

Full Length Research Paper

Antimicrobial activities of different solvents extracted samples of *Linum usitatissimum* by disc diffusion method

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This research work was carried out to investigate the antimicrobial activities of different solvents extracted samples of *Linum usitatissimum* against seven bacterial and one fungal pathogen. Three concentrations (0.66, 1.00 and 1.33 mg discs⁻¹) each of ethyl acetate, n-hexane, butanol and distilled water were used. Analysis of the data revealed that the growth of *Staphylococcus aureus* was inhibited equally by butanol and ethyl acetate extracted samples that is, 56% when tested by disc diffusion method. Similarly, ethyl acetate and butanol extracted samples reduced the growth of *Bacillus cereus* by 44 and 64% respectively. The data further suggest that *Erwinia carotovora* was inhibited 42% by ethyl acetate and 65% by butanol extracted samples.

Key words: Antimicrobial, solvent, *Linum*, disc diffusion.

INTRODUCTION

The use of plants as a source of remedies for the treatments of many diseases is prehistoric. Plants have been a valuable source of natural products for many years in maintaining human health, especially in the last decade where more intensive studies were devoted to natural therapies. Traditional medicine is used in all parts of the world with rapidly growing economic importance (Ates and Erdogru, 2003; Rehman et al., 2004; Dash et al., 2005; Agra et al., 2007a; Ushimaru et al., 2007). Researchers in different parts of the globe are using extracts of plants for their antibacterial, antifungal and antiviral activities. It is reported that more than 400,000 species of tropical flowering plants possess medicinal properties (Yildirim et al., 2000; Kumar et al., 2005; Odugbemi, 2006). Despite remarkable progress in synthetic organic chemistry of the twentieth century, over 25% of prescribed medicines in the industrialized countries are directly derived from plants (Newman et al., 2002). However, plants used in traditional medicines are

still not well studied (Kirby, 1996). It is reported that most antibiotics are derived from microorganisms, and one to three antibiotics are launched every year (Clark, 1996). In developing countries where medicines are quite expensive, investigation on antimicrobial activities from ethno medicinal plants is the need of the day. It is obvious that these phytochemicals will find their way in the arsenal of antimicrobial drugs prescribed by physicians (Cowan, 1999). Antibiotics has a limited effective life and the public is becoming increasingly aware of these problems with the over prescription of these antibiotics. In addition, many people principally in the developed countries are interested in having more autonomy over their medicinal care, so self-medication is a common place (Eisenberg et al., 1993).

Antimicrobial compounds from plants may inhibit the growth of microorganisms by different mechanism than those presently used. Antimicrobials therefore, may have a significant clinical value in the treatment of resistant microbial strains (Eloff, 1998). Antimicrobial activities of plant oils and extracts in particular have formed the basis of many applications including raw and processed food preservation, pharmaceuticals, alternative medicine, and

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Table 1. Composition of nutrient agar.

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

natural therapies (Hammer et al., 1999). Natural products can be selected for biological screening based on ethnomedical use of plants, because many infectious diseases are known to have been treated with herbal remedies throughout the history of mankind. Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries (Zakaria, 1991). Bakht et al., 2011 a, b, c).

The plant botanically known as *Linum usitatissimum* belongs to the family Liniaceae. More than 200 species are present in the genus *Linum* including cultivated specie *L. usitatissimum* (Tutin et al., 1968). Barbary et al. (2010) reported that lignan extracts from *L. usitatissimum* showed antimicrobial activity of different selectivity and different minimum inhibitory concentrations (MICs) for each microorganism. Flaxseed lingans extracts also showed moderate (between 70 and 90%) antifungal activity at 2.5 to 3.0 mg ml⁻¹ for both *Aspergillus flavus* and *Aspergillus niger*, respectively. *In vitro* anti-HCV screening of flaxseed lignans revealed significant effect on RNA replication with concentration at 100 mg ml⁻¹. However, the lignan extracts showed negative results when used at concentrations at 5, 25, and 50 mg ml⁻¹. Keeping in view the role of plants in traditional medicine, this study was carried out with the objectives to determine the antibacterial and antifungal activities of *L. usitatissimum* using different solvents.

MATERIALS AND METHODS

The research work was carried out in the Institute of Biotechnology and Genetic Engineering (IBGE), and KPK Agricultural University Peshawar. Flax (*L. usitatissimum*) seeds used in this research work were collected from the fields in Peshawar KPK. Seeds were then washed with distilled water to remove dirt and soil particles. The seeds were dried at room temperature in a shaded region for a period of one week. The dried seeds were grinded with ordinary grinder.

Preparation of crude extract

One kilogram of dried powdered seed material of *L. usitatissimum* specie was taken into round bottom flask and dipped in 95% methanol. The semisolid extract was distilled off on rotary and dried in a China dish through water bath at about 50 °C. About 110 g of the dried crude (methanol) extract was prepared in this manner.

Fractionation of crude extract

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 into a glass beaker for fractionation with different solvents. The crude extract for fractionation was suspended into 200 ml of distilled water and methanol (water: methanol at the ratio of 8:2) and then extracted with 200 ml n-hexane. The separatory funnel was shaken and compounds soluble in n-hexane phase were collected and the aqueous phase was extracted thrice with n-hexane. All fractions of n-hexane were combined and poured into round bottom flask of rotary evaporator and n-hexane was isolated from the fraction leaving behind semisolid n-hexane fraction. The semisolid n-hexane fraction was dried in a China dish through water bath at about 50 °C and was stored in the glass vials until used. Solvent extraction methodology was carried out for ethyl acetate, distilled water and butanol.

Culture media

For culturing of the microorganisms, nutrient agar medium (Table 1) was used. Nutrient broth (Table 2) was used for the incubation and standardization of the microorganisms, mentioned in Table 3.

Preparation of media

The required quantities of nutrient agar (2.8 g 100 ml⁻¹) and nutrient broth (1.3 g 100 ml⁻¹) were prepared by dissolving it in distilled water in conical flasks. Some of the nutrient broth (approximately 20 ml test tube⁻¹) was also poured into the test tubes. Media was sterilized in an autoclave at 15 psi pressure and 121 °C for 15 min. After sterilization, nutrient agar media was poured aseptically into sterilized petri plates in a laminar flow hood. All the steps were performed in sterile environment in order to prevent contamination. The media was allowed to be solidified in petri plates for about an hour and then placed in an incubator at 37 °C for 24 h. The next day, uncontaminated plates were used for culturing of the microorganisms.

Microorganisms used

Antimicrobial activity was tested against the bacterial and fungal strains shown in Table 3.

Disc diffusion susceptibility method

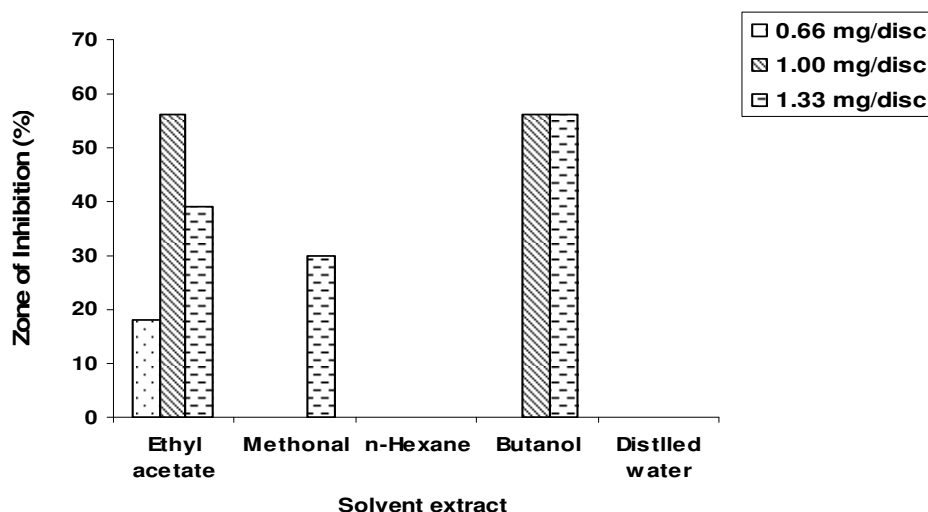
In this method, nutrient agar media plates were seeded with 18 to

Table 2. Composition of nutrient broth.

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

Table 3. Microbial strains used during the experiment.

Microbial species	Strain
<i>Bacillus cereus</i>	Clinical isolate obtained from Microbiology laboratory Quaid-E-Azama University Islamabad Pakistan
<i>Candida albicans</i>	Clinical isolate obtained from Hayatabad Medical Complex Peshawar KPK Pakistan
<i>Erwinia carotovora</i>	Plant Pathology Department of KPK AUP
<i>Escherichia coli</i>	ATCC # 25922
<i>Kleibsiella pneumoniae</i>	Clinical isolate obtained form Microbiology laboratory Quaid-E-Azama University Islamabad Pakistan
<i>Pseudomonas aeruginosa</i>	ATCC # 9721
<i>Salmonella typhi</i>	Clinical isolate obtained from Microbiology laboratory Islamabad Pakistan
<i>Staphylococcus aureus</i>	ATCC # 6538

**Figure 1.** . Antibacterial activity of ethyl acetate, methanol, n hexane, butanol and distilled water extracted samples from *L. usitatissimum* against *S. aureus*.

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 different plant extracts in volume of 4 μ l (0.66 mg discs⁻¹), 6 μ l (1 mg discs⁻¹) and 8 μ l (1.33 mg discs⁻¹) were applied on the discs. Antibiotics (6 mg discs⁻¹) as positive control and DMSO (6 mg discs⁻¹) were also applied on the discs. Media plates were incubated at 37°C for 18 to 24 h in an incubator. The chemicals diffused from the discs into the agar media thus preventing the growth of microorganisms (if susceptible) in the area around the disc known as zone of inhibition. The next day, zones of inhibition were calculated from each treatment.

Positive controls

For Gram positive bacteria, azithromycin (50 μ g 6 μ l⁻¹) was used; for Gram negative bacteria, ciprofloxacin (30 μ g 6 μ l⁻¹) was used and for *Candida albicans*, clotrimazole (50 μ g 6 μ l⁻¹) was used.

RESULTS AND DISCUSSION

Data shown in Figure 1 indicated the antimicrobial activity of ethyl acetate, methanol, n-hexane, butanol and

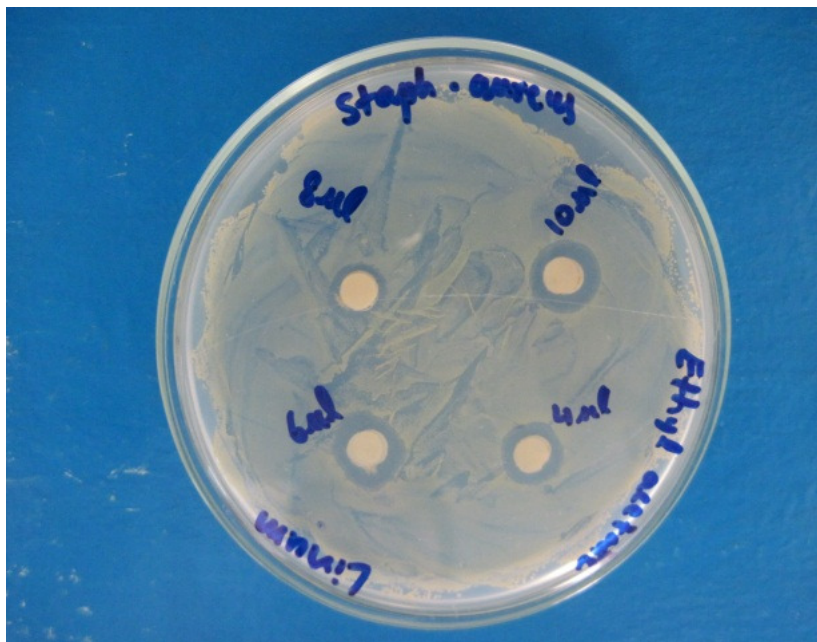


Plate 1. Antibacterial activity of ethyl acetate extracted samples of *L. usitatissimum* against *St. aureus*

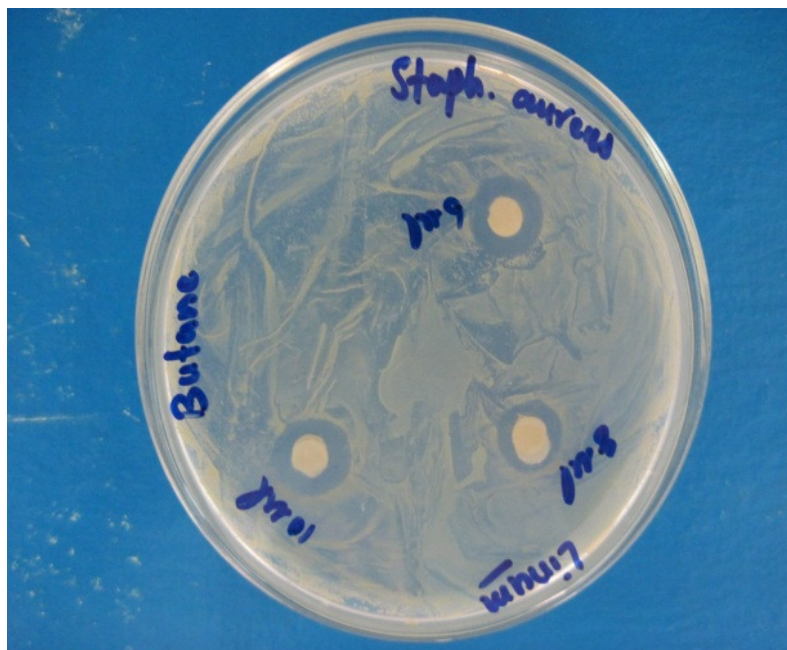


Plate 2. Antibacterial activity of butanol extracted samples of *L. usitatissimum* against *S. aureus*.

distilled water extracted samples from *L. usitatissimum* against *S. aureus*. Analysis of the data revealed that ethyl acetate and butanol extracted samples had a profound inhibition effect against *S. aureus*. In the case of ethyl acetate extracted samples, maximum growth reduction occurred at a concentration of 1 mg disc⁻¹ (56% Z.I) while

butanol extracted samples inhibited growth by 56% (Z.I) at maximum concentration (1.33 mg discs⁻¹). Methanol extracted samples reduced growth by 30% at 1.33 mg disc⁻¹ (Plates 1 and 2). The data also suggest that n-hexane and distilled water extracted samples did not show any antimicrobial activity against *S. aureus*. Similar

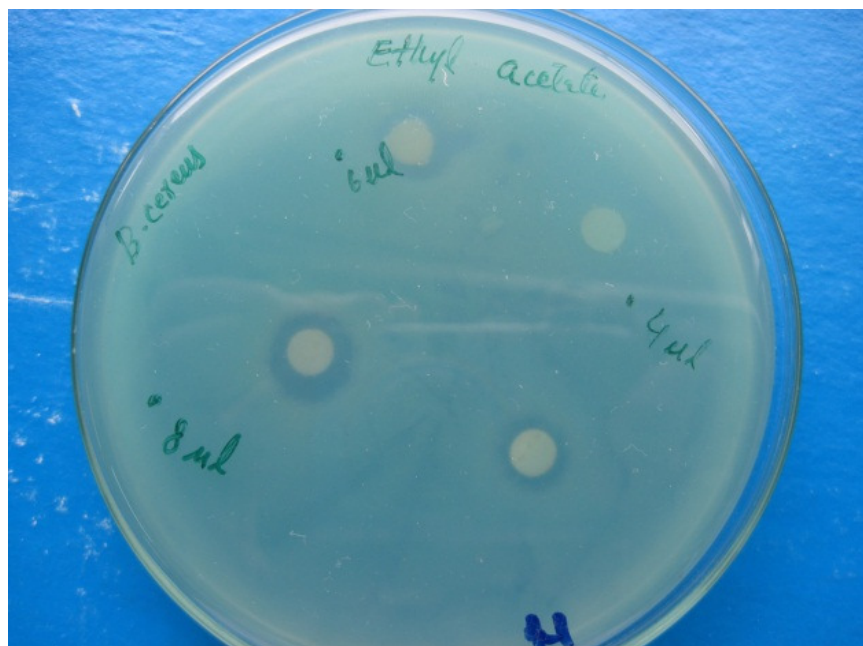


Plate 3. Antibacterial activity of ethyl acetate extracted samples of *Linum usitatissimum* against *Bacillus cereus*.

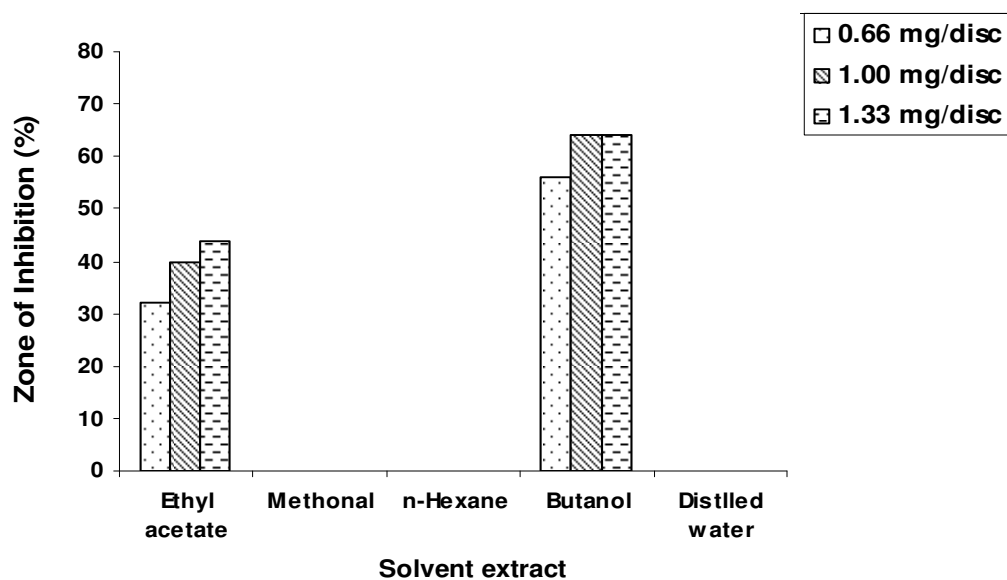


Figure 2. Antibacterial activity of ethyl acetate, methanol, n-hexane, butanol and distilled water extracted samples from *L. usitatissimum* against *B. cereus*.

results were also reported by Seher et al. (2006), Borchard et al. (2009) and Kaithwas et al. (2011).

The data revealed that ethyl acetate and butanol extracted sample were more effective against *B. cereus*. In the case of ethyl acetate extracted samples, the highest inhibition of 44% occurred at concentration of 1.33 mg disc⁻¹ while butanol extracted samples reduced growth of *B. cereus* by 64% at maximum concentration of

1.33 mg disc⁻¹ (Plate 3). It is also clear from the data that n-hexane, methanol and distilled water extracted samples did not inhibit the activity of *B. cereus* (Figure 2). Seher et al. (2006) reported that metonalic extract from linseed was effective against Gram positive and Gram negative bacteria.

Analysis of the data indicated that distilled water, ethyl acetate and butanol extracted samples were more

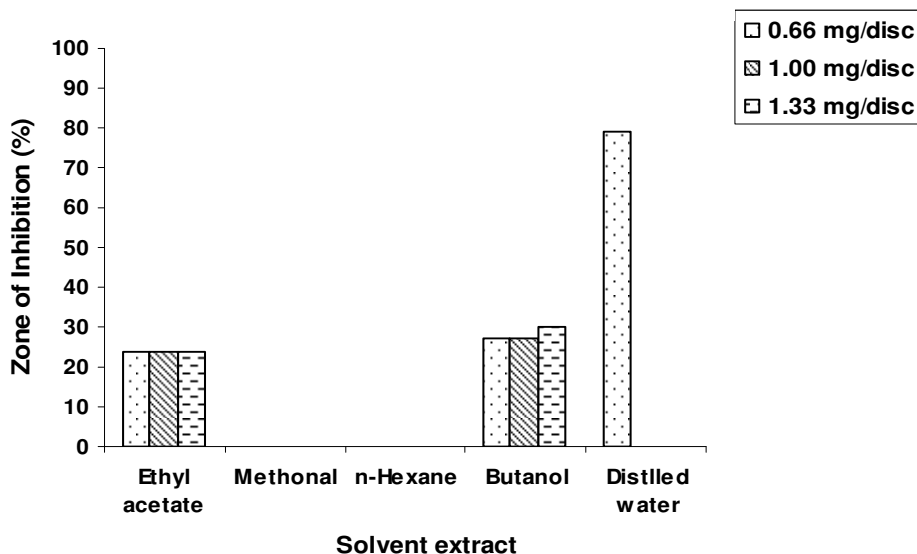


Figure 3. Antibacterial activity of ethyl acetate, methanol, n- hexane, butanol and distilled water extracted samples from *L. usitatissimum* against *S. typhi*.

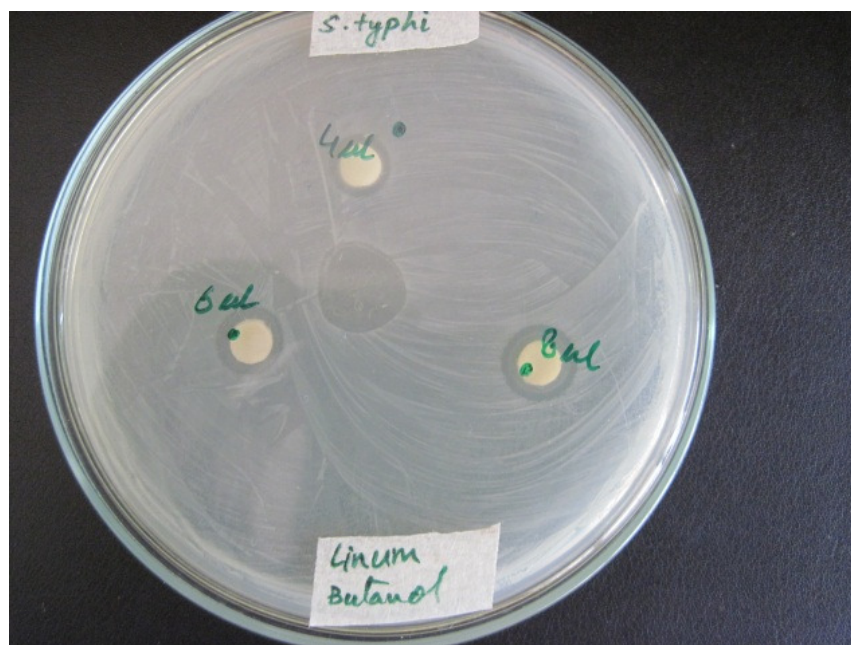


Plate 4. Antibacterial activity of butanol extracted samples of *L. usitatissimum* against *S. typhi*.

efficient against *S. typhi* when compared with other solvent extracted samples (Figure 3). Ethyl acetate extracted samples equally inhibited the growth of *S. typhi* at all concentrations. Similarly, butanol extracted samples reduced the growth of *S. typhi* by 30% at 1.33 mg disc⁻¹. The data further suggested that distilled water extracted samples reduced growth by 79% at 0.66 mg disc⁻¹ (Plates 4 and 5). These results agree with those reported by Gopalakrishnan et al. (2000). Ethyl acetate and butanol

extracted samples had a profound inhibition effect against *Klebsiella pneumoniae*. In the case of ethyl acetate, maximum inhibition (34% Z.I) occurred at 1.33 mg disc⁻¹. Similarly, butanol extracted samples reduced the growth of *K. pneumoniae* by 45% at 1.33 mg disc⁻¹ (Plates 6 and 7). It is also evident from the data that n-hexane, methanol and distilled water extracted samples did not inhibit the growth of *K. pneumoniae* (Figure 4).

These findings are in agreement with Vidya and

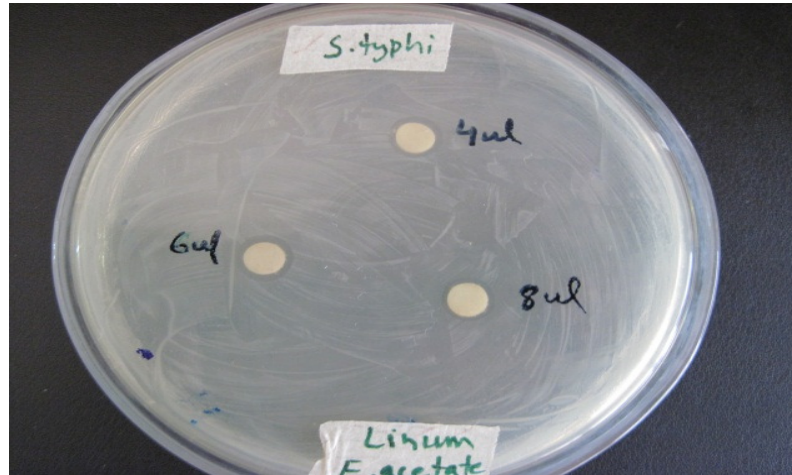


Plate 5. Antibacterial activity of ethyl acetate extracted samples of *L. usitatissimum* against *S. typhi*.



Plate 6. Antibacterial activity of butanol extracted samples of *L. usitatissimum* against *K. pneumonia*.



Plate 7. Antibacterial activity of ethyl acetate extracted samples of *L. usitatissimum* against *K. pneumonia*.

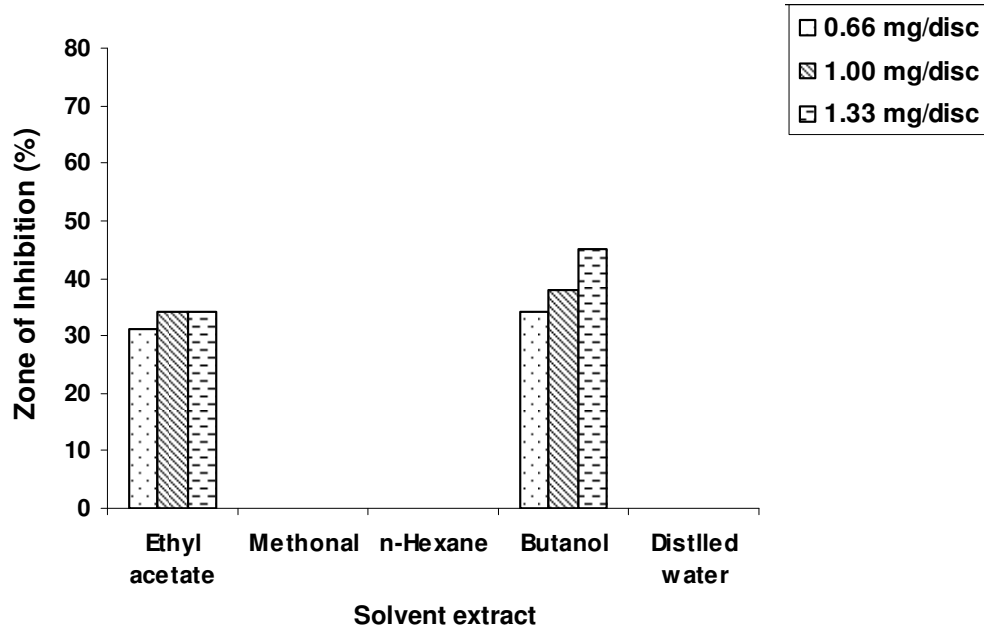


Figure 4. Antibacterial activity of ethyl acetate, methanol, n- hexane, butanol and distilled water extracted samples from *L. usitatissimum* against *K pneumoniae*.

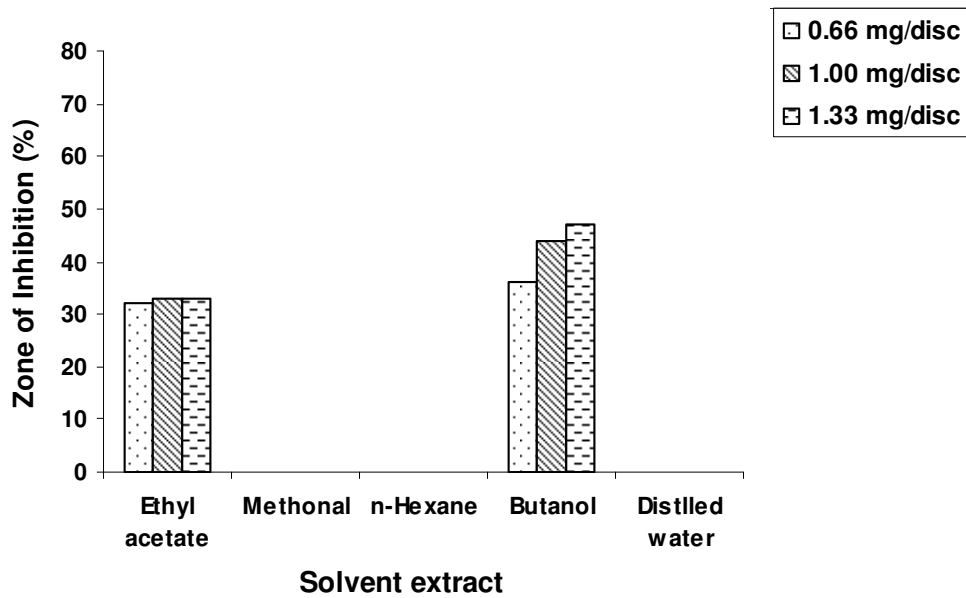


Figure 5. Antibacterial activity of ethyl acetate, methanol, n- hexane, butanol and distilled water extracted samples from *L. usitatissimum* against *P. aeruginosa*.

Parimala (2000). It can be seen from the data shown in Figure 5 that ethyl acetate and butanol extracted samples showed inhibition against *Pseudomonas aeruginosa*. Maximum inhibition of 47% occurred at 1.33 mg disc⁻¹ by ethyl acetate extracted samples while butanol extracted samples recorded 33% reduction in growth (Plate 8). Borchard et al. (2009) revealed that methonal extracts

from linseed inhibited the growth of *P. aeruginosa*. The data further reveal that n-hexane and distilled water extracted samples were ineffective against *P. aeruginosa*. Afolayan et al. (2002) observed that distilled water and acetone extracted samples did not inhibit the growth of Gram-negative bacterium.

Analysis of the data also revealed that ethyl acetate

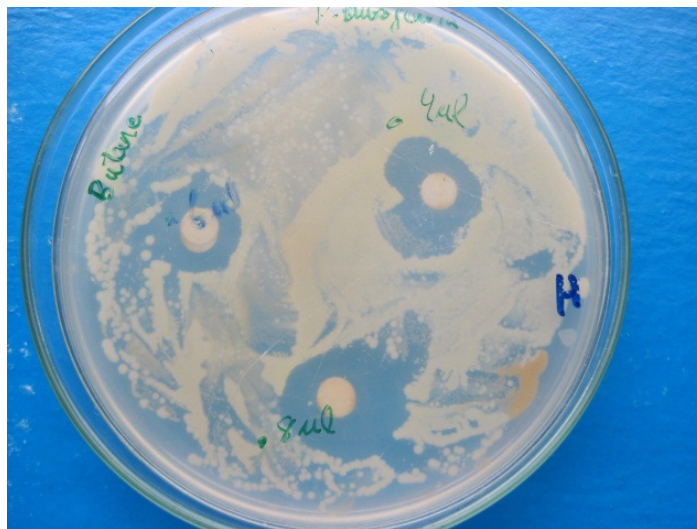


Plate 8. Antibacterial activity of butanol extracted samples of *L. usitatissimum* against *P. aeruginosa*

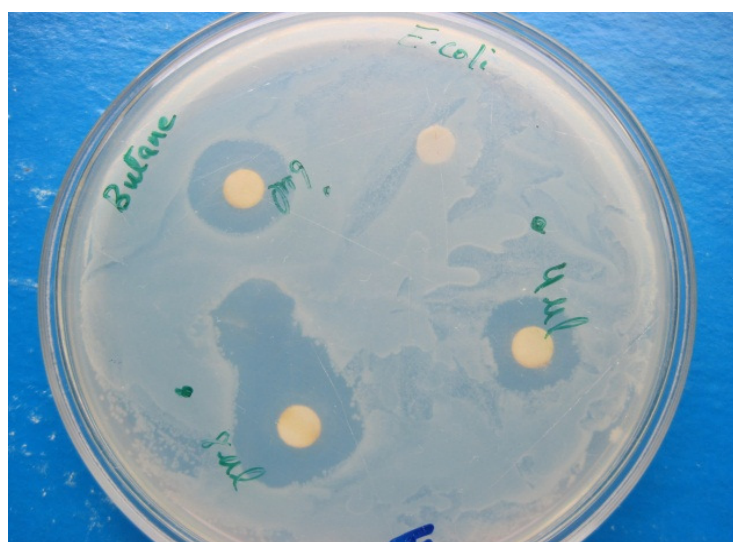


Plate 9. Antibacterial activity of butanol extracted samples of *L. usitatissimum* against *E. coli*.

and butanol extracted samples were effective against *Escherichia coli*. Ethyl acetate extracted samples had a profound inhibitory effect at $1.33 \text{ mg disc}^{-1}$ (37% Z.I) when compared with their profound positive and negative controls. In the case of butanol extracted samples, maximum inhibition was noted at concentration of $1.33 \text{ mg disc}^{-1}$ (47% Z.I; Plates 9 and 10). Methanol, n-hexane and distilled water extracted samples did not showed any reduction in the growth of *E. coli*, as shown in Figure 6. Our results are contradictory to those of Borchard et al. (2009) who revealed that methonal extracts from linseed inhibited the growth of *E. coli*. The data also reveal that only ethyl acetate and butanol extracted samples showed

inhibition against *C. albicans* when compared with other samples. Ethyl acetate extracted samples reduced the growth of *C. albicans* by 37% at $1.33 \text{ mg disc}^{-1}$ when compared with their respective positive and negative controls. In the case of butanol, maximum reduction in growth of *C. albicans* occurred at $1.33 \text{ mg disc}^{-1}$ (41% Z.I). The data further suggest that n-hexane, methanol and distilled water extracted samples did not inhibit the growth of *C. albicans* at any concentrations (Figure 7). On the other hand, Borchard et al. (2009) reported that methonal extracts from linseed inhibited the growth of *C. albicans*. Ethyl acetate and butanol extracted samples inhibited the growth of *E. carotovora* by 42 and 65%

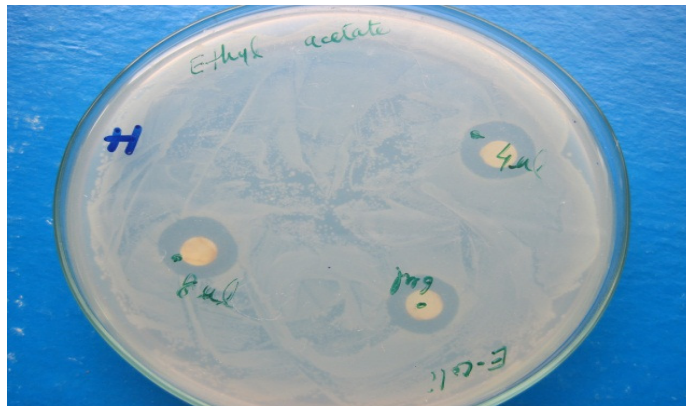


Plate 10. Antibacterial activity of ethyl acetate extracted samples of *L. usitatissimum* against *E. coli*

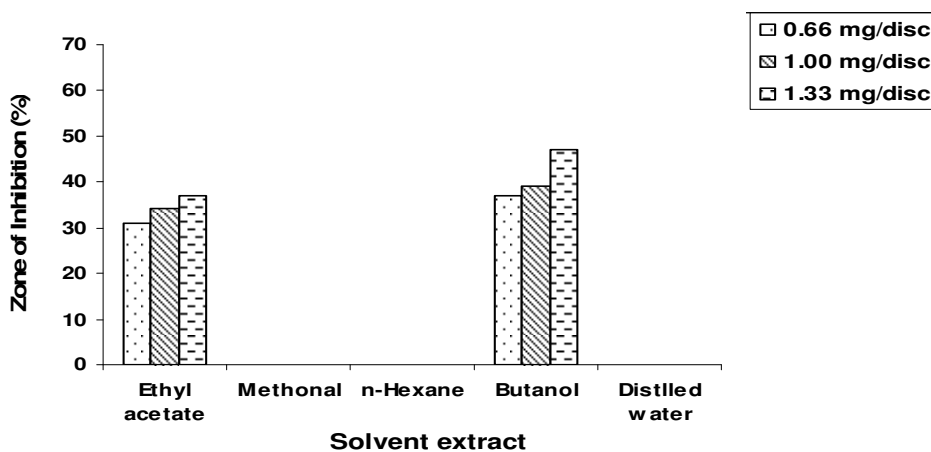


Figure 6. Antibacterial activity of ethyl acetate, methanol, n- hexane, butanol and distilled water extracted samples from *L. usitatissimum* against *E. coli*.

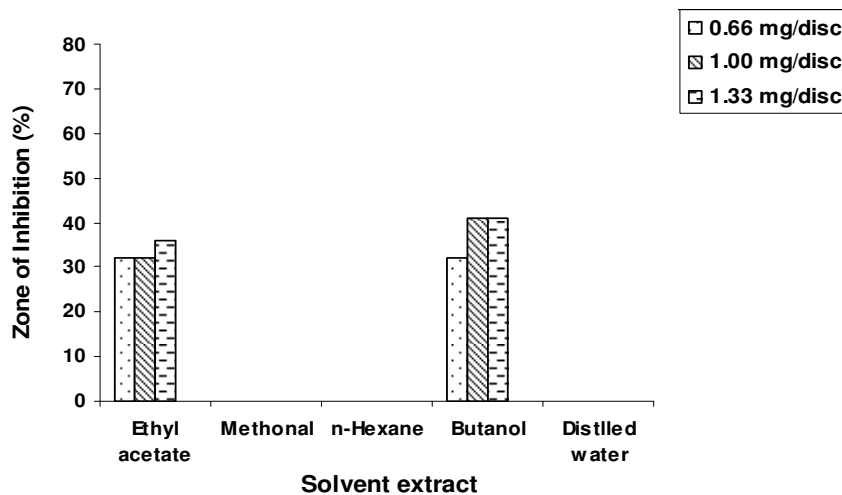


Figure 7. Antibacterial activity of ethyl acetate, methanol, n- hexane, butanol and distilled water extracted samples from *L. usitatissimum* against *C. albicans*

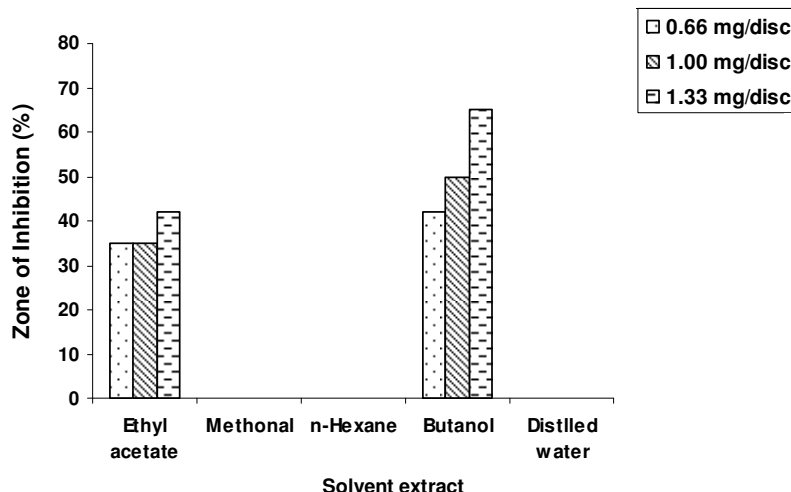


Figure 8. Antibacterial activity of ethyl acetate, methanol, n-hexane, butanol and distilled water extracted samples from *Li. usitatissimum* against *E. carotovora*.

respectively at concentration of 1.33 mg disc⁻¹. It can also be seen from the data that n-hexane, methanol and distilled water extracted samples did not inhibit the growth of *E. carotovora* at any concentrations (Figure 8).

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