

Full Length Research Paper

Antimicrobial potentials of *Eclipta alba* by disc diffusion method

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This study was conducted to evaluate the antimicrobial potentials of methanol, petroleum ether, dichloromethane, ethyl acetate, butanol and water extracted samples from the aerial parts of *Eclipta alba* against nine microbial species. The antimicrobial (antibacterial and antifungal) susceptibility was screened by disc diffusion assay. The tested microbial species were *Bacillus cereus*, *Bacillus subtilis*, *Candida albicans*, *Erwinia carotovora*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Staphylococcus aureus*. The extracts were applied in different concentrations of 1, 2 and 3 mg disc⁻¹. Analysis of the data revealed that all the six extracts from *E. alba* showed different ranges of antimicrobial activities. Butanol fraction showed inhibitory activities against all the nine microbial species. Ethyl acetate fraction showed inhibitory effects against all the tested microbial species except *B. cereus* and *S. typhi*. Petroleum ether, dichloromethane, methanol and water extracted samples had varying levels of inhibitions against some of these microorganisms. The most resistant microbial strain was *S. typhi* and was not controlled by petroleum ether, dichloromethane, methanol and water extracted samples. The most susceptible Gram positive bacterium was *B. subtilis* and was inhibited by all the six extracts from *E. alba* while the most resistant Gram positive bacterium was *B. cereus*. *E. carotovora* was the most susceptible Gram negative bacterium while *S. typhi* and *E. coli* were highly resistant among the Gram negative bacteria.

Key words: Antimicrobial, potential, *Eclipta alba*, disc diffusion.

INTRODUCTION

Almost every plant has some medicinal value but herbs have innumerable applications in human health. Herbs are characterised by their low toxicity and lack of accumulation in the body. Many plant natural products have served as a major source of medicine for centuries and about quarter of today's drugs are derived from medicinal plants. The World Health Organization (2008) estimated that 75 to 80% of the world's population used plant medicines either in part or entirely for health care. The World Health Organization has encouraged herbal treatments, especially in developing countries, as a source of providing affordable primary health care and of creating agricultural markets for those economies. The practice of traditional medicine is widespread in China,

India, Japan, Pakistan, Srilanka and Thailand. In China, about 40% of the total medicinal consumption is attributed to traditional tribal medicines (Hoareau and DaSilva, 1999). Unani system is dominant in Pakistan but the ethno-medicinal plants use is also seen in the remote areas (Ahmad et al., 2003). India is the largest producer of medicinal herb and is appropriately called the botanical garden of the world (Seth and Sharma, 2004).

The resistance of pathogenic bacteria to various antibiotics has been reported (Seddik et al., 2010). Most important resistant bacteria include both Gram-positive (*Staphylococcus aureus*), Gram-negative (*Pseudomonas aeruginosa*) bacteria and Mycobacterium tuberculosis (Eloff et al., 2005). The increasing rate of resistance to commonly use antibiotics have led to search for newer, more effective, affordable and easily available drugs (Adekunle and Adekunle, 2009). Medicinal plants are the richest bio-source of drugs of traditional systems of

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medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). However, these plants need to be further investigated for their possible bioactive compounds. The beneficial medicinal effects of plant materials typically result from the combinations of secondary metabolites present in the plant (Briskin, 2000). This has involved the isolation and identification of secondary metabolites produced by plants and their use as active principles in medicinal preparations (Taylor et al., 2001).

Eclipta alba Linn. is one of the important medicinal herbs that have important roles in the traditional medicine of the East. It is reported to possess antiseptic, analgesic, antipyretic, antispasmodic, antimicrobial and antiviral properties. *E. alba* is reported to be effective for the retrieval of memory (Banji et al., 2007), and as a hepatoprotective (Tabassum and Agrawal, 2004; Malhotra and Singh, 2007), anti-inflammatory (Arunachalam et al., 2009) and anti-malarial agent (Bapna et al., 2007; Chenniapan and Kadarkarai, 2010). This plant is considered rejuvenative and good for hair and a blackening dye for hair is obtained from this plant. The leaves of *E. alba* are used against snake bites and scorpion stings. This plant is an important constituent of the polyherbal cardioprotective drug called abana (Baliga et al., 2004). *E. alba* is also reported with antiana-phylactic (Patel et al., 2010), antihyperglycemic (Ananthi et al., 2003) and antioxidant properties (Karthikumar et al., 2007; Veeru et al., 2009). Important chemical constituents of this plant include resin, ecliptine, wedelolactone, nicotine, wedelic acid, demethylwedelolactone, etc.

E. alba plant extract is traditionally used to cure skin problems like athlete foot, eczema, dermatitis etc, and for preventing hair loss. Leaves of this plant are used against snake bite in China, India and Brazil while leaf juice after mixing with honey is used to cure catarrh in infants. The root extract of this plant is considered as emetic and purgative. The entire *E. alba* plant is used for the treatment of bleeding, haemoptysis, hematuria, hepatitis, itching, diphtheria and diarrhoea in Taiwan. This plant extract is used against dysentery, eye-diseases, anaemia, asthma and liver cirrhosis, and as alternative medicine as expectorant, antispasmodic, antipyretic, liver tonic, deobstruent in hepatic and spleen enlargement, etc. Keeping in view the medicinal value of *E. alba*, this study was initiated to investigate the inhibitory effect of different solvents extract from this plant on different micro-organisms.

MATERIALS AND METHODS

Plant material

Aerial parts of *E. alba* were collected from different localities of Peshawar including the University of Peshawar Campus,

Hayatabad Peshawar residential area and University Town Peshawar KPK Pakistan. The collected plants were thoroughly washed with tap water to remove the dirt and soil particles. The clean aerial parts of *E. alba* were then placed in a shaded room for a period of 7 days.

Crude extract preparation

Shade dried aerial parts of *E. alba* were first chopped and then grinded with ordinary grinder to obtain dried fine powder. About 700 g of dried powder from the aerial parts of the plant were taken in to extraction drums containing methanol till the powder was completely dipped in. These extraction drums were then kept at room temperature for about 5 to 6 days. During this period, shaking of the drums was performed twice daily. The methanol soluble compounds were then filtered using double filter paper. Fresh methanol was added into the used plant material and the process was repeated three times. The filtered methanolic solution containing plant compounds was subjected to the rotary evaporator for drying. The methanolic solution was taken in the round bottom flask of rotary evaporator and methanol was isolated from the solution below 45°C under vacuum pressure. The semisolid extract was distilled off and dried in a china dish via water bath at about 45°C. About 110 g of dried crude (methanol) extract was prepared in this manner.

Fractionation of crude extract

The crude extract prepared was divided into two portions; one portion (10 g) was taken into glass vials to be tested as crude methanol extract for antimicrobial activity while the second portion (100 g) was taken in to 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 is 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 a 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. Solvent extraction methodology was carried out for dichloromethane (heavier than water), ethyl acetate (lighter than water) and butanol (lighter than water) respectively resulting in dichloromethane, 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. In this way six different extracts, that is, crude methanol extract, petroleum ether fraction, dichloromethane fraction, ethyl acetate fraction, butanol fraction and aqueous fraction were prepared from the dried aerial parts of *E. alba*.

Culture media

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

Preparation of media

The required quantities of nutrient agar (2.8 g 100 ml⁻¹) and nutrient

Table 1. Composition of nutrient agar used for culturing.

Nutrient agar modified (QUELAB QB-39-3504)	
Composition	Concentration (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	Concentration (g L ⁻¹)
Gelatin peptone	5
Beef extract	1
Yeast extract	2
Sodium chloride	5
Total	13

broth (1.3 g 100 ml⁻¹) were prepared in distilled water and poured into conical flasks. Some of the nutrient broth (approximately 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 be solidified in Petri plates for about an hour and then these Petri plates were 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 the flasks (approximately 20 ml flask⁻¹) were used for shaking incubation of the microorganisms while nutrient broth in test tubes were used for standardization of microbial cultures.

Microorganisms used

Antimicrobial activity of different solvent extracted samples of *E. alba* was tested against different bacterial and fungal strains (Table 3).

All the microbial stock cultures were freshened by streaking using 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 first day 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

In this method, nutrient agar media plates were seeded with 18 to 24 h cultures of microbial inoculums (a standardized inoculums of 1

to 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 dimethyl sulfoxide (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. The next day, zones of inhibition were recorded in millimetre around the discs in each plate.

The positive controls for Gram positive bacteria was azithromycin (50 µg 6 µl⁻¹); for Gram negative bacteria it was ciprofloxacin (30 µg, 6 µl⁻¹), and for *Candida albicans* it was clotrimazole (50 µg, 6 µl⁻¹).

The same procedure was followed thrice for each microorganism to get the best possible results and clear any uncertainty regarding the results.

RESULTS AND DISCUSSION

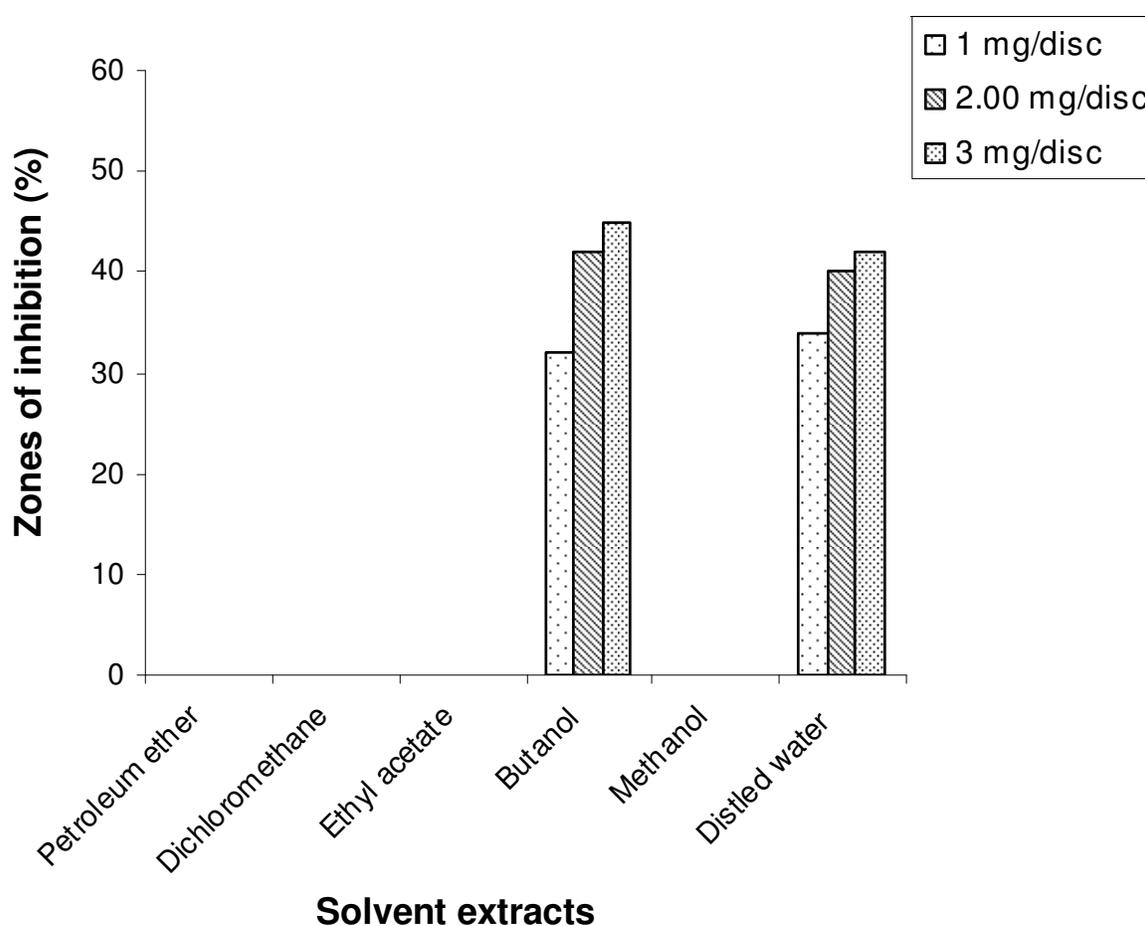
Figure 1 shows the antibacterial activities of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *Bacillus cereus* using the disc diffusion susceptibility assay. It is clear from the data that petroleum ether, dichloromethane, ethyl acetate and methanol extracted samples did not inhibit the growth of *B. cereus* at any concentration when compared with inhibition by azithromycin (positive control). All these extracts exhibited zero percent inhibition of *B. cereus* grown on nutrient agar media. Butanol and water extracted samples, on the other hand, inhibited the growth of *B. cereus* significantly. Moreover, butanol extracted sample was more effective in inhibiting the growth of *B. cereus* at maximum concentration (45% at 3 mg disc⁻¹) when compared with water extracted sample where inhibition was 42% at the same concentration (3 mg disc⁻¹). These results confirm the findings of Rahman and Rashid (2008) and Karthikumar et al. (2007).

The antibacterial activities of six different solvents extracted samples from *E. alba* against *B. subtilis* (Gram positive) by disc diffusion method is shown in Figure 2. The data indicated that *B. subtilis* was susceptible to all the six different solvents extracted samples from *E. alba*. The data suggested that petroleum ether, dichloromethane, ethyl acetate, butanol and water extracted samples at all levels inhibited the growth of *B. subtilis*. The data further revealed that the highest growth inhibition of *B. subtilis* was recorded for butanol (63%) and ethyl acetate (54%) at the highest concentrations of 3 mg disc⁻¹. The lowest inhibition of *B. subtilis* was noted for water extracted samples when compared with other samples activity. These results confirm the findings of Rahman and Rashid (2008).

Analysis of the data also indicated that petroleum ether, dichloromethane and water extracted samples did not inhibit the growth of *C. albicans* and were ineffective in controlling the growth of *C. albicans* at any concentration (Figure 3). The data further revealed that butanol, ethyl

Table 3. Microbial strains used in this research work.

Microbial species	Gram strain type	Details of the microbial strain used
<i>B. cereus</i>	Positive	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
<i>B. subtilis</i>	Positive	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
<i>C. albicans</i>	Fungus	Clinical isolate obtained from Hayatabad Medical Complex Peshawar KPK Pakistan
<i>E. carotovora</i>	Negative	Plant Pathology Department KPK AUP Pakistan
<i>E. coli</i>	Negative	ATCC # 25922
<i>K. pneumoniae</i>	Negative	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
<i>P. aeruginosa</i>	Negative	ATCC # 9721
<i>S. typhi</i>	Negative	Clinical isolate obtained from Microbiology lab. QAU Islamabad Pakistan
<i>S. aureus</i>	Positive	ATCC # 6538

**Figure 1.** Antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *B. cereus* by disc diffusion assay

acetate and methanol extracted samples were more effective in controlling the growth of *C. albicans* when compared with the positive (clotrimazole) and negative (DMSO) controls. The zone of inhibition for butanol extracted sample was 57% (at 1 mg disc⁻¹), 72% (at 2 mg disc⁻¹) and 89% (at 3 mg disc⁻¹) while for ethyl acetate

extracted samples, reduction in the growth were 40, 57 and 83%, respectively. The crude methanol extract was also found effective in inhibiting the growth of *C. albicans* and the recorded inhibitions were 38, 48 and 59% at 1, 2 and 3 mg disc⁻¹ concentrations, respectively. Similar results were also reported by Phongpaichit et al. (2005).

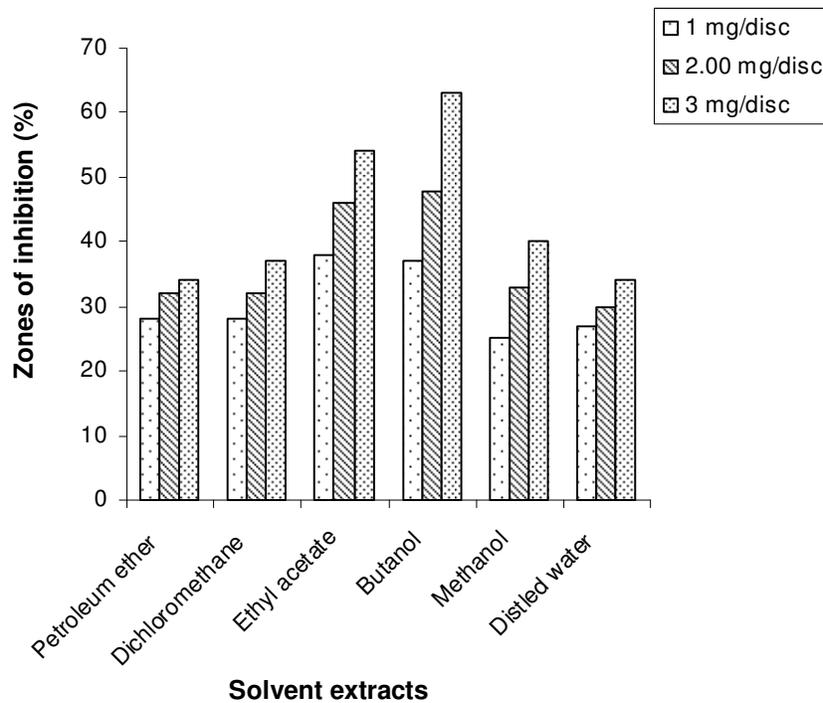


Figure 2. Antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *B. subtilis* by disc diffusion assay.

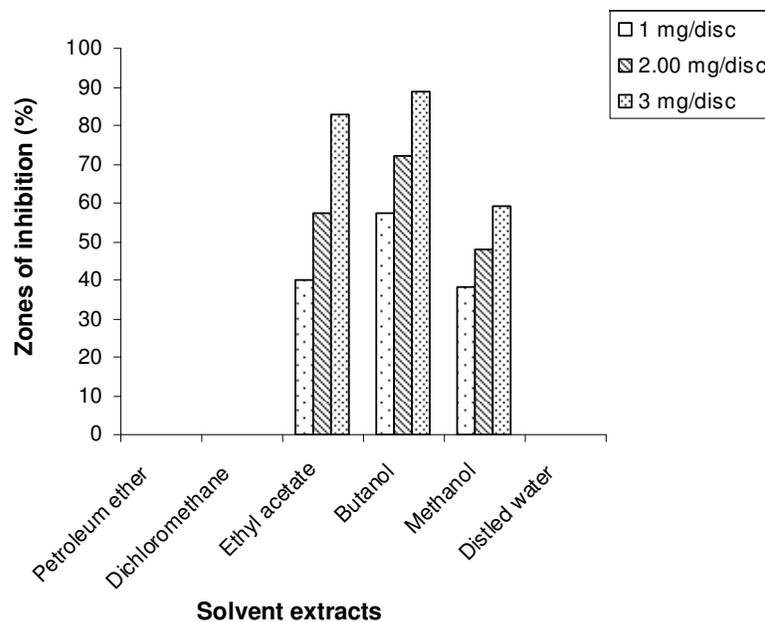


Figure 3. Antifungal activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *C. albicans* by disc diffusion assay.

Figure 4 presents the antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol

and water extracted samples from *E. alba* against *Erwinia carotovora* (Gram negative). *E. carotovora* was

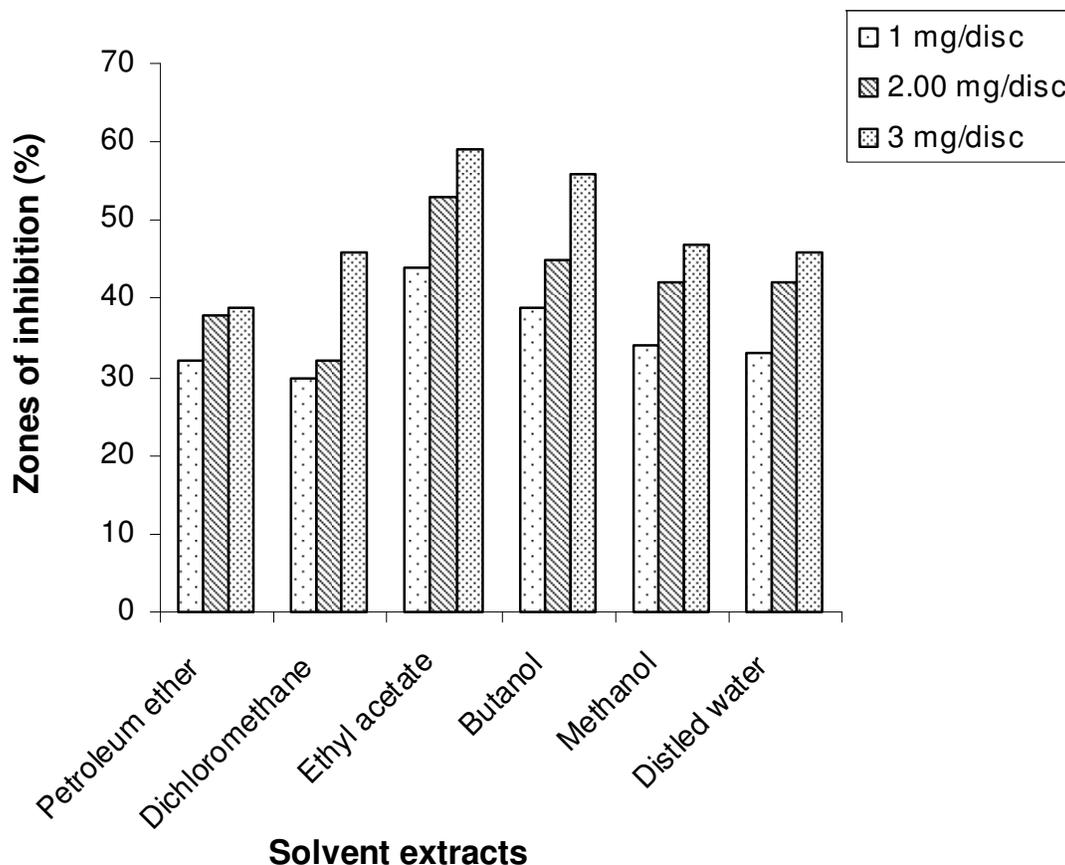


Figure 4. Antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *E. carotovora* by disc diffusion assay.

susceptible to all the six different solvents extracted samples from *E. alba* measured by the disc diffusion method. Petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples were all effective in inhibiting the growth of *E. carotovora* at all concentrations used. The degree of inhibition was higher at the increased concentrations of the samples. Ethyl acetate extracted sample showed the highest inhibition of *E. carotovora* growth (59% at 3 mg disc⁻¹ concentration), followed by butanol extracted sample (56% ZI), dichloromethane extracted sample (46% ZI) and water extracted sample (46% ZI). The lowest inhibitory activity against *E. carotovora* was noted for petroleum ether extracted sample at all concentrations.

The data also revealed that *Escherichia coli* showed resistance to petroleum ether, dichloromethane, methanol and water extracted samples of *E. alba*. These extracts did not inhibit the growth of *E. coli* even when used in higher concentrations and recorded zero percent zone of inhibition when compared with ciprofloxacin (positive control). Ethyl acetate and butanol extracted samples showed some degree of inhibition of *E. coli*. The zones of inhibition by ethyl acetate fraction against *E. coli*

were 24 and 35% at 1 and 2 mg disc⁻¹. However, when ethyl acetate fraction was used in 3 mg disc⁻¹ concentration, there was non significant inhibition of *E. coli* growth. The butanol extracted sample, on the other hand, had 37% reduction in *E. coli* growth when used at 3 mg disc⁻¹ (Figure 5). Similar results were also reported by Lenza et al. (2008) and Rahman and Rashid (2008).

Figure 6 shows the data pertaining to petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples against *Klebsiella pneumoniae* (Gram negative); was susceptible to ethyl acetate, butanol, methanol and water extracted samples. These extracts effectively inhibited the growth of *K. pneumoniae*. Percent inhibition of these samples increased with increasing concentration. The data also suggested that inhibitions by ethyl acetate extracted samples were 28, 43 and 56% while for butanol fraction the percentages of inhibition were 28, 38 and 49% at 1, 2 and 3 mg disc⁻¹, respectively. The same levels of inhibitory activities against *K. pneumoniae* were shown by methanol and water extracted samples with increasing concentrations. Petroleum ether and dichloromethane did not inhibit the growth of *K. pneumoniae* at any

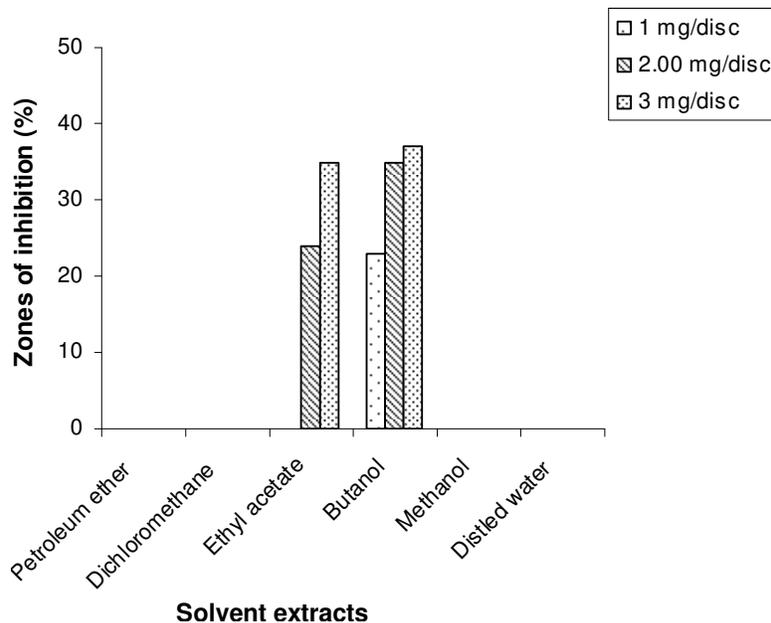


Figure 5. Antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *E. coli* by disc diffusion assay.

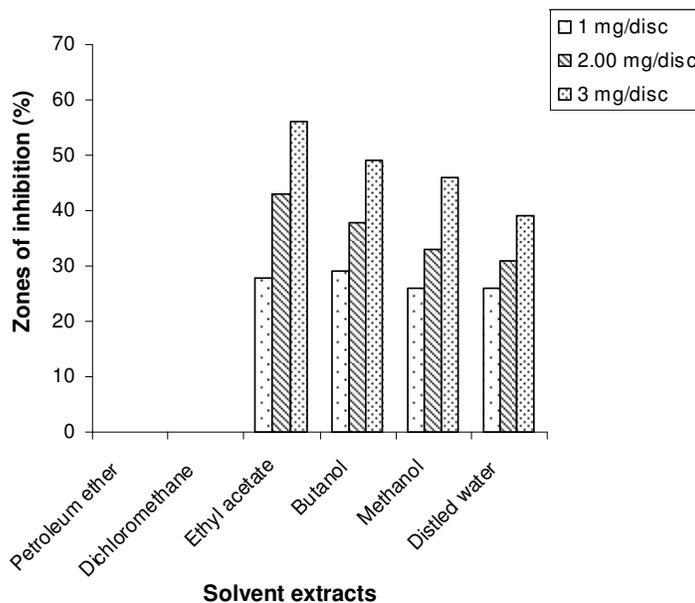


Figure 6. Antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *K. pneumoniae* by disc diffusion assay.

concentration used. This strain of *K. pneumoniae* was resistant to petroleum ether and dichloromethane extracts. These results agree with those reported by Lenza et al. (2008).

The antibacterial activities of six different solvents

extracts from *E. alba* aerial parts against *P. aeruginosa* (Gram negative) is presented in Figure 7. Analysis of the data showed that all the six different solvents extracted samples had inhibitory activities against *P. aeruginosa*. Among the six different extracted samples, ethyl acetate

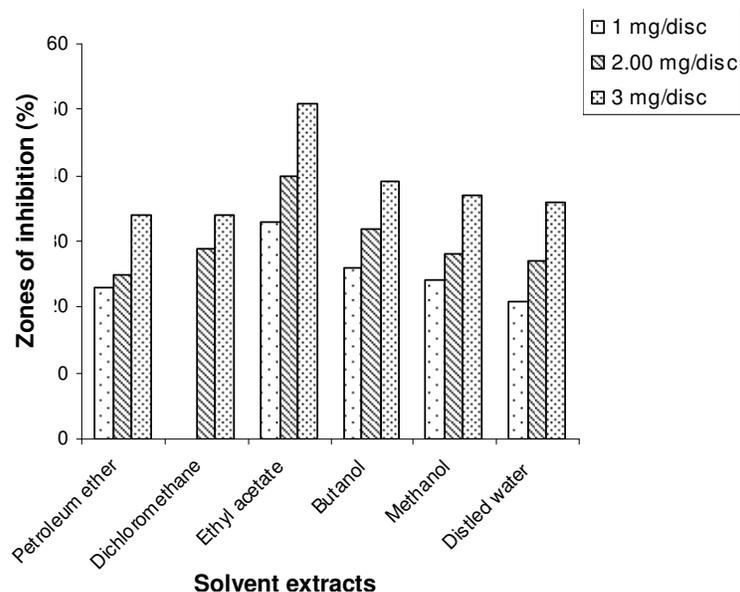


Figure 7. Antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *P. aeruginosa* by disc diffusion assay.

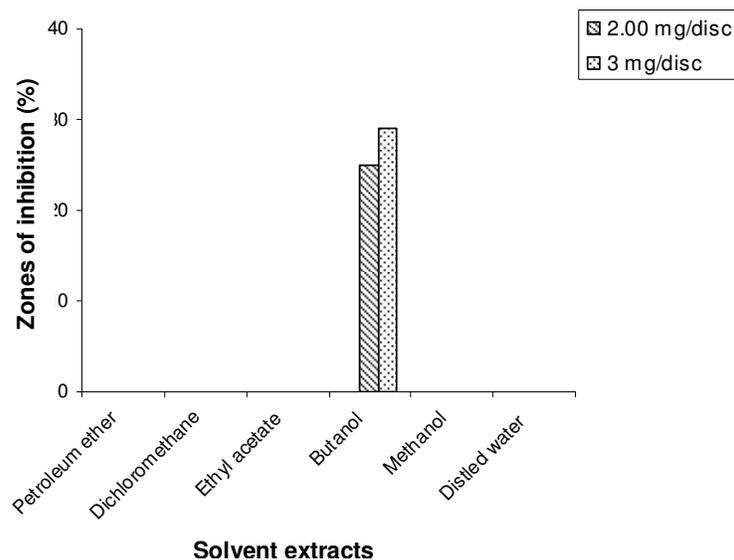


Figure 8. Antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *S. typhi* by disc diffusion assay.

samples had the highest inhibitory activities causing of 33, 40 and 51 at 1, 2, 3 mg disc⁻¹ concentrations, respectively. Butanol fraction was second in its efficiency to inhibit the activity of *P. aeruginosa*, that is, 26, 32 and 39% inhibitions at 1, 2 and 3 mg disc⁻¹ concentrations, respectively. The data further suggested that methanol extracted sample reduced the growth of *P. aeruginosa* by 24, 28 and 37% at 1, 2 and 3 mg disc⁻¹ concentration.

Petroleum ether and water extracted samples were equally effective in controlling the growth of *P. aeruginosa*. The lowest inhibitory activity against this bacterium was shown by dichloromethane extracted samples compared with the other samples. These results agree with those reported by Karthikumar et al. (2007) and Devi et al. (2009).

Data presented in Figure 8 indicated that *S. typhi* was

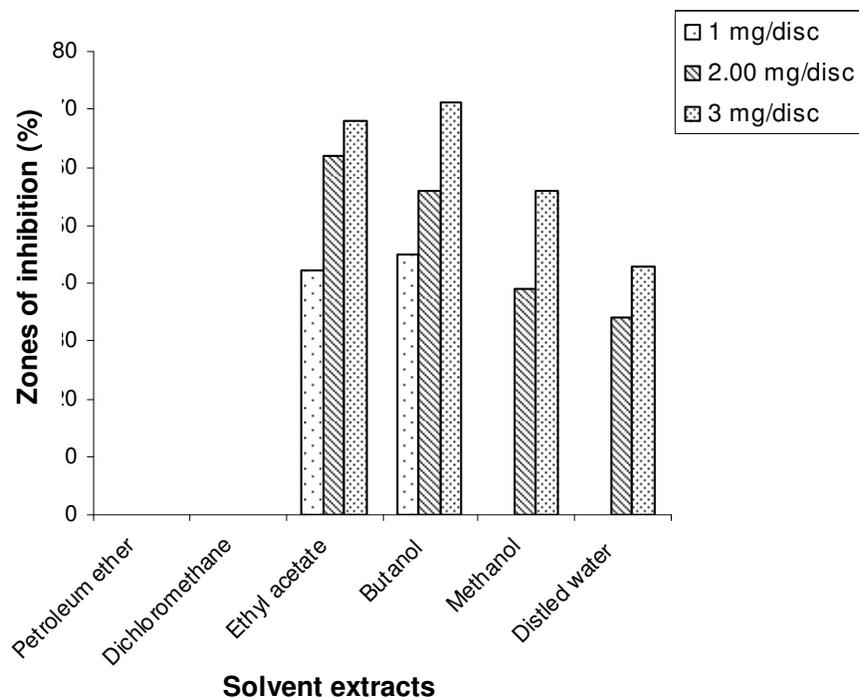


Figure 9. Antibacterial activity of petroleum ether, dichloromethane, ethyl acetate, butanol, methanol and water extracted samples from *E. alba* against *S. typhi* by disc diffusion assay.

resistant to different *E. alba* extracts. Petroleum ether, dichloromethane, ethyl acetate, methanol and water extracted samples were ineffective to control the growth of *S. typhi* by the disc diffusion method. None of these extracts showed any kind of inhibitory activity against this bacterium. The data further showed that butanol extract inhibited the growth of *S. typhi* growth but only to limited extent. Butanol extract inhibited the growth of *S. typhi* only at higher concentrations, that is, 26 and 29% at 2 and 3 mg disc⁻¹, respectively. These results agree with those reported by Lenza et al. (2008).

The data also revealed that petroleum ether and dichloromethane extracted samples were ineffective in inhibiting the growth of *S. aureus* even at higher concentrations (Figure 9). Methanol and water extracted samples showed varying degree of inhibitory activity. Methanol extract did not inhibit the growth of *S. aureus* at 1 mg disc⁻¹ concentration but the same extract when used in 2 and 3 mg disc⁻¹ concentrations, exhibited 39 and 56% of inhibitions, respectively. The water extracted sample did not inhibit the growth of *S. aureus* at 1 mg disc⁻¹ concentration but had 34 and 43% of inhibitory activities at 2 and 3 mg disc⁻¹ concentrations, respectively. Analysis of the data further revealed that ethyl acetate and butanol extracted samples were more effective against *S. aureus*. Ethyl acetate reduced the growth of *S. aureus* by 42, 62 and 68% at 1, 2 and 3 mg disc⁻¹ concentrations, respectively. Similarly, butanol

fraction recorded the highest inhibition of 71% in the growth of *S. aureus* at highest concentration (that is, 3 mg disc⁻¹) when compared with other samples at different concentrations. These results confirm the findings of Wiart et al. (2004), Rahman and Rashid (2008) and Dalal et al. (2010).

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