

In vitro* Antimicrobial Potential of Samples From the Leaves and Fruits of Medicinally Important *Melia azedarach

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(received October 3, 2018; revised May 11, 2019; accepted June 20, 2019)

Abstract. Different solvent extracted samples obtained from the leaves and fruits of *Melia azedarach* were investigated for their antibacterial and antifungal activities. All the extracts from both tissues sources showed varying degrees of antimicrobial activities. Ethyl acetate and n-butanol extracts of both tissues inhibited the growth of the tested micro-organisms at all concentrations. N-hexane extracted fractions of the leaves reduced the activity of *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* at all concentrations, while no growth inhibition was recorded against *Klebsiella pneumonia* and *Candida albicans*. Crude methanolic extract from the leaves tissues showed activity against all the tested microbial species and highest susceptibility was noted against *Bacillus subtilis*. In case of leaves, the most susceptible bacteria were *Bacillus subtilis* (gram positive) and *Klebsiella pneumonia* (gram negative) was the most resistant one. In case of fruits extracts, the most susceptible bacteria were *Klebsiella pneumonia* (gram negative) and *Escherichia coli* (gram negative) was the most resistant one.

Keywords: antibacterial, antifungal, zone of inhibition, *Melia azedarach*

Introduction

The use of plants as herbal remedies has been documented by Chinese, Greeks, Romans, Egyptians, Syrians and Indians since 2800 B.C (Nessler *et al.*, 1985). More than 50% of the deaths in tropical countries are resulted from various infectious diseases (WHO, 2005). Over the years, various pharmaceutical industries are constantly engaged to develop different antibiotics to cure these infectious diseases. However, in response to these synthetic drugs, microbes are also adopting different strategies and developing fast resistance (Hart and Karriuri, 1998). Therefore, these days the main goal of many pharmaceutical companies is to search plant based novel and cheap bioactive compounds with fewer side effects. About 25 to 45 percent of current prescriptions possess important bio-compounds obtained from plants as a major source of ingredients (Subramanian *et al.*, 2009). Research shows that plants possess therapeutic effects due to different bio-active compounds produced by the plants (Khan and Khan, 2003). Hundreds of plants species have been investigated for their medicinal properties, however, most of them have not been scientifically studied (Mahesh and Satish, 2008). Different parts of a number of medicinally important plants are employed as extract for raw drugs

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having number of curative characteristics including antimicrobial activity (Bakht *et al.*, 2018; Bilal *et al.*, 2018; Ayaz *et al.*, 2018; 2017; Wajid *et al.*, 2017).

Melia azedarach, a member of family *Meliaceae*, is an important therapeutic plant present in Pakistan, Bangladesh, Southeast Asia, Australia, India and Indochina. Leaves are used to cure leprosy and as anthelmintic, diuretic, insecticide etc. Roots of the same plant are used as de-obstruent and resolvent. It is also used as antiseptic against ulcers and sores, rheumatism, skin diseases and malaria, analgesic, anti-cancerous, antiviral and antispasmodic (Biswas *et al.*, 2002).

Materials and Methods

Plant material. The present study was conducted at IBGE, The University of Agriculture Peshawar Pakistan. Plant materials (leaves and fruits) of *Melia azedarach* were obtained from the research farms of the University of Agriculture Peshawar, KPK, Pakistan. The plant specimen was identified at the Department of Botany, University of Peshawar. After collection, the plant materials were washed thoroughly with distilled water, shade dried and grounded with electric grinder.

Crude extract preparation, fractionation and antimicrobial activity. Crude extract preparation, fractionation

and antimicrobial activity (Table 1) was carried out as described by Bakht *et al.* (2018).

Statistical analysis. Data are presented as mean values of three replications. MSTATC computer software was used to carry out statistical analysis (Russel and Eisensmith, 1983). Least Significant Difference (LSD) test was employed to compare significant difference among means (Steel *et al.*, 1997).

Table 1. Microbial strains used during the experiment

Microbial species	Gram strain type	Details of the microbial strains used
<i>Klebsiella pneumonia</i>	Negative	Clinical isolate obtained from Department of the Microbiology, Quaid-I-Azam University Islamabad Pakistan
<i>Pseudomonas aeruginosa</i>	Negative	ATCC # 9721
<i>Staphylococcus aureus</i>	Positive	ATCC # 6538
<i>Bacillus subtilis</i>	Positive	Clinical isolate obtained from the Department of Microbiology, Quaid-I-Azam University Islamabad Pakistan
<i>Escherichia coli</i>	Negative	ATCC # 25922
<i>Candida albicans</i>		ATCC # 10231. Plant Pathology Department, The University of Agriculture Peshawar KPK Pakistan

Results and Discussion

Antimicrobial activity of samples from leaves. *Bacillus subtilis* showed highest susceptibility to crude methanolic extract (Fig. 1). Crude methanolic extract revealed highest inhibitory activity (70% ZI) at concentration of 3 mg/disc followed by 63% and 57% ZI at 2 and 1 mg/discs respectively when compared with control (DMSO). N-hexane extracted fractions showed 57% inhibitory zone at 3 mg/discs. Aqueous extracted sample was active at 3 mg/discs, however, showed no activity against *B. subtilis* at lower concentrations of 1 and 2 mg/discs. N-butanol and ethyl acetate samples showed the lowest activity of 22% ZI and 28% ZI at 1 mg/discs concentrations respectively. *Staphylococcus aureus* showed highest susceptibility (67% ZI) to n-butanol samples followed by ethyl acetate and n-hexane fractions (63% and 51% ZI respectively) at 3 mg/discs. Aqueous extracted fractions showed no inhibitory activity, while crude methanolic extracts obtained from the leaves

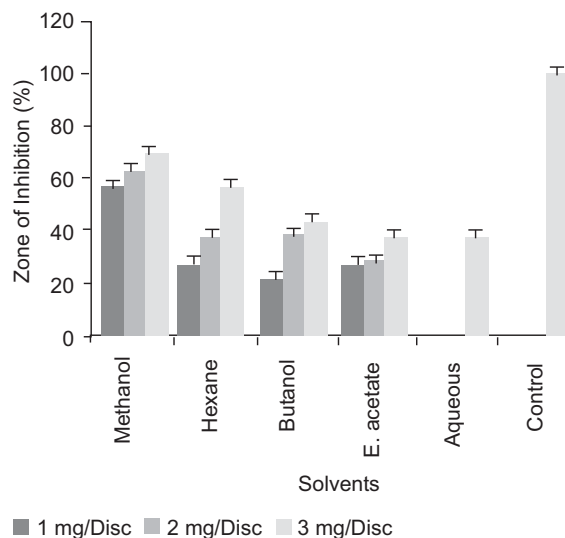


Fig. 1. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the leaves of *Melia azedarach* against *B. subtilis* by disc diffusion assay (Control = DMSO; Bar shows LSD at $P < 0.05$).

showed minimum inhibitory activity of 28% at 1 mg/discs (Fig. 2).

N-hexane samples showed highest inhibitory zones of 48% and 47% against *Escherichia coli* at 3 and 2 mg/disc respectively. Crude methanol, ethyl acetate and n-butanol samples revealed 39% each inhibitory activity at concentration 3 mg/disc against the same microbe. No inhibition was recorded in response to aqueous extracts, while lowest inhibitory zone was measured by n-butanol extracted samples (22% ZI each) at 1 and 2 mg/disc (Fig. 3). All the extracts showed inhibitory activity against *Klebsiella pneumonia* except n-hexane and aqueous samples. *Klebsiella pneumonia* showed maximum susceptibility to n-butanol samples (55% ZI) at 3 mg/disc followed by 44% inhibition measured by n-butanol at 2 mg/disc. Minimum inhibitory zone of 21% was revealed by crude methanolic extract at 1 mg/disc (Fig. 4). n-butanol and ethyl acetate samples reduced the growth of *Pseudomonas aeruginosa* by 52% ZI each at 3 mg/disc (Fig. 5). Crude methanolic extracts showed least inhibitory activity of 23% at 1 mg/disc and no activity by aqueous extracted fractions at all the tested concentrations. Similarly, inhibitory activity of 41% was recorded by methanol extracted samples at 3 mg/disc. *Candida albicans* was highly susceptible to ethyl acetate samples (54% ZI each) at 2 and 3 mg/disc followed by

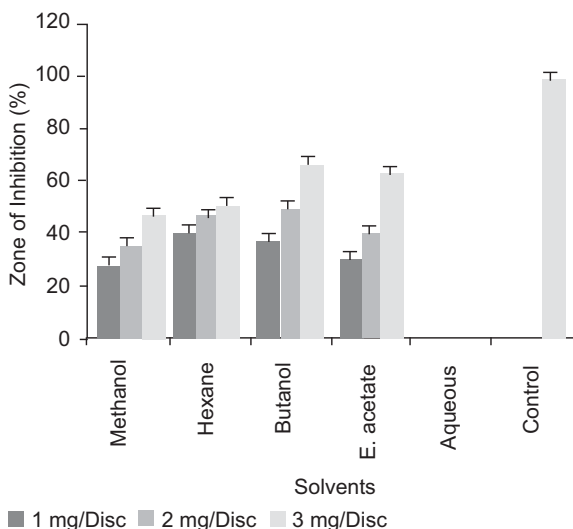


Fig. 2. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the leaves of *Melia azedarach* against *S. aureus* by disc diffusion assay (Control = DMSO; Bar shows LSD at P<0.05)..

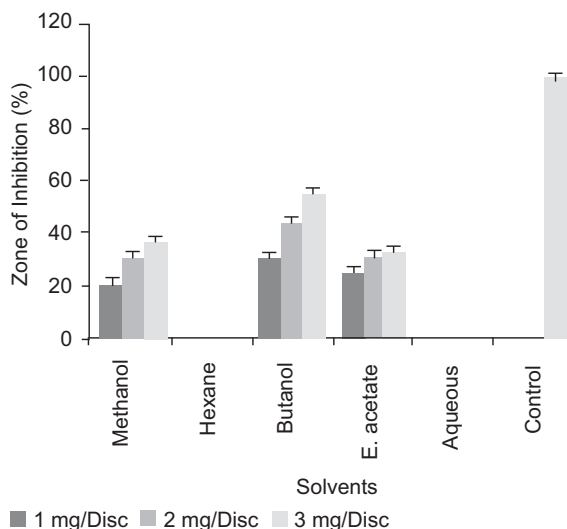


Fig. 4. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the leaves of *Melia azedarach* against *K. pneumoniae* by disc diffusion assay (Control = DMSO; Bar shows LSD at P<0.05).

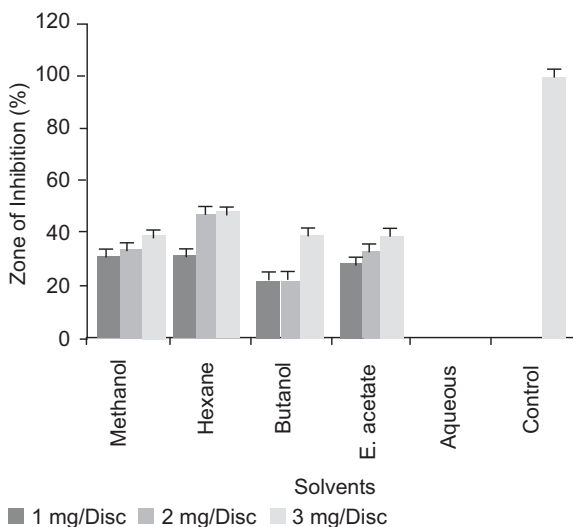


Fig. 3. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the leaves of *Melia azedarach* against *E. coli* by disc diffusion assay (Control = DMSO; Bar shows LSD at P<0.05).

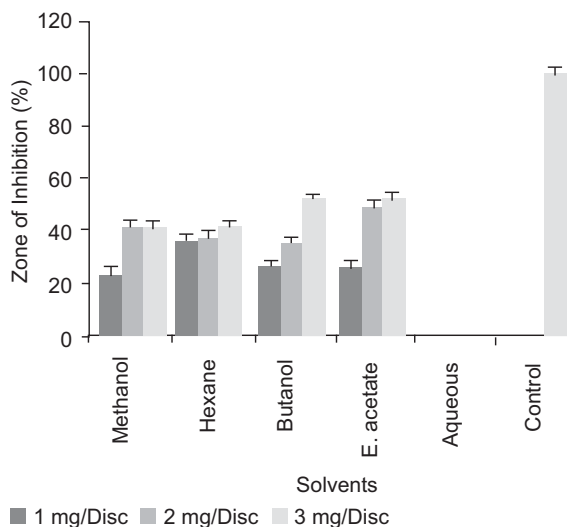


Fig. 5. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the leaves of *Melia azedarach* against *P. aeruginosa* by disc diffusion assay (Control = DMSO; Bar shows LSD at P<0.05).

n-butanol and crude methanolic extracts (47% and 41 % ZI respectively) at the concentration. N-hexane and aqueous samples did not control the growth of *C. albicans* at all the tested concentrations (Fig. 6).

Antimicrobial activity of samples from the fruits. All the solvent extracted samples were effective against *B. subtilis* and inhibited its growth at all concentrations (Fig. 7). Ethyl acetate extracted fraction reduced the

growth of *B. subtilis* by 63% at highest concentration. Crude methanolic extracts and ethyl acetate fractions inhibited the growth of the same microbe by 57% each at 3 and 2mg/disc. n-butanol reduced the growth of the

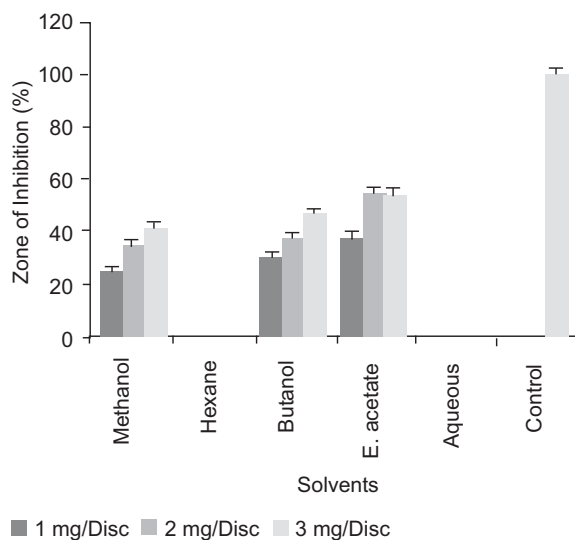


Fig. 6. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the leaves of *Melia azedarach* against *C. albicans* by disc diffusion assay (Control = DMSO; Bar shows LSD at $P<0.05$).

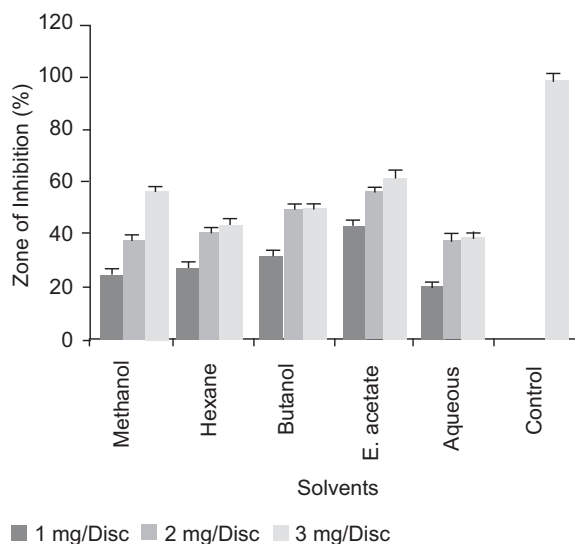


Fig. 7. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the fruits of *Melia azedarach* against *B. subtilis* by disc diffusion assay (Control = DMSO; Bar shows LSD at $P<0.05$).

tested microbe by 50% each at 2 and 3 mg/disc. The lowest inhibitory zone was measured for aqueous extracted samples (20% ZI) at 1 mg/disc. Ethyl acetate fraction was effective to control the growth of *S. aureus* by 60% and 57% ZI at 3 and 2 mg/disc respectively. n-butanol fractions reduced the growth of *S. aureus* by 54% at 3 mg/disc. Lowest inhibitory zone was measured by n-hexane and aqueous samples (23% ZI each) at concentration of 1 mg/disc and crude methanolic extract did not control the growth at lower concentrations of 1 and 2mg/disc (Fig. 8).

Ethyl acetate fractions exhibited the highest inhibitory activity against *E. coli* (60% ZI) at 3 mg/disc followed by 59% activity at 2 mg/disc. n-butanol samples showed 48% and 42% inhibitory activity at 3 and 2 mg/disc respectively. No activity was shown by aqueous samples. Crude methanolic extract though reduced the growth of the tested bacterium at 2 and 3 mg/disc, however, did not affect the growth at lower concentration of 1 mg/disc. Minimum of 22% ZI each was recorded for hexane crude methanolic and ethyl acetate samples at lower concentration of concentration of 1mg/disc (Fig. 9). All the extracts obtained from the fruits inhibited the growth of *K. pneumonia* at different degrees. n-butanol extracted samples reduced the growth of the tested microbe by 80% and 64% at 3 and 2 mg/disc respectively.

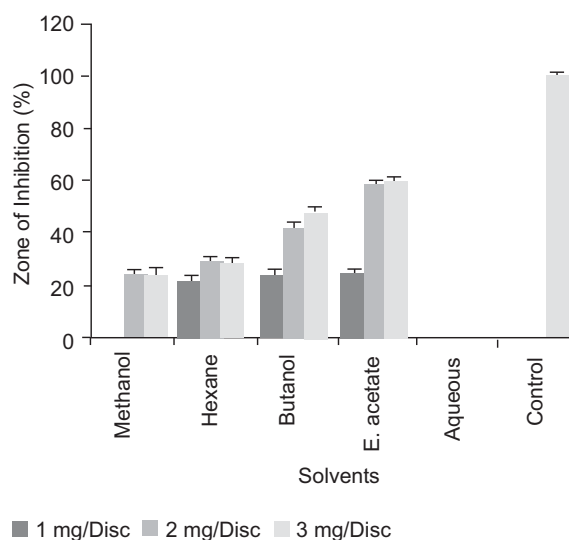


Fig. 8. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the fruits of *Melia azedarach* against *S. aureus* by disc diffusion assay (Control = DMSO; Bar shows LSD at $P<0.05$).

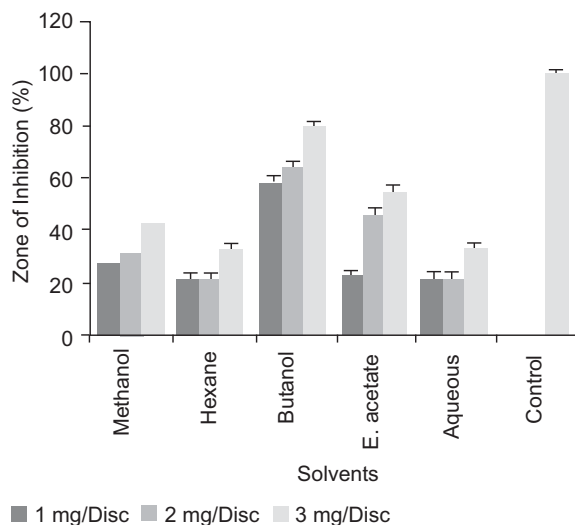


Fig. 9. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the fruits of *Melia azedarach* against *E. coli* by disc diffusion assay (Control = DMSO; Bar shows LSD at $P<0.05$).

Ethyl acetate samples also showed good activity of 55% ZI when applied in concentration of 3 mg/disc. Minimum growth inhibition of 21% was measured for aqueous and n-hexane samples at 1 and 2 mg/disc respectively (Fig. 10). *P. aeruginosa* was highly susceptible to n-butanol extracts (58% ZI) at 3 mg/disc. Ethyl acetate samples showed 52% inhibitory zone each at higher concentration of 2 and 3 mg/disc. n-hexane samples showed minimum inhibitory zone of 27% at 1 mg/disc (Fig. 11) Ethyl acetate fractions showed maximum inhibitory activity of 88% at 3 mg/disc followed by 60% inhibitory activity at lower concentrations. Fig. 12. The lowest inhibitory activity against the same fungal specie was measured for crude methanolic extracts (23% ZI) at 1 mg/disc.

The anti-bacterial activity of different solvent extracted samples from the leaves of *Melia azedarach* revealed that crude methanolic extracts effectively reduced the growth of *B. subtilis* at highest concentrations. N-hexane extracted fraction showed good activity at concentration of 3 mg/discs Though aqueous extracted fractions was active at highest concentrations of 3 mg/discs, however, no activity was recorded against *B. subtilis* at lower concentrations. n-butanol and ethyl acetate samples showed the lowest activity at 1 mg/discs. Khan *et al.* (2008) reported that ethyl acetate samples exhibited highest inhibitory activity followed by crude

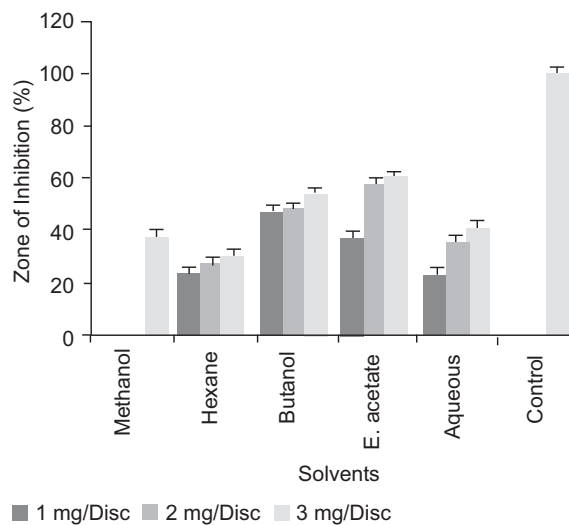


Fig. 10. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the fruits of *Melia azedarach* against *K. pneumoniae* by disc diffusion assay (Control = DMSO; Bar shows LSD at $P<0.05$).

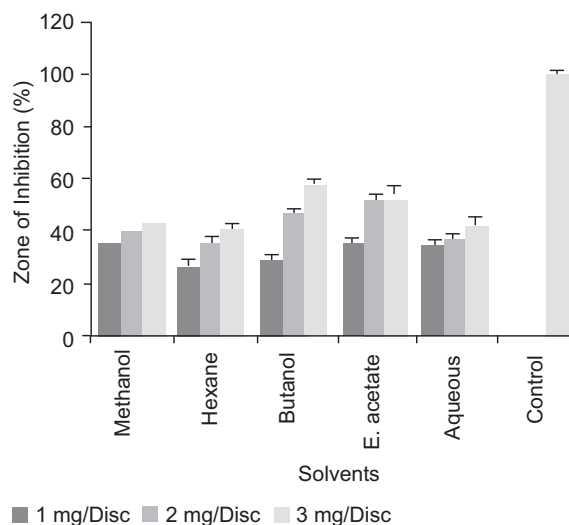


Fig. 11. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the fruits of *Melia azedarach* against *P. aeruginosa* by disc diffusion assay (Control = DMSO; Bar shows LSD at $P<0.05$).

methanolic extract at different concentrations. *S. aureus* had more susceptibility to n-butanol extracted samples followed by ethyl acetate and n-hexane extracted fractions at highest concentrations. Aqueous extracted

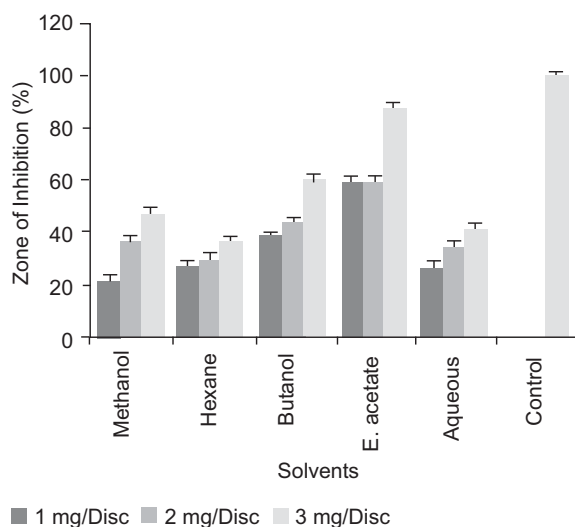


Fig. 12. Antibacterial activity of crude methanol, n-hexane, butanol, ethyl acetate and water extracted samples from the fruits of *Melia azedarach* against *C. albicans* by disc diffusion assay (Control = DMSO; Bar shows LSD at $P < 0.05$).

fractions measured no zone of inhibition and crude methanolic extracts showed minimum inhibitory activity at lowest concentration. Similar results are also reported by Ramya *et al.* (2009) and Khan *et al.* (2011). n-hexane samples measured maximum zone of inhibition against *E. coli* at 3 mg/disc. Ethyl acetate, methanol and n-butanol samples also reduced the growth of the tested microbe at the highest concentrations. No inhibition was measured by aqueous extracts and minimum activity was recorded by n-butanol samples at lower concentrations. All samples showed inhibitory activity against *K. pneumonia* except n-hexane and aqueous extracted samples and measuring no activity at all the tested concentrations. *K. pneumonia* was more susceptible to n-butanol extracted samples at highest concentrations. These results agree with Khan *et al.* (2008) and Ramya *et al.* (2009). Analysis of the data indicated that n-butanol and ethyl acetate extracted samples were very effective to control activity of *P. aeruginosa*. Crude methanolic extracts on the other hand, measured least inhibitory activity at lowest and no activity was recorded by aqueous extracted fractions at all the tested concentrations. *C. albicans* was highly susceptible to ethyl acetate samples at higher concentrations followed by n-butanol and crude methanolic extracts. n-hexane and aqueous samples

were ineffective against *C. albicans* at all the tested concentrations.

Antimicrobial activity of different solvent extracted samples from the fruits of *Melia azedarach* against *B. subtilis* indicated that increasing concentration of different extracts increased the activity against the tested microbe. All the solvent extracted samples were effective to reduce the growth of *Bacillus subtilis* at all the three concentrations. Ethyl acetate extracted fraction was more effective to control the growth of *B. subtilis* 3 mg/disc concentrations. Crude methanolic extracts n-butanol and, n-butanol extracted fractions also control the activity of the same microbe at 3 and 2 mg/disc concentrations. The lowest inhibitory zone was noted for aqueous extracted samples at concentration of 1 mg/disc) when compared with control. Ethyl acetate extracted fraction was more effective to reduce the activity of *S. aureus* at 3 and 2 mg/disc concentrations. N-butanol extracted fractions also reduced the growth of the *S. aureus* applied in concentration of 3 mg/disc. Lowest inhibitory zone was noted for n-hexane and aqueous extracted samples at concentration of 1 mg/disc. Crude methanolic extract was did not control the growth at lower concentrations of 1 and 2 mg/disc concentration showing 0% ZI.

Ethyl acetate fractions from the fruits measured highest inhibitory activity at highest concentration against *E. coli* and n-butanol samples also reduced the activity of the same microbe at higher concentrations. No activity was shown by aqueous extracted samples. Crude methanolic extract though reduced the growth of the tested microbe at higher concentration however, did not show any activity at lowest concentration. n-butanol samples effectively controlled the growth of *K. pneumonia* compared with other fractions and controls n-butanol samples also reduced the growth of the tested microbe at higher concentrations. Ethyl acetate samples also showed good activity when applied in concentration of 3 mg/disc. Minimum growth inhibition was measured for aqueous and n-hexane extracted samples at lower concentration. All the samples from the fruits caused maximum reduction in the growth of *P. aeruginosa* at all concentrations. *P. aeruginosa* was highly susceptible to n-butanol extracts at highest concentration. Ethyl acetate samples also showed good activity at higher concentration. Antimicrobial activity of n-hexane, ethyl-acetate, n-butanol, methanol and aqueous extracted samples from the fruits of *Melia azedarach* against *C. albicans* suggested that ethyl acetate extracted fractions

showed maximum antimicrobial activity at highest concentration. The lowest inhibitory activity against the same fungus was noted for crude methanolic extracts at lowest concentration. These results agree with of the findings Khan *et al.* (2008).

Conclusion

The tested micro-organisms showed high sensitivity to n-butanol and ethyl acetate samples derived from the leaves and fruits. Against *B. subtilis*, crude methanolic samples of leaves showed the highest activity as compared to other extracts. All the tested microbes were resistant to aqueous extract of leaves and moderate sensitivity was recorded in response to aqueous extract of the fruits. Ethyl acetate samples obtained from the fruits showed high antimicrobial activity. Samples from fruits showed maximum activity as compared to leaves. *K. pneumonia* and *C. albicans* were resistant to n-hexane and aqueous extracts of leaves and all the tested microbes showed moderate sensitivity to n-hexane extracts of fruits.

Conflict of Interest. The authors declare no conflict of interest.

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