

Impact of different solvent extracts from leaves and fruits of *Eucalyptus globulus* on growth of different bacteria and fungi

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Abstract: The present research investigates the antimicrobial activities of the samples extracted from the leaves and fruits of *Eucalyptus globulus* through disc diffusion susceptibility assay using 1, 2 and 3mg disc⁻¹ concentrations. Different extracted samples from the leaves and fruits of *Eucalyptus globulus* exhibited different degrees of antimicrobial. The data indicated that n-butanol and ethyl acetate extracted fraction of both the leaves and fruits inhibited the growth of all microorganisms at all the tested concentrations. Aqueous extracted sample of the leaves inhibited the growth of *Candida albicans* while the same fraction from the fruits showed no activity against *Bacillus subtilis* at any concentration. N-hexane extracted samples of the leaves inhibited the growth of *Bacillus subtilis*, *E. coli* and *Pseudomonas aeruginosa* at the tested concentrations while no activity was recorded against *Klebsiella pneumonia*, *Candida albicans* and *Staphylococcus aureus*. N-butanol extracted samples from the leaves and fruits showed activity against *Pseudomonas aeruginosa* at the tested concentrations. In case of leaves, the most susceptible bacteria was *Bacillus subtilis* (gram positive) and *Staphylococcus aureus* (gram positive) was the most resistant one. In case of fruits the most susceptible bacteria was *Staphylococcus aureus* (gram positive) and *E. coli* (Gram negative) was the most resistant one.

Keywords: Antibacterial activity, anti-fungal activity, disc diffusion assay, *Eucalyptus globulus*.

INTRODUCTION

Plants produce a wide range of compounds which give them characteristic pigment, odor, flavor and may also have antimicrobial properties (Cowan, 1999). For many years, medicines derived from traditional plants have been used in many parts of the world (Chariandy *et al.*, 1999; Bhavnani and Ballow, 2000). Major portion of the research work on the use of traditional medicinal plants are under way on plants of Asian (Patwardhan, 2005), African (Hostettmann *et al.*, 2000) and South America (Paz *et al.*, 1995) origin. Medicines derived from plants are important therapeutic weapons to cure human diseases (Bakht *et al.*, 2013 a, b, c; Bilal *et al.*, 2018, Khaleeq *et al.*, 2018). Medicinal plants having pharmacological properties are used in developing unique therapeutic agents. Therefore, worldwide attention has been moved towards the identification and isolation of novel bio-molecules for the development of new drugs. Herbal medicines are cost effective and with less side effects (Verpoorte, 1998; Ayaz *et al.*, 2017, 2018). Medicinal plant contains different bio-active compounds and can be used as such or mixed with other bio-active compound for their effectiveness. Presently herbal products and extracts are extensively used to control various human diseases (Srinivasan *et al.*, 2006). Drugs derived from medicinal plants owe the advantage of being simple, effective and exhibit broad spectrum activity (Chin *et al.*, 2006). The worldwide attention in therapeutic potential of

phytochemicals during the last decades is therefore, quite obvious. Bacterial resistance is increasing to the presently available antibiotics day by day so it is important to search for new antimicrobial agents.

Eucalyptus is botanically known as *Eucalyptus globules* belong to the family Myrtaceae. More than 700 species of this family are native to Australia and a small number of species of the same family are found in New Guinea, Indonesia and the Phillipines. *Eucalyptus* are important for their wood and some are also valuable sources of proteins, tannins, gum, and dyes, though their most valuable product is the eucalyptus oil that is readily distilled from their leaves (Trivedi and Hotchandani, 2004; Sartorelli *et al.*, 2007). The oil is antiseptic, so it is used medicinally as a decongestant for treating catarrh, bronchitis and influenza. It is also used in liniments for bruises, sprains and muscular pains, and to make herbal tea infusions.

Eucalyptus is used to control numerous diseases resulting from microbial infections. The Aborigines (native Australians) usually use eucalyptus leaves to heal wounds and fungal infections. Leaf extracts of eucalyptus have been permitted as food additives. These extracts are also currently used in cosmetic formulations. Research data has demonstrated that the extracts exhibited various biological effects, such as antibacterial, anti-hyperglycemic (Gray and Flatt, 1998) and antioxidant (Lee and Shibamoto, 2001) activities. It has been reported that macrocarps from *E. macrocarpa* and grandinoli

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from *E. perriniana* were effective against Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*) (Yamakoshi *et al.*, 1992). The present study was carried out with the objectives to investigate the antimicrobial potential of different solvent extracted samples from the leaves and fruits of *Eucalyptus globules*.

MATERIALS AND METHODS

Collection of plant material

The present research work was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, Pakistan. The plant material (Leaves, fruits) of *Eucalyptus globulus* was collected from the trees planted in front of the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, Pakistan during 2015. The plant material was identified at Botany Department University of Peshawar, KPK Pakistan. Three kg of leave and two kg of fruit material was carefully washed with the tap water in order to remove dust particles, kept under shade and dried for two weeks. Dried leaves and fruits were separately ground to fine powder by electric grinder.

Crude extract preparation

The powdered material of both the samples (leaves and fruits) were separately soaked in 5 liters each of methanol for 7 days and agitated three times a day for complete dissolution of the bioactive compounds. The mixture was filtered through Whatmann filter paper No.1 and the residue was mixed with three liters of fresh methanol and the whole process was repeated three times. All the filtered methanolic extracts obtained were dried at 45°C under vacuum pressure through rotary evaporator. The extract was divided into two parts; one part to be used as crude methanolic extract and the other portion was further fractionated with various solvents.

Fractionation of crude extract

Hundred gram of methanolic crude extract was mixed in five hundred ml of sterilized distilled water, mixed with 300 ml of hexane and gradually shaken. The mixture was allowed to stand for 15 minutes for separation of two phases, upper layer being hexane and the lower is aqueous phase. The upper n-hexane phase was collected and the lower aqueous phase was re-extracted thrice with fresh n-hexane. All the n-hexane fractions were pooled together and dried in rotary evaporator at 45°C in order to obtain dried extracts. The same process of fractionation was followed for ethyl acetate and n-butanol. Aqueous phase left at the end was dried using rotary evaporator at 45°C to be used as aqueous extract. The same procedure was adopted for both plant materials (leaves and fruits).

Disc diffusion susceptibility assay

Nutrient agar media was used for the culturing and growth and nutrient broth for shaking incubation and

standardization of different microorganisms. Media was prepared as described by Bakht *et al.* (2013a). To examine the antimicrobial activity of different solvent extracted samples of leaves and fruits, disc diffusion method was employed. Antibacterial activity of the extracts was evaluated by the methods of Bauer *et al.* (1966) and anti-fungal activity by Ramdas *et al.* (1998). The plates were inoculated using microbial inoculums (a standardized inoculums 1-2×10⁷ CFU/ml-1 0.5 McFarland Standard) under sterile conditions. Stock solutions of the different extracts were prepared in sterile DMSO. Three discs of Whatmann No. 1 filter paper (6 mm diameter) were placed on the Petri plates using sterilized forceps. Three volumes i.e., 6, 12 and 18µl from each stock solution were applied on discs respectively corresponding to three concentrations of the extracts i.e. 1, 2 and 3 mg per disc. The plates were labeled, properly sealed by parafilm and incubated at 37°C for 24 hours. Antibiotics were used as positive control while DMSO as negative control. After incubation, zone of inhibition were measured in millimeters in comparison with positive control. The same procedure was repeated for each micro-organism. The percent (%) inhibition was finally calculated by the following formula:

$$\% \text{ inhibition} = \frac{\text{Zone of inhibition of sample (mm)}}{\text{Zone of inhibition of standard (mm)}} \times 100$$

Standard (mm)

Positive controls

For Gram-positive bacteria; Ciprofloxacin 50µg per 6µl
For Gram negative-bacteria; Ciprofloxacin 50µg per 6µl
For Fungal strain; Fluconazole 50µg per 6µl

Negative control

DMSO was used as negative control.

Microbial strains used

Different microbial strains under study were *Klebsiella pneumonia*, *Pseudomonas aeruginosa* ATCC #9721, *Staphylococcus aureus* ATCC #6538, *Bacillus subtilis*, *Escherichia coli* ATCC #25922 and *Candida albicans* ATCC #10231.

STATISTICAL ANALYSIS

The experiment was repeated in triplicate and MSTAT computer software was used for the analysis of the data. Least Significant Difference (LSD) test was employed upon obtaining significant difference at p<0.05 (Steel *et al.*, 1997).

RESULTS

Antibacterial and antifungal activity of different solvent extracted samples from leaves of *Eucalyptus globulus*

Fig. 1 shows the anti-bacterial activity of crude methanolic, n-hexane, n-butanol, ethyl-acetate, and

aqueous extracted samples from the leaves of *Eucalyptus globulus* against *Bacillus subtilis* by disc diffusion assay. *Bacillus subtilis* showed highest susceptibility towards ethyl acetate extract. Ethyl acetate extracted fraction measured highest inhibitory activity (60% ZI) at concentration of 3mg disc⁻¹ followed by 52% and 43% ZI at 2 and 1mg discs⁻¹ respectively compared with other samples and controls. N-hexane extracted fractions on the other hand measured 30% inhibitory zone at concentration of 2 or 3mg discs⁻¹. Aqueous extracted samples showed 41% ZI each at both 3 and 2mg discs⁻¹. N-butanol and crude methanolic extracts showed the lowest activity of 22% ZI and 30% ZI at 1mg discs⁻¹ concentration respectively. The data also revealed that *Staphylococcus aureus* showed susceptibility (33% ZI) to n-ethyl acetate extracted samples followed by n- butanol and crude methanolic extracts fractions measuring 32% and 30% zone of inhibitions respectively at 3mg discs⁻¹ concentrations N-hexane fractions showed no inhibitory activity while aqueous extracted fractions obtained from the leaves reduced the growth of the same microbe 18% at 3mg disc⁻¹ when compared with controls and other samples. The data also suggested that n-hexane, n-butanol and aqueous extracted samples did not shown any activity recording 0% ZI (fig. 2).

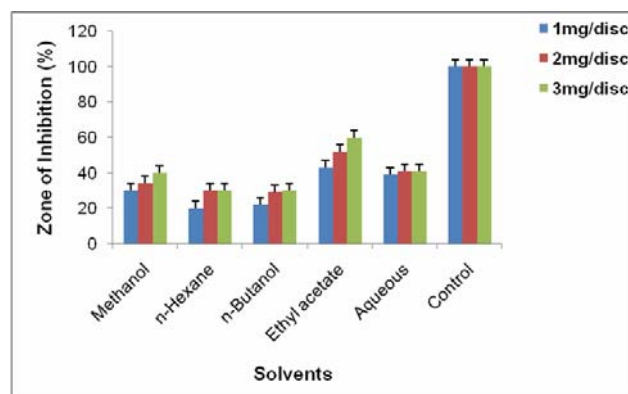


Fig. 1: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Bacillus subtilis* (Bar shows LSD values at P<0.05).

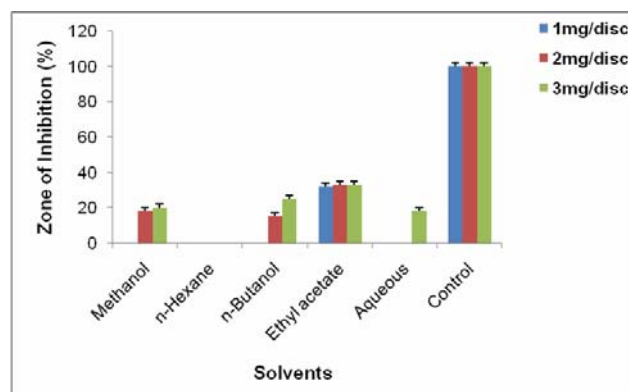


Fig. 2: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against

Staphylococcus aureus (Bar shows LSD values at P<0.05).

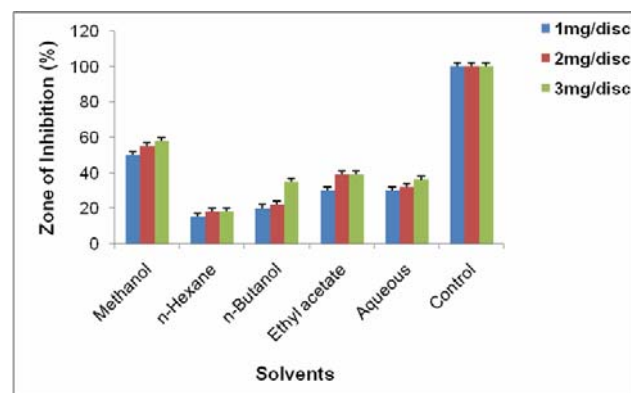


Fig. 3: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Escherichia coli* (Bar shows LSD values at P<0.05).

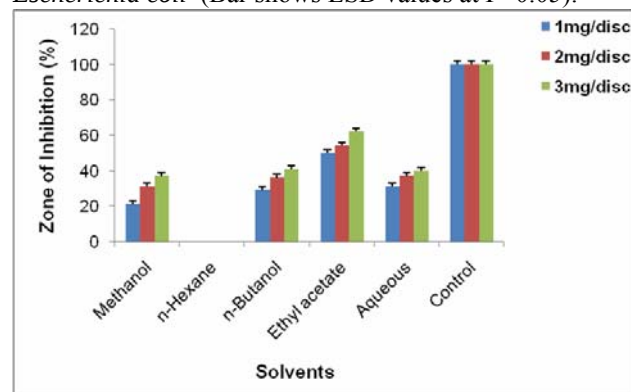


Fig. 4: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Klebsiella pneumonia* (Bar shows LSD values at P<0.05).

The results revealed that crude methanolic extract showed highest activity (58% and 55% ZI) against the same bacterium at 3 and 2mg disc⁻¹ respectively. Ethyl acetate indicated 39% inhibitory activity at concentration of 3 mg disc⁻¹ against the same microbe while aqueous extracted fraction reduced the activity by 36% at the same concentration compared with other samples and controls. The data further suggested that lowest inhibitory zone was measured by n-hexane and by n-butanol extracted samples at (15% and 20% ZI) at 1mg disc respectively compared with controls (fig. 3). All the extracts showed inhibitory activity against *Klebsiella pneumonia* except n-hexane extracted samples measuring no activity at 1, 2 and 3mg disc⁻¹ concentrations when compared with controls. According to the results, *Klebsiella pneumonia* showed maximum susceptibility to ethyl acetate extracted samples by measuring 62% ZI at 3mg disc⁻¹ followed by 41% inhibition zone by n-butanol at the same concentration compared to controls. Minimum inhibitory zone of 37% was revealed by crude methanolic extract at 3mg disc⁻¹ concentration. The data also suggested that

water extracted samples reduced the growth of the tested microbe at all the concentrations. N-hexane on the other hand did not inhibit the growth of *Klebsiella pneumonia* at any concentration measuring 0% zone of inhibition when compared with other samples and controls (fig. 4).

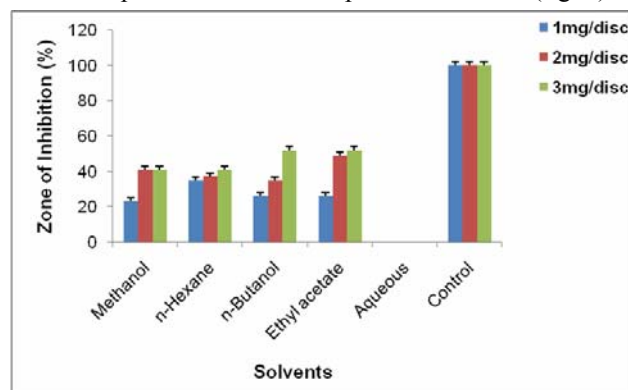


Fig. 5: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Pseudomonas aeruginosa* (Bar shows LSD values at $P < 0.05$).

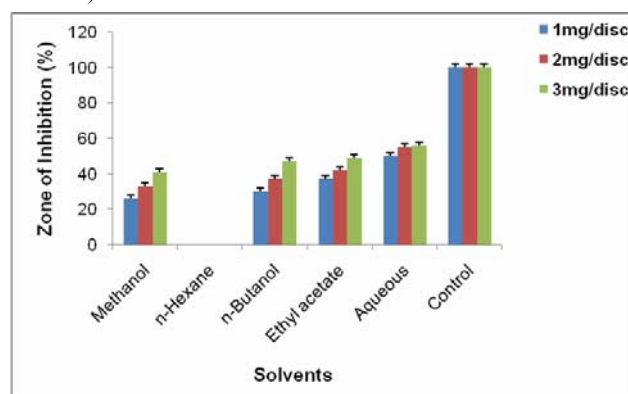


Fig. 6: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Candida albicans* (Bar shows LSD values at $P < 0.05$).

Fig. 5 reveals the anti-bacterial potentials of different solvent extracted samples obtained from the leaves of *Eucalyptus globulus* against *Pseudomonas aeruginosa*. N-butanol and ethyl acetate extracted samples efficiently reduced the growth of *Pseudomonas aeruginosa* by measuring 52% ZI each at 3 mg disc⁻¹ concentration when compared with controls and other samples. Crude methanolic extracts, on the other hand, showed least inhibitory activity of 23% at 1mg disc⁻¹ concentration while no activity was recorded by aqueous extracted fractions at all the tested concentrations measuring 0% ZI. The data also suggested that crude methanolic extract and n-hexane extracted fraction reduced the growth of the tested bacterium by 41% each at 3mg disc⁻¹ compared to controls and other samples. The data shown in fig. 5 indicated that increasing concentration of the extracts increased its activity against the tested microbe. *Candida albicans* was highly susceptible to aqueous extracted

samples and inhibited its growth by 56% at 3mg disc⁻¹ concentration followed by n-butanol and crude methanolic extracts by measuring 41% and 47 % ZI at 3 mg disc⁻¹ concentration compared with other samples and controls. N-hexane did not control the growth of *Candida albicans* and showed no activity at all the tested concentrations when compared with controls and other samples (fig. 6).

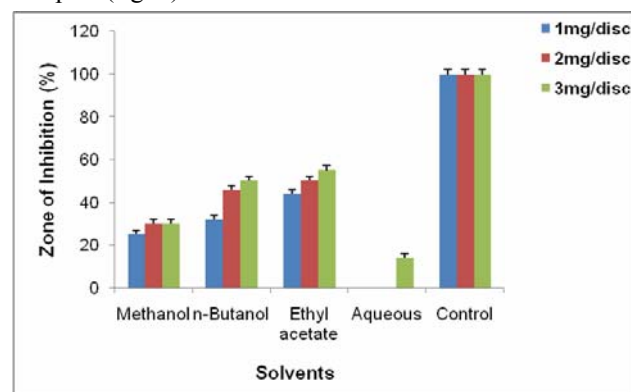


Fig. 7: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Bacillus subtilis* (Bar shows LSD values at $P < 0.05$).

Antibacterial and antifungal activity of different solvent extracted samples from fruits

Antibacterial activity of different solvent extracted samples from the fruits of *Eucalyptus globulus* against *Bacillus subtilis* is presented in fig. 7. The data indicates that by increasing concentration of different extracts results in greater degree of inhibition. All the solvent extracted samples were effective against *Bacillus subtilis* except aqueous which shows inhibition activity of (14%) only at 3 mg disc. Ethyl acetate extracted fraction reduced the growth of *Bacillus subtilis* by 63% at highest concentration followed by 55% and 50% inhibitory activity at 3 and 2mg disc⁻¹ concentrations. Crude methanolic extracts and n-butanol extracted fractions inhibited the growth of the same microbe by 50% and 30% at 3 mg disc⁻¹ concentrations. Fig. 8 reveals the anti-bacterial activity of different solvent extracted samples obtained from the fruits of *Eucalyptus globulus* against *Staphylococcus aureus*. The data showed that crude methanolic extract and n-butanol measured at par activity (55%) at 3mg disc⁻¹. The data also indicated that ethyl acetate and aqueous extract inhibited the growth of microbe at 44% and 30% at 2mg disc. Lowest inhibitory zone was measured in aqueous extracted samples (23% ZI each) at concentration of 1mg disc⁻¹.

Our results revealed that n-butanol extracted fractions exhibited the highest inhibitory activity of 48% against *Escherichia coli* at 3mg disc⁻¹ concentration followed by 42% ZI at 2mg disc⁻¹ concentration when compared with other samples and controls under study (fig. 9). Ethyl acetate and aqueous extracted fractions on the other hand

showed 21% and 14% inhibitory activity at 3mg disc⁻¹ concentrations respectively. The data indicated that n-hexane extracted samples did not reduce the activity of *Escherichia coli* at all the tested concentration measuring no zone of inhibition. The data also suggested that no activity was shown by crude methanolic extract samples at 1 and 2 mg discs measuring 0% ZI, however, minimum activity of 12% was recorded for the same extract at concentration of 3mg disc⁻¹. The data also indicated that all the extracts obtained from the fruits inhibited the growth of *Klebsiella pneumonia* at different degrees. The data suggested that ethyl acetate extracted samples were more effective against *Klebsiella pneumonia* compared with other fractions. The data suggested that ethyl acetate extracted fractions reduced the growth of *Klebsiella pneumonia* by 40, 42 and 55% at 1, 2 and 3mg disc⁻¹ concentrations respectively. N-butanol extracted samples inhibited the activity of the tested microbe by 38% and 43% at 2 and 3mg disc⁻¹ concentrations respectively. Minimum growth inhibition of 22% was measured for aqueous extracted samples at 3mg disc⁻¹ concentration respectively when compared with other samples and controls. The data also suggested that crude methanolic extract reduced the activity of the same bacterium by 24, 31 and 32% at 1, 2 and 3mg disc⁻¹ concentrations respectively (fig. 10).

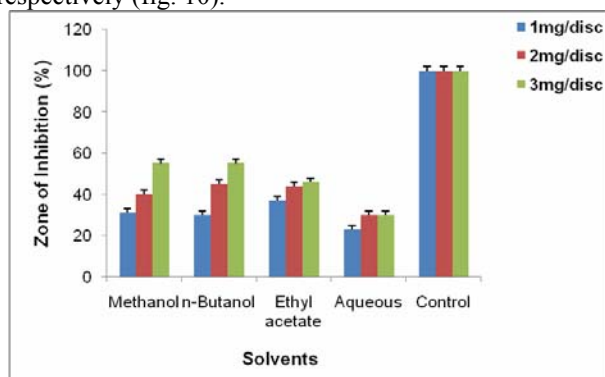


Fig. 8: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Staphylococcus aureus* (Bar shows LSD values at $P < 0.05$).

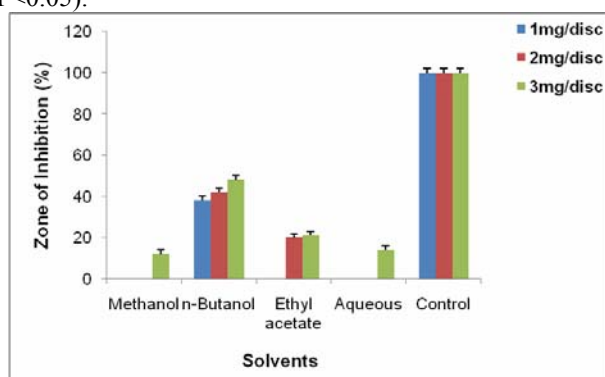


Fig. 9: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Escherichia coli* (Bar shows LSD values at $P < 0.05$).

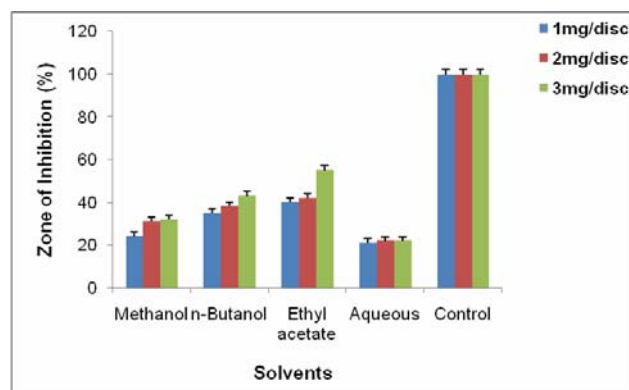


Fig. 10: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Klebsiella pneumonia* (Bar shows LSD values at $P < 0.05$).

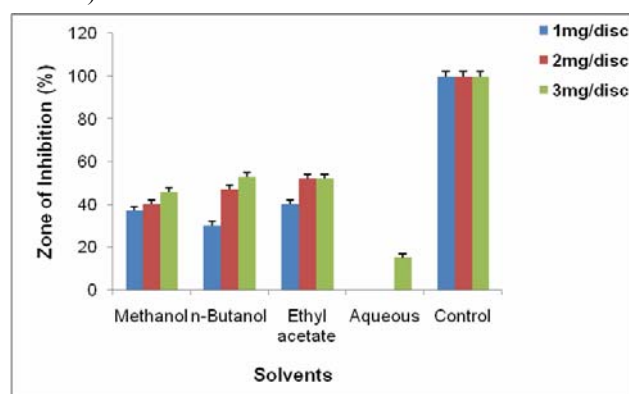


Fig. 11: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Pseudomonas aeruginosa* (Bar shows LSD values at $P < 0.05$).

The data shown in fig. 11 indicated that all the solvent extracted samples from the fruits were effective against *Pseudomonas aeruginosa* at all the three concentrations used except aqueous extracted sample, which shows no activity at 1 and 2mg discs when compared with controls. The data showed that *Pseudomonas aeruginosa* was highly susceptible to n-butanol extracts by measuring 53% inhibitory zones at 3mg disc⁻¹ concentration. Ethyl acetate extracted samples measured 52% inhibitory zone each at higher concentration of 3 and 2mg disc⁻¹. The data also revealed that aqueous extracted samples showed minimum inhibitory zone of 15% at 3mg disc⁻¹ concentration. The data suggested that n-butanol extracted fractions showed maximum inhibitory activity of 53% against *Candida albicans* at concentration followed by 40% inhibitory activity of methanolic samples at 3mg disc⁻¹ concentration. The lowest inhibitory activity against the same fungal specie was measured for ethyl acetate extracts (15% ZI) at concentration of 2mg disc⁻¹ compared with controls. The data also suggested that aqueous extracted fraction did not show any activity at all the three concentrations measuring 0% ZI (fig. 12).

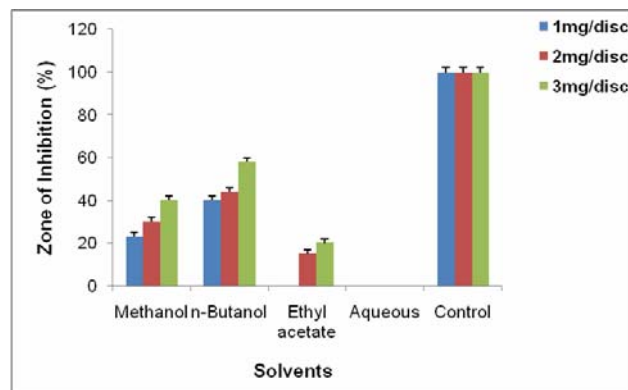


Fig. 12: Antibacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* against *Candida albicans* (Bar shows LSD values at $P < 0.05$).

DISCUSSION

The anti-bacterial activity of different solvent extracted samples from the leaves of *Eucalyptus globulus* revealed that ethyl acetate extracted fractions effectively reduced the growth of *Bacillus subtilis* at highest concentrations when compared with other samples and controls. These results are in agreement with Premanath *et al.* (2011) who reported maximum activity against *B. subtilis* by ethyl acetate extracted samples. Khan *et al.* (2008) concluded that ethyl acetate extracted samples exhibited highest inhibitory activity at different concentrations. N-hexane extracted fractions and n-butanol extracted samples revealed similar inhibitory activity at concentration of 3mg discs⁻¹ against *Bacillus subtilis*. The data also indicated that *Staphylococcus aureus* was more susceptible to n-butanol extracted samples followed by ethyl acetate and n-hexane extracted fractions at 3 mg discs⁻¹ compared with other samples and controls. N-hexane extracted sample measured no zone of inhibition and aqueous extracts showed minimum inhibitory activity at lowest concentration. Similar results are also reported by Takahashi *et al.* (2004) and Premanath *et al.* (2011). Antibacterial activity of different solvent extracted samples from the leaves tissues showed that crude methanolic extracted samples measured maximum zone of inhibition against *Escherichia coli* at highest concentration of 3mg discs⁻¹ compared with controls and other samples. Ethyl acetate, aqueous and n-butanol extracted samples also reduced the growth of the tested microbe at the same concentration of 3mg discs⁻¹. The data also revealed that all the extracted samples showed inhibitory activity against *Klebsiella pneumoniae*. The data further indicated that minimum activity was recorded by n-hexane extracted samples at concentration of 2 and 3 mg discs⁻¹. Similar results are also reported by Khan *et al.* (2012). *Klebsiella pneumoniae* was more susceptible to crude methanolic extracts at 3mg discs⁻¹ followed by n-butanol extracted samples at the same concentration.

Minimum inhibitory zone was revealed n-hexane extract at 1 and 2 mg discs⁻¹ concentration when compared with other samples and controls. Similar results also reported by Khan *et al.* (2008). Analysis of the data also indicated that n-butanol and ethyl acetate extracted samples were very effective to control the activity of *Pseudomonas aeruginosa*. No activity was recorded by aqueous extracted fractions at all the tested concentrations when compared with controls and other samples. Similar inhibitory zones were measured by crude methanolic extracts and ethyl acetate extracted samples at highest concentration of 3 mg discs⁻¹ while similar activity was noted by crude methanol extract at 2 mg discs⁻¹. The data further revealed that *Candida albicans* was highly susceptible to aqueous extracted samples and resulted in maximum inhibition of growth of the same fungus at highest concentrations of 3 mg discs⁻¹ followed by n-butanol and crude methanolic extracts at the same concentration. The present results are in agreement with Carpinella *et al.* (2003).

Antibacterial activity of different solvent extracted samples from the fruits of *Eucalyptus globulus* against *Bacillus subtilis* indicated increasing concentration of different extracts increased the activity against the tested microbe resulting in greater degree of inhibition. The data also showed that all the solvent extracted samples were effective against *Bacillus subtilis* except aqueous which shows growth inhibition at 3 mg per disc only. Ethyl acetate extracted fraction was more effective to control the growth of *Bacillus subtilis* 3 mg discs⁻¹ concentrations. The lowest inhibitory zone was noted for aqueous extracted samples at concentration of 1 milligram discs⁻¹ when compared with controls and other samples. Crude methanolic extracts, ethyl acetate and n-butanol extracted fractions also reduced the activity of the same microbe at different concentrations. The lowest inhibitory zone was noted for aqueous extracted samples at concentration of 1 milligram discs⁻¹ when compared with controls and other samples. Crude methanolic extracts was more effective to reduce the activity of *Staphylococcus aureus* at 3 and 2mg discs⁻¹ concentrations. Similar results were also obtained by Khan *et al.* (2011). n-butanol extracted fractions also reduced the growth of *Staphylococcus aureus* when applied in different concentrations compared with other extracts and controls. The data further suggested that lowest inhibitory zone was measured by aqueous extracted samples at concentration of 1mg discs⁻¹. The data presented also indicated that aqueous extracted samples also inhibited the growth of the same microbe at both 2 and 3 mg discs.

The results revealed that all the tested extracts reduced the activity of *Klebsiella pneumoniae* measuring at different degree of inhibition. The data suggested that ethyl acetate extracted samples were more effective against *Klebsiella pneumoniae* compared with other fractions. Minimum growth inhibition was measured by aqueous extracted

samples at highest concentrations of 3mg disc⁻¹. N-butanol extracted samples reduced the growth of the tested microbe both at 2 and 3mg disc⁻¹ concentrations respectively. Ethyl acetate extracted samples also showed good activity when applied in concentration of 3 mg disc⁻¹. The data also indicated that all the solvent extracted samples from the fruits were effective against *Pseudomonas aeruginosa* at all the three concentrations used except aqueous extracted sample, which shows no activity at 1 and 2 mg discs. The data further showed that *Pseudomonas aeruginosa* was highly susceptible to n-butanol extracted fractions at 3mg disc⁻¹ concentration. The data also revealed that aqueous extracted samples showed minimum inhibitory zone at 3mg disc⁻¹ concentration. Ethyl acetate extracted samples also reduced the growth of the same bacteria at higher concentration of 3 and 2mg disc⁻¹. Data regarding the antifungal activity of ethyl-acetate, n-butanol, methanol and aqueous extracted samples from the fruits of *Eucalyptus globulus* against *Candida albicans* suggested that n-butanol extracted fractions showed maximum inhibitory activity at highest concentration followed by crude methanolic extracts while aqueous extracted fractions shows no activity at all the three concentrations. These results agreed with Khan *et al.* (2008). When antimicrobial activity of leaves and fruits were compared, the data indicated that extracts from fruits showed more activity as compared to leaves. These results suggested that *Eucalyptus globulus* may contain certain bio-active compounds which resulted in the antimicrobial activity against different microbes as revealed by Djenane *et al.* (2011) and Sartorelli *et al.* (2007) who reported the presence of γ -terpinene (94.48%), 1,8-cineole (46.98%) and carvacrol (46.10%) in its essentials oils. Our results were innovative when compared with the findings of other researchers. We first isolated the crude extracts and then fractionated the crude with different solvents in ascending polarity starting from less polar to more polar which demonstrated effective isolation of different bioactive compounds whereas other researchers used crude extract in different solvent.

CONCLUSION

All the tested microbes were resistant to n-hexane extracted samples from the leaves and fruit. Samples extracted from fruits showed maximum activity as compared to leaves. *Klebsiella pneumonia* and *Candida albicans* were resistant to n-hexane extract of leaves and all the tested microbes showed moderate sensitivity to aqueous extract of fruits.

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