chloroform were more effective against *B. subtilis* at both concentrations. Our results suggested that ethanol extracted samples did not inhibit the growth of *B. subtilis* at any concentration showing 0% ZI (Zone of Inhibition). Similar results were also reported by Hughes and Lawson (1991), Bekenblia (2004), Santas *et al.* (2010) and Chathradhyunthi *et al.* (2009).

The data further revealed that petroleum ether, butanol and ethanol extracted samples were not effective in controlling the growth of *C. albicans* at low concentrations. However, at higher concentrations (2 mg disc⁻¹) these samples showed more inhibition against *C. albicans*. Ethyl acetate and chloroform extracted samples were more effective against *C. albicans* as compared to other samples. Water extracted samples did not reduce the growth of *C. albicans* at any concentration recording 0% ZI. These results agree with those reported by Chathradhyunthi *et al.* (2009) and Irikin and Korukluoglu (2007). Chloroform and butanol extracted samples effectively reduced the growth of *E. carotovora* at both concentrations whereas ethyl acetate extracted samples were more effective to reduce the growth of *E. carotovora* at high concentration only. Ethanol extracted samples showed zero percent inhibition at both concentrations. Similar results were also reported by Hughes and Lawson (1991), Bekenblia (2004), Santas *et al.*, (2010) and Chathradhyunthi *et al.* (2009).

E. coli were susceptible to ethyl acetate, chloroform and water extracted samples at both concentrations. *E. coli* were highly susceptible to ethyl acetate extracted samples followed by chloroform and water at 2 mg disc⁻¹. The data also indicated that petroleum ether, butanol and ethanol extracted samples did not inhibit the growth of *E. coli* at any concentration and measured 0% ZI. Similar results were also concluded by Hughes and Lawson (1991), Bekenblia (2004), Santas *et al.* (2010) and Chathradhyunthi *et al.* (2009). Chloroform and butanol extracted samples revealed inhibitory effect against *Klebsiella pneumonia* at both concentrations when compared with positive controls. Petroleum ether, ethyl acetate and water extracted samples were effective only at 2 mg disc⁻¹ concentration. Ethanol extracted samples did not inhibit the growth of *K. pneumonia* at any concentration showing 0% ZI. Petroleum ether, chloroform, butanol, ethanol and water extracted samples were ineffective to inhibit the growth of *P. aeruginosa* at any concentration showing 0% zone of inhibition when

compared with positive control (azithromycin). These results suggested that *P. aeruginosa* was highly resistant to all these fractions. Ethyl acetate, however, showed varying degree of inhibition i.e. 0% inhibition at 1 mg disc⁻¹ concentration and 53% inhibition when concentration was increased to 2 mg disc⁻¹.

S. typhi was resistant to petroleum ether, butanol, ethanol and water fractions of onion and measured zero percent zone of inhibition. Ethyl acetate inhibited the growth of *S. typhi* at higher concentrations only. Chloroform fraction was effective in reducing the growth of *S. typhi* at both concentrations. Similar results were also shown by Hughes and Lawson (1991), Bekenblia (2004), Santos *et al.* (2010) and Chathradhyunthi *et al.* (2009). Butanol, ethanol and water extracted samples were not effective to control the growth of *S. aureus* at any concentration. Petroleum ether, ethyl acetate and chloroform extracts effectively reduced the growth of *S. aureus* at both concentrations.

CONCLUSION

From the present study it can be concluded that most of the antimicrobial compounds of *Allium cepa* are soluble in ethyl acetate and chloroform followed by butanol as compared to other solvents. Also crude ethanol extract and other fractions showed effective antifungal activity suggesting a potential use of this plant as antifungal agent.

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