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ANTIMICROBIAL POTENTIALS OF FRESH *ALLIUM CEPA* AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA AND FUNGI

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Abstract

Study of the antimicrobial potentials of *Allium cepa* revealed that all the extracts from both fresh and old samples showed different ranges of antimicrobial activities. Ethyl acetate fraction showed inhibitory activities against all the 8 microbes tested including bacteria and a fungus. Chloroform followed by butanol fraction also inhibited the activity of all the microbes except *Pseudomonas aeruginosa* which was highly resistant. Petroleum ether fraction was effective at both lower and higher concentration. Ethanol and water sub-fractions were found least effective or ineffective. Among gram positive microbes, *Bacillus subtilus* was the most susceptible bacteria inhibited by all extracts while the most resistant gram positive bacteria was *Staphylococcus aureus. Erwinia caratovora* and *Klebsella pneumonia* were the most susceptible gram negative bacteria while *Pseudomonas aeurginosa* and *Salmonella typhi* were the most resistant bacteria.

Introduction

Medicinal plants have been in use by both ancient and modern man of all cultures for treating different ailments (Caceres *et al*., 1991; Nweze *et al*., 2004; Vineela & Elizabeth, 2005; Shinwari & Qaisar, 2011). A single plant processed in different formulations can be used to cure a wide range of diseases and may show excellent results against different pathogens (Kuroyanagi *et al*., 2012). The universality and efficacy of traditional medicine /medicinal herbs is evident from their continued use by a significant portion of the world's population (Mathews *et al*., 1999, Gilani *et al*., 2010). However, the historic role of medicinal herbs in the treatment and prevention of diseases do not assume their safety for uncontrolled use by an uninformed public (Mathews *et al*., 1999). Injuries and even death from the misuse, contamination and/or adulteration of medicinal herbs have been reported (De Smet *et al*., 1997). The use of traditional medicine and medicinal plants in most developing countries for the maintenance of good health has been widely observed (Anon., 1996).

Effort has been devoted over the years to the search for new antimicrobial materials from natural sources for food preservation (Topal, 1989; Paster *et al*., 1995; De *et al*., 1999; Yin & Tsao, 1999; Nielsen & Rios, 2000). Naturally derived compounds and other natural products may have applications in controlling bacteria in foods (Delaquis & Mazza, 1995; Bowles & Juneja, 1998). Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural herbal remedies (Anon., 1998). Moreover, in these societies, herbal remedies have become more popular in the treatment of minor ailments. Systematic screening of plant materials reveals an all-important effort to find new bioactive compounds with the needed therapeutic potential to fight against pathogenic microorganisms, particularly hospital based (Parekh & Chanda, 2007; Bakht *et al*., 2011 a, b, c, d; Bakht *et al*., 2012). The elucidation of the chemical structures of some of these compounds had led to the synthesis and production of more potent and safer drugs.

Since ancient times onions (*Allium cepa, L.*) have been an important dietary resource and have also been of interest for medical purposes (Rose *et al*., 2005). Onions (*Allium cepa*) is a monocotyledon belongs to the family *Liliaceae.* The onion is one of the world's leading vegetable crops, believed to have been domesticated in central and western Asia. Onions were used as early as 5,000 years ago in Egypt (Anon., 1999). Onions were consumed throughout Europe during the Middle Ages and were later thought to guard against evil spirits and the plague, probably because of their strong odor (Blumenthal *et al.,* 1998). Folk healers traditionally used onions to prevent infections is among the oldest cultivated plants used both as a food and for medicinal applications (Lanzotti *et al.,* 2006). Compounds derived from onion have exerted anti-inflammatory and antihistamine effects *in vitro* and in animal models (Anon., 1999; Griffiths *et al*., 2002). *In vitro* studies have shown onion to possess 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 plant, the present study was conducted to investigate the antimicrobial activity of different solvent extracted samples of onion (*Allium cepa*).

Materials and Methods

Plant material: The present study was conducted at the Institute of Biotechnology and Genetic Engineering KPK Agricultural University Peshawar Pakistan. Fresh plant materials were collected from local market of Peshawar KPK. Onion bulbs were first chopped and then shaded dried. The dried chopped bulbs were subjected to grinding to make fine powder. The following procedure was followed for crude extract in different solvents.

Crude extract preparation: About one Kg of dried powder was stirred into extraction drums containing five liters of methanol. These extraction drums were kept at room temperature for 6 days. During this period, the drums were shaken twice daily. The methanol-soluble compounds were filtered using Whatman filter paper No.1. Twenty five hundred ml fresh methanol was added to the solid residue and the whole process was repeated three times. The

filtered methanolic solution was dried in a rotary evaporator by removing methanol from the solution below 45ºC under vacuum pressure. The semisolid extract was removed and dried in a china dish at 45ºC.

Fractionation of crude extract: Crude extract was divided into two parts. One part (10 g) was kept in glass vials to be tested as crude ethanol extract for antimicrobial activity while the second part (100 g) was transferred to a glass beaker for fractionation with different solvents. The second portion was dissolved in water and poured into a separation funnel. Distilled petroleum ether (20 ml) 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 45ºC and stored in glass vials until used. The same process of fractionation was carried out with dichloromethane, ethyl acetate and butanol resulting in dichloromethane, ethyl acetate and butanol fractions. The lower aqueous phase at the end of the process was dried via rotary evaporator at 45ºC. At the end of the process, six different extracts, i.e., crude ethanol extract, petroleum ether, chloroform, ethyl acetate, butanol and aqueous fractions were prepared for antimicrobial testing (Fig. 1).

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

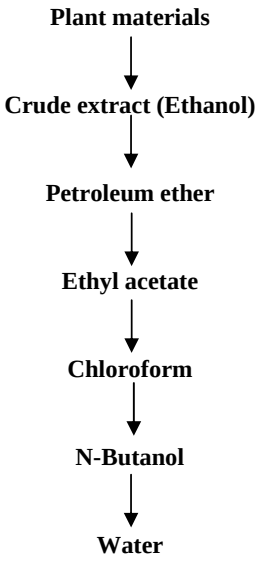

Fig. 1. Flow chart showing crude extract preparation and different fractions by various solvents.

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 before the petri plates were placed in an inverted position (to avoid evaporation of water from the medium within the plates) in an incubator at 37ºC 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.

Microorganisms used: Antimicrobial activity of different solvent extracted samples of onion was tested against the following 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, incubated at 37 ºC for 24 hrs. After 24 hrs, the streaked cultures were again sub-cultured on medium plates and incubated at 37 ºC for 24 hrs. The second streaked cultures were inoculated into nutrient broth in flasks and subjected to shaking incubation for 18 hrs at 37 ºC (200 rpm).

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 × 107 CFU ml-1 0.5 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, 2 and 3 mg disc-1 in 6, 12 and 18 µl volume were applied on the discs. Antibiotics (6 µl $disc^{-1}$) as positive control and DMSO (6 µl disc-1) as negative control were also applied on the discs. Inoculated plates were then incubated at 37 ºC for 18-24 hrs. The next day zones of inhibition were recorded in mm around the discs in each plate.

Positive controls

For Gram-positive bacteria;	Azithromycin 50 µg per 6µl
For Gram negative-bacteria;	Ciprofloxacin 30 µg per 6µl
For *Candida albicans*;	Clotrimazole 50 µg per 6µl

Results and Discussion

The present study investigates the antimicrobial (antibacterial and antifungal) activities of fresh form of *Allium cepa* (onion) against 8 different microbial species. Among them 7 were bacteria (gram positive and negative) and one was fungus. Fig. 2 represents the antimicrobial activity of 6 different solvent extracted samples of fresh onion bulbs against *Bacillus subtilus*. The data indicated that *Bacillus subtilus* was highly susceptible to petroleum ether, ethyl acetate, chloroform and butanol extracted samples both at 1 and 2 mg $disc^{-1}$. All extracted samples showed significant reduction in the growth of *Bacillus*

subtilus. Among these samples, chloroform fraction showed highest zone of inhibition (71% at 1 mg disc^{-1} and 82% at 2 mg disc^{-1}) as compared to other samples. Water extracted samples, however, inhibited the growth of the microbe only at 2 mg disc^{-1}, while ethanol extracted sample did not inhibit the growth of *Bacillus subtilus* at any concentration. Similar results were given by Walter *et al*., (2011), Hughes & Lawson (1991), Benkeblia (2004), Santas *et al.,* (2009) and Chathradhyunthi *et al.*, (2009).

Antifungal activity of different solvents samples of fresh onion against *Candida albicans* is indicated in Fig. 3. Petroleum ether, ethyl acetate, chloroform and butanol extracted samples were effective in inhibiting the growth of *Candida* at both concentrations (1 and 2 mg disc^{-1}). Degree of inhibition increased with increasing concentration. Chloroform extracted samples showed highest inhibition of *Candida* growth i.e. 95% at 2 mg disc^{-1} concentration, followed by ethyl acetate extracted samples (84% ZI) and butanol fraction (42% ZI) at the same concentration. Ethanol extracted sample, however, showed inhibitory effect only at 2 mg disc^{-1} concentration. Water extracted sample on the other hand did not inhibit the growth of *Candida albicans* at any of concentration. These results agree with those reported by Sarwat *et al*., (2012), Chathradhyunthi *et al.*, (2009) and Irkin & Korukllugin (2009).

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

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

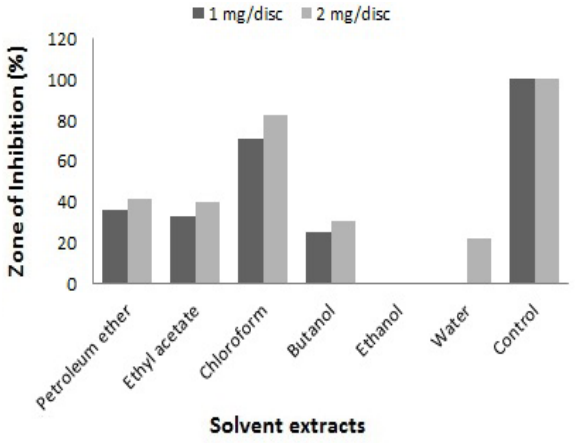

Fig. 2. Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* aginst *Bacillus subtilis* by disc duffusion assay.

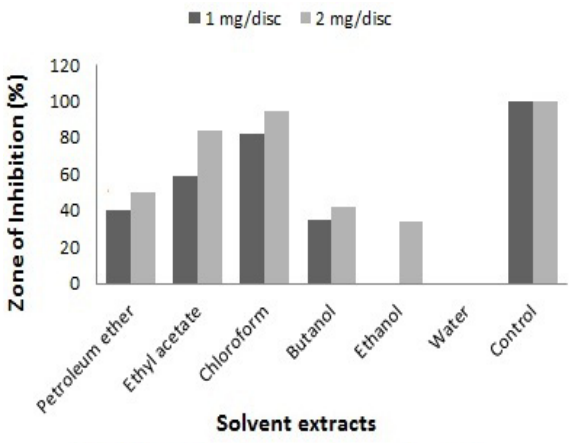

Fig. 3. Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* aginst *Candida albicans* by disc duffusion assay.

Fig. 4 suggested that antibacterial activity of petroleum ether, ethyl acetate and chloroform extracted samples from fresh onion bulbs against *Erwinia caratovora* showed significant inhibitory effect at both 1 and 2 mg disc^{-1} concentrations. Among different solvent samples, chloroform showed highest zone of inhibition i.e., 90% ZI at 2 mg disc^{-1}. Butanol and ethanol extracted samples reduced the growth of *Erwinia caratovora* only at high concentration (42% by butanol and 33% by ethanol at 2 mg disc^{-1}). The data further suggested that *Erwinia caratovora* is resistant to water extracted samples at both concentrations. Similar results are also reported by Irikin & Korukllugin (2008).

The data also revealed that *E. coli* was totally resistant to the petroleum ether, ethanol and water extracted samples showing no zone of inhibition at all. Chloroform, ethyl acetate and butanol on the other hand showed varying degree of inhibition at both concentrations when compared with positive control (Fig. 5). The highest zone of inhibition was given by chloroform i.e. 91% at 2 mg disc^{-1} concentration followed by ethyl acetate 67% and butanol 31% at the same

concentration. Similar results were given by Hughes & Lawson (1991), Bekenblia (2004), Santas *et al.*, (2009) and Chathradhyunthi *et al.*, (2009).

The antibacterial activity of six different solvent extracted samples from fresh *Allium cepa* against *Klebsella pneumonia* is given in Fig. 6. *Klebsella pneumonia* was resistant to water extracted samples while ethanol extracted samples did not inhibit the growth of *Klebsella pneumonia* at lower concentration of 1mg disc^{-1}, however, the same extract had 25% inhibition of *Klebsella pneumonia* at 2mg disc^{-1} concentration. The data further suggested that petroleum ether, ethyl acetate, chloroform and butanol extracted samples exhibited effective inhibitory effect against *Klebsella pneumonia* at both concentrations. Chloroform extracted samples showed highest inhibition of 62% at 2 mg disc^{-1} when compared with positive control. Similar results were reported by Hughes & Lawson (1991), Bekenblia (2004), Santas *et al.*, (2009) and Chathradhyunthi *et al.*, (2009). *Pseudomonas aeruginosa* was resistant to ethanol, chloroform, petroleum ether and water extracted samples and showed zero percent zone of inhibition at both concentrations. Ethyl acetate and butanol fraction showed no reduction in the growth of *Pseudomonas aeruginosa* growth at 1 mg disc^{-1} concentration but showed significant inhibitory zones on higher concentration of 2 mg disc^{-1} , i.e., ethyl acetate showed 53% ZI and butanol 46% ZI (Fig. 7).

The data also indicated that only chloroform extracted sample showed effectiveness against *Salmonella typh*i at both 1 and 2 mg disc^{-1} concentrations showed 27% and 39% ZI. Ethyl acetate exhibited 31% inhibition only at 2 mg disc^{-1} concentration. Petroleum ether, butanol, ethanol and water sub fractions were resistant to *Salmonella typhi* at both 1 and 2 mg disc^{-1} concentrations (Fig. 8). Similar results were also concluded by Hughes & Lawson (1991), Bekenblia (2004), Santas *et al.*, (2009) and Chathradhyunthi *et al.*, (2009). *Staphylococcus aureus* was resistant to fresh *Allium cepa* extracts. Butanol, ethanol and water extracted samples were ineffective to control the growth of *Staphylococcus aureus* at any concentration. The data further suggested that petroleum ether, ethyl acetate and chloroform extracts inhibited the growth of *Staphylococcus aureus* at both lower and higher concentration. Petroleum ether fraction reduced the growth of *Staphylococcus aureus* by 61% and 77% at 1 and 2 mg disc^{-1} respectively, ethyl acetate inhibited the growth by 51% and 60% and chloroform reduced the growth by 44% and 48% at the same concentration respectively (Fig. 9).

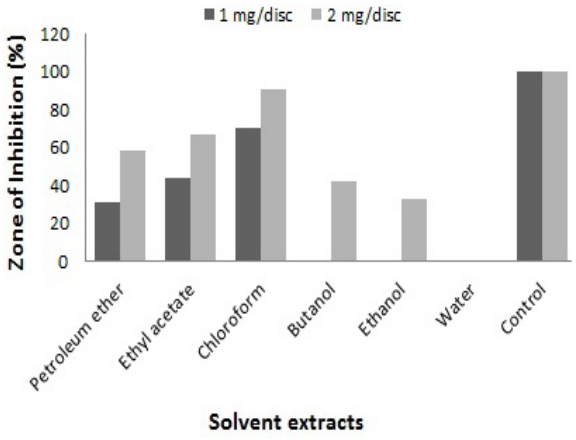

Fig. 4. Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* aginst *Erwinia carotovora* by disc duffusion assay.

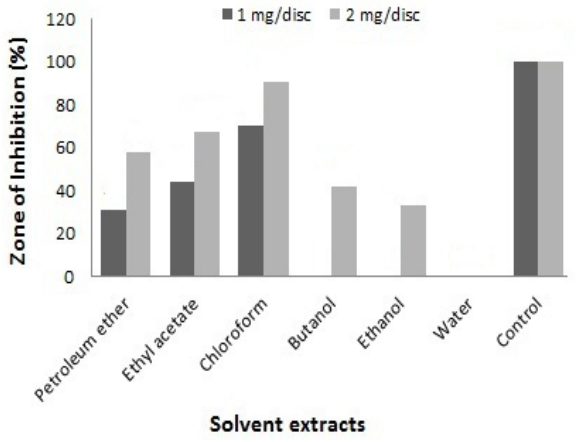

Fig. 5. Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* aginst *Escherichia coli* by disc duffusion assay.

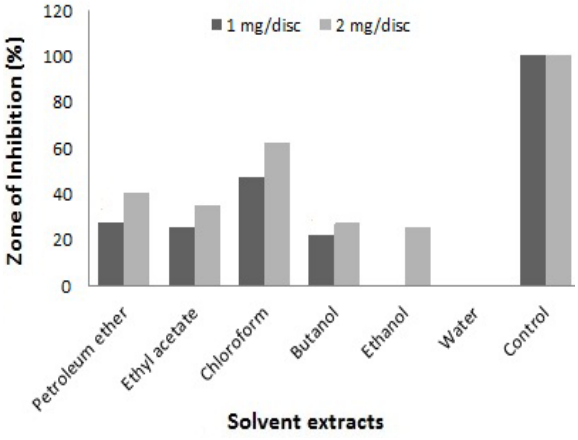

Fig. 6. Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* aginst *Klebsiella pneumoniae* by disc duffusion assay.

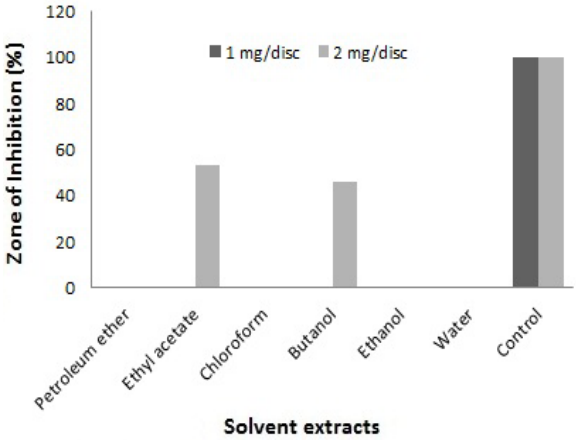

Fig. 7. Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* aginst *Pseudomonas aeruginosa* by disc duffusion assay.

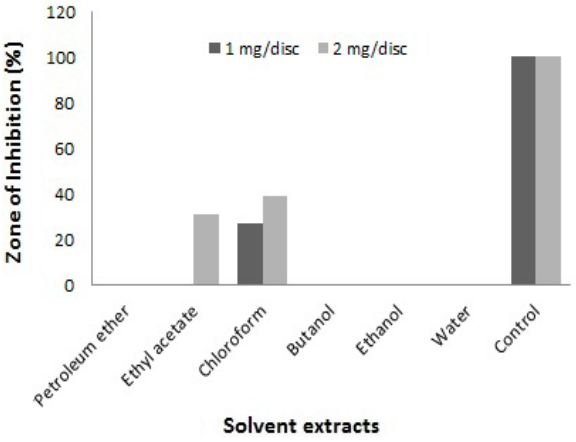

Fig. 8. Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* aginst *Salmonella typhi* by disc duffusion assay.

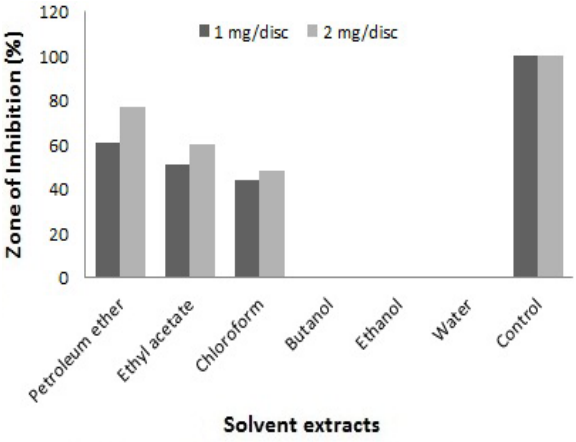

Fig. 9. Antibacterial activity of petroleum ether, ethyl acetate, chloroform, butanol, ethanol and water extracted samples from *Allium cepa* aginst *Staphylococcus aureus* by disc duffusion assay.

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