Full Length Research Paper

Effect of different solvent extracted sample of *Allium sativum* (Linn) on bacteria and fungi

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This study was aimed to evaluate the antimicrobial activity of garlic-extracted samples against bacteria and fungi at different concentration, in various polar solvents. For this purpose, six different extracts were prepared, using five different polar solvents (methanol, ethyl acetate, petroleum ether, chloroforms and butanol) and water. Two different concentrations (1 and 2 mg disc^{-1}) of each extract were subjected for preliminary antibacterial screening against seven pathogenic bacteria by Kirby-Bauer disk diffusion method. The result of *in vitro* antibacterial screening showed that 6 extracts from garlic had different ranges of antibacterial activities. When garlic extracts were studied for their antibacterial potential against Gram-positive bacteria and Gram-negative bacteria, the butanol extracted samples showed the highest inhibitory effect against *B. cereus* (76% ZI at 2 mg disc^{-1} concentration). Water extracted samples indicated a good range of inhibitory effect against *Salmonella typhi* (73% ZI at 2 mg disc^{-1}) and butanol extracted sample showed highest activity against *Erwinia carotovora* (75% ZI). The data also showed that of petroleum ether, methanol and water did not show any inhibitory effect against the tested microbes.

Key words: Solvent, bacteria, fungi, *Allium sativum*.

INTRODUCTION

The use of higher plants and their extracts to treat infections is an ancient practice in traditional medicine. Human are using natural products of animals, plants and microbial sources for thousands of years either in the pure forms or crude extracts (Parekh and Chanda, 2007). About 80% of individuals from developed countries use traditional medicine, with origin from plants. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Prusti et al*.,* 2008). Traditional medical practice has been continued for centuries in almost in every part of the world (Sofowora, 1993). This practice varies from country to country. Numerous plants and herbs are used by traditional medicine practitioners. The use of herbs as medicine is the most ancient approach in the known history of mankind. The herbal medicines may be in the form of powders, liquids, or mixtures, which may be raw, or boiled, ointments, liniments, and incisions. Roots, barks and leaves of various plants are employed in ethno-medicine. More than 70% of the people living in Nigeria depend on various forms of concoction and herbal decoctions for the treatment of some diseases (Kimbi and Fagbenro-Beyioku, 1996). Many investigators have reported the antimicrobial activity of the constituents of some higher plants. (Akobundu and Agyakara, 1987; Misra et al*.,* 1992; Hablemariam et al., 1993) and quite a number of chemical compounds of plant origin have been shown to possess antimicrobial activities (Corthout et al., 1992).

Bioactive compounds from diverse sources have been isolated and characterized around the world. Systematic screening of plant materials represent an important effort to find new bioactive compounds with the needed therapeutic potential to fight against pathogenic microorga-

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Abbreviation: **ZI**, Zone of inhibition.

nisms (for example, *Salmonella typhae, Klebsella pneumonia, Streptococus aureus,* etc.) particularly with respect to those that are hospital based. The clarification of the chemical structures of some of these compounds had led to the synthesis and production of more potent and safer drugs. However, within the last few decades, microbial resistance has emerged for most of the available agents, thus, necessitating the search for newer drugs (Bhattacharjee et al., 2005). The increasing reliance on drugs from natural sources has led to the extraction and development of several drugs and chemotherapeutic agents from traditional herbs which are present in abundance in the tropics (Falodun et al., 2006). In fact, the use of medicinal plants to treat diseases of varying etiology is part of the African tradition, although, it has been used for thousands of years. None of these bioactive plant compounds have been exploited for clinical uses as antibiotics. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are chiefly synthesized during secondary metabolism of the plant. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Prusti et al., 2008).

Garlic is one of those plants that were seriously investigated over the years. It has been used for centuries to fight infections (Onyeagba et al., 2006). The early Egyptians used it to treat diarrhea; the ancient Greeks used it to treat intestinal and extra-intestinal diseases, while the ancient Japanese and Chinese used it to treat headache, flu, sore throat and fever. In Africa, particularly in Nigeria, it is used to treat abdominal discomfort, diarrhea, otitis media and respiratory tract infections (Ankri and Mirelman, 1999; Jaber and Al-Mossawi, 2007). The phyto-chemical constituents of garlic have been established in previous studies (Farbman et al., 1993; Cavallito and Bailey, 1994; Ankri and Mirelman, 1999; Prados-Rosales et al., 2003). The aim of this study was to study the effectiveness of garlic extracts against Gram positive and negative bacteria and fungi. Garlic is a strong antibacterial agent and acts as an inhibitor on both Gram-positive and Gram-negative bacteria including such species as *Escherichia*, *Salmonella*, *Streptococcus*, *Staphylococcus*, *Klebsiella*, *Proteus* and *Helicobacter pylori* (Ankri and Mirelman, 1999). The antimicrobial activity of this diminished at high temperature, because high temperature will denature its active compound allicin (Sato and Miyata, 2000).

MATERIALS AND METHODS

This study was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), Khyber Pakhtunkhwa Agricultural University Peshawar, Pakistan during 2010. This study investigated the antimicrobial activity of crude extract of garlic (*Allium sativum)* against different micro-organisms. Plant materials (bulb) were collected from different localities from Charsadda and Peshawar valley.

Crude extract preparation

Bulbs of garlic were first peeled to make the drying process faster. Bulbs of garlic were then kept in a shaded room for a period of 5 to 7 days for drying. The bulbs were first chopped followed by grinding to obtain dried powder material. The powder were then placed in a tank and methanol added to the tank till the powder material was completely dipped in. Shaking of the tank for 7 to 10 days was performed twice a day. The solution (methanol + extract) were then subjected to the rotatory evaporator to separate methanol leaving only semisolid extract solution (crude extract). For further study, crude extract was fractioned with different solvents that is, butanol, ethyl acetate, chloroform, petroleum ether and water.

Fractionation of crude extract

The prepared crude extract was divided into two portions; one portion (10 g) was poured into the glass vials to be tested as crude methanol extract for antimicrobial activity, while the second portion (100 g) was taken in a glass beaker for fractionation with different solvents. The second portion was dissolved in water, poured into a separatory funnel and then distilled petroleum ether was added into it. The separatory funnel was shaken to separate the two phases as petroleum ether being immiscible with water. Compounds soluble in the upper petroleum ether phase (petroleum ether being lighter than water) were collected and the lower aqueous phase was extracted thrice with petroleum ether. All fractions of petroleum ether were combined and poured into round bottom flask of rotary evaporator and petroleum ether was isolated from the fraction leaving behind semisolid petroleum ether fraction. The semisolid petroleum ether fraction was dried in a China dish via water bath at about 45°C and was stored in the glass vials until used. The same process of fractionation was carried out for chloroform (heavier than water), ethyl acetate (lighter than water) and butanol (lighter than water), respectively resulting in chloroform, ethyl acetate and butanol fractions. The lower aqueous phase at the end of the process was taken and dried via rotary evaporator and water bath.

Culture media

Nutrient agar media was used for the culturing and growth of all microorganisms used in this study. Nutrient broth was used for shaking incubation and standardization of these microorganisms (Tables 1 and 2).

Preparation of media

The required quantities of nutrient agar (2.8 g l^{-1}) and nutrient broth (1.3 g l^{-1}) media were prepared in distilled water and poured into conical flasks. Some of the nutrient broth (approx. 20 ml test tube^{-1}) was also poured into the test tubes. All the media flasks and test tubes were plugged with cotton wool and then sterilized in an autoclave at 1.5 pounds pressure and 121°C for 15 min. After sterilization, nutrient agar media was poured aseptically into sterilized Petri plates in a laminar flow hood. A sterile environment was maintained during pouring to avoid contamination. The media was allowed to become solid in petri plates for about an hour and

Table 1. Composition of nutrient agar used for culturing different microbes.

Nutrient agar modified QUELAB QB-39-3504	
Composition	g l^{-1}
Beef extract	1
Yeast extract	2
Gelatin extract	5
Sodium chloride	5
Agar	15
Total	28

Table 2. Composition of nutrient broth used for shaking incubation and standardization.

Nutrient broth modified QUELAB QB-39-3504	
Composition	g l^{-1}
Gelatin peptone	5
Beef extract	1
Yeast extract	2
Sodium chloride	5
Total	13

then placed in inverted position (to avoid evaporation of water from the media within the plates) in an incubator at 37°C for 24 h. After 24 h, uncontaminated plates were used for culturing of bacteria and fungi. The nutrient broth in flasks (approx. 20 ml flask^{-1}) were used for shaking incubation of microorganisms, while nutrient broth in test tubes were used for standardization of microbial cultures.

Microorganisms used

Antimicrobial activity of different solvent extracted samples were tested against the following different bacterial and fungal strains (Table 3).

All the microbial stock cultures were freshened by sterile inoculation loop on nutrient agar media plates in a laminar flow hood and incubated at 37°C for 24 h. The next day, the streaked cultures were again subcultured 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).

Disc diffusion susceptibility method

Nutrient agar media plates were seeded with 18 to 24 h cultures of microbial inoculums (a standardized inoculums 1-2 10^7 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 and 2 mg disc^{-1} in 6 and 12 µl volumes 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°C for 18 to 24 h. After 24 h, zones of inhibition were recorded in mm around the discs in each plate.

Positive controls

For Gram positive bacteria, azithromycin 50 µg 6 µl^{-1}; for Gram negative bacteria, ciprofloxacin 30 µg 6 µl^{-1}; for *Candida albicans*, clotrimazole 50 µg 6 µl^{-1}.

RESULTS AND DISCUSSION

Figure 1 presents the antibacterial activities of local garlic extracted samples in petroleum ether, chloroform ethyl acetate, methanol, butanol and water against *B. cereus* (Gram positive) using the disc diffusion method. Analysis

of data indicated that petroleum ether, aqueous and methanol extracted samples did not inhibit the growth of *B. cereus* at any concentration when compared with the controls. All these extracts exhibited zero percent inhibition of *B. cereus* grown on nutrient agar media. Butanol, chloroform and ethyl extracted samples, on the other hand, inhibited the growth of *B. cereus* at both concentrations. Butanol extracted samples were more effective in inhibiting the growth of *B. cereus* at maximum concentration (76% at 2 mg disc^{-1}) when compared with chloroform and ethyl acetate extracted sample where inhibition was 44 and 40% at the same concentration, respectively (2 mg disc^{-1}). Similar results are also reported by EL-mahmood Muhammad Abubakar (2009). Data regarding the antimicrobial activity of six different solvents extracted samples from local garlic against *E. coli* (Gram negative) is presented in Figure 2. The data showed that *E. coli* was resistance to petroleum ether, chloroform, methanol and ethyl acetate extracted samples of local garlic.Those samples did not inhibit the growth of *E. coli* at any concentrations and recorded zero percent zone of inhibition when compared with controls. Aqueous and butanol extracted samples showed some degree of activity against *E. coli*. The zones of inhibition by aqueous fraction against *E. coli* were 27% at 2 mg disc^{-1}. When butanol fraction was used at 2 mg disc^{-1} concentration, 45% reduction in *E. coli* growth was noted (Figure 2). These results agree with those reported by Roy et al. (2006) and Jabbar and Mossawi (2007), but contrasted with that of Debnath (2005).

Data presented in Figure 3 indicated the antimicrobial activity of the different solvents extracted samples against *Salmonella typhi* (Gram negative). Analysis of the data revealed that *S. typhi* was very much susceptible (76% ZI) to aqueous extracts of local garlic. Petroleum ether and methanol extracted samples were ineffective to control the growth of *S. typhi*. None of these extracts showed any inhibitory activity against this bacterium. The data further suggested that butanol extractad samples inhibited the growth of *S. typhi* by 32 and 35% at 1 and 2 mg disc^{-1}, respectively. Similarly, ethyl acetate and chloroform recorded 38 and 20% inhibition in the growth of *S. typhi*. These results agreed with those reported by Jabbar and Mossawi (2007) and EL-mahmood Muhammad

Table 3. Microbial strains tested in the present study.

Bacterial strain	Gram strain type	Detail of the microbial strain used
B. cereus	Positive	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
P. aeruginosa	Negative	ATCC # 9721
C.albicans	Fungus	Clinical isolate obtained from Hayatabad Medical Complex Peshawar Pakistan
E. carotovora	Negative	Plant Pathology department of KPK AUP Pakistan
E. coli	Negative	ATCC # 25922
K. pneumoniae	Negative	Clinical isolate obtained form Microbiology lab. QAU Islamabad Pakistan
S. typhi	Negative	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
S. aureus	Positive	ATCC # 6538

Figure 1. Antibacterial activity of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *Bacillus cereus.*
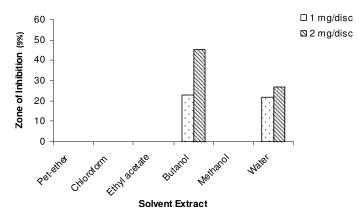

Figure 3. Antibacterial activity of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *Salmonella typhi.*

Figure 2. Antibacterial activity of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *Escherichia coli.*

Abubakar (2009). Analysis of the data also indicated that *K. pneumoniae* was susceptible to butanol, chloroform and water extracted samples from local garlic at both concentrations (Figure 4). It is clear from the data that chloroform extracted samples inhibited the growth of *K. pneumoniae* by 26 and 33%, while butanol fraction
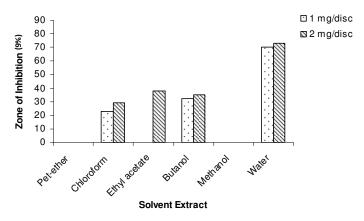
reduced the activity by 36 and 41%, respectively at 1 and 2 mg disc^{-1}. Aqueous extracted samples reduced the growth by 34% at highest concentration (2 mg disc^{-1}). Data also revealed that petroleum ether, methanol and ethyl acetate extracted samples did not inhibit the growth of *K. pneumoniae* at any concentration. The aqueous extract was more potent than the organic extracts, similar to observations of Roy et al. (2006), Jaber and Al-Mossawi (2007) and EL-mahmood Muhammad Abubakar (2009), but contrasted with that of Debnath (2005).

The antibacterial activities of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *S. aureus* (Gram positive) is indicated in Figure 5. The data showed that petroleum ether, chloroform, ethyl acetate and butanol extracted samples were effective in controlling the growth of *S. aureus.* The highest activity was recorded by petroleum ether (61% ZI) followed by butanol (45% ZI) and chloroform (43% ZI). Methanol and water extracted samples showed no inhibitory activity against *S. aureus* and recorded zero percent inhibition. These results also agree with those concluded by EL-mahmood Muhammad Abubakar (2009). The data further revealed that *Erwinia carotovora* was susceptible to all extracted samples except methanol (Figure 6). Butanol extracted
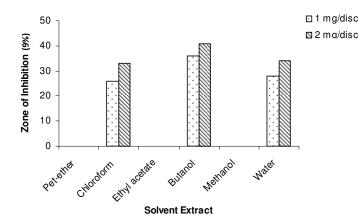
Figure 4. Antibacterial activity of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *Klebsiella pneumoniae.*
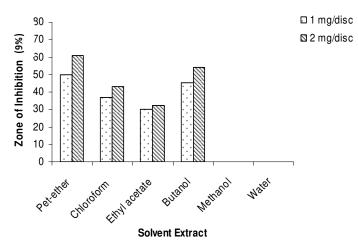

Figure 6. Antibacterial activity of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *Erwinia carotovora.*

Figure 5. Antibacterial activity of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *Staphylococcus aureus.*
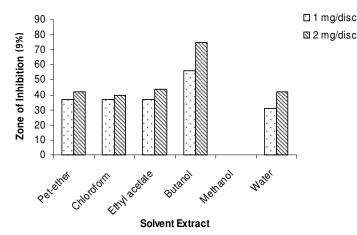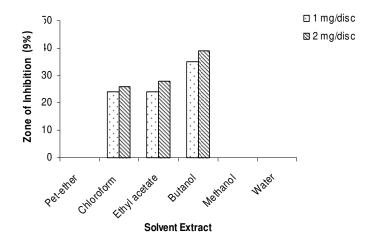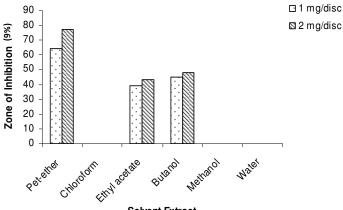

Figure 7. Antibacterial activity of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *Pseudomonas aeruginosa.*

sample showed highest inhibition of *E. carotovora* growth that is, 75% at 2 mg disc^{-1} concentration compared with other solvent extracted samples. Methanol extracted samples were ineffective to control the growth of *E. carotovora* and recorded zero percent ZI. Similar results were also found by EL-mahmood Muhammad Abubakar (2009) and Deresse (2010).

The antibacterial activities of six different solvents extracts from local garlic against *P. aeruginosa* (Gram negative) is presented in Figure 7. Analysis of the data showed that chloroform, butanol and ethyl acetate extracted samples had inhibitory activities against *P. aeruginosa.* Butanol extracted samples reduced the growth of *P. aeruginosa* by 39% at 2 mg disc$^{-1.}$ Ethyl acetate and chloroform extracted samples reduced the growth of *P. aeruginosa* by 28 and 26% at highest concentration (2 mg disc^{-1}). The data further suggested

that methanol, petroleum ether and water extracted samples were not effective in controlling the growth of *P. Aeruginosa* and recorded zero percent inhibition at both concentrations (Figure 7). These results are supported by EL-mahmood Muhammad Abubakar (2009). Analysis of the data further indicated that petroleum ether had the highest inhibitory activity (77% ZI) against *C. albicans* at 2 mg disc^{-1} concentrations followed by petroleum ether extracted samples (64% ZI) at 1 mg disc^{-1} concentration when compared with other solvent extracted samples and controls. The data also showed that butanol extracted samples were also effective (48% ZI) in controlling the growth of *C. albicans* at 2 mg disc^{-1} concentration (Figure 8). Chloroform, methanol and water extracted samples were ineffective to control the growth of *C. albicans* at both concentrations and recorded zero percent inhibition.

Figure 8. Antibacterial activity of petroleum ether, chloroform, ethyl acetate, butanol, methanol and water extracted samples from local garlic against *Candida albicans.*

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