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ANTIMICROBIAL ACTIVITY OF DIFFERENT SOLVENTS EXTRACTS OF *Acacia cyanophylla*

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ABSTRACT

The present research work was carried out to investigate the antimicrobial activity of leaves of a weedy plant Acacia cyanophylla. Three different solvent extracts viz., methanol, ethanol and water were tested against 2 gram-positive bacterial strains i.e, Stephylococcus aureus and Bacillus subtilis and 4 gram-negative bacterial strains i.e, Pseudomonas aeruginosa, Xanthomonas, Escherichia coli and Cirtobacter. All the extracted samples showed significant results against all the tested microbes. Methanol extracted sample showed almost same results against gram-positive and gram-negative bacteria with zone of inhibition between 9 and 8 mm against Stephylococcus aureus and Bacillus subtilis using concentration of 1 and 2 mg disc^{-1}, respectively; whereas against Pseudomonas aeruginosa 8 mm each, Xanthomonas 8 and 9 mm, E.coli 8 and 7 mm and 8 and 7.5 mm zone of inhibition for Citrobacter. Ethanol extracted samples are equally effective. Against S. aureus, it showed 7 and 8 mm ZI. The same results were shown for Bacillus subtilis, E. coli and Citrobacter. Against Pseudomonas aeruginosa,7 mm each ZI was recorded and 7 and 9 mm for Xantomonas. The water extracted sample exhibited significant results against the tested bacteria. Against gram positive bacteria, 8 and 7 mm ZI were shown for S. aureus whereas 8 and 9 mm for Bacillus subtilis. Among gram negative bacteria 7 and 8 mm ZI were shown against Pseudomonas aeruginosa, 9 and 8mm in Xanthomonas, 9 and 10 mm in E.coli and 9 and 8 mm ZI were shown against Citrobacter. It is concluded that A.cyanophylla leaves possess good antimicrobial activity and can be used for medicinal purposes.

Key words: Anti-bacterial activity, disc diffusion method, microorganisms and weed.

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INTRODUCTION

Plants are important due to the synthesis of secondary products (Prusti *et al.,* 2008) and their inhibitory effects against various growing human pathogens (Silver, 1993). An increasing number of infectious agents have become highly resistant to various latest commercial antimicrobial compounds (Hancock *et al.,* 2012). However, the production of secondary products through different medicinal plants requires varying strategies that largely depend on the need of new drugs.

Medicinal plants have been used by local communities for centuries (Shinwari, 2010). Pakistan has more than 6000 species of higher plants, out of which 12% are used for medicinal purposes (Shinwari and Qaiser, 2011). Ethno-botanical studies not only document local knowledge about the use of plants in the field of drug development (Hussain *et al.,* 2005), but also provide useful information, thus saving time and money. Through ethno-botanical census, indigenous knowledge of the locals and practitioners has been documented in order to identify plants that may provide drugs against infectious diseases (Shinwari, 2010). Different plant parts are used for the physiotherapy of various forms of diseases and infections (Nweze *et al.,* 2004). Medicinal plants might represent an alternative treatment i.e. they can be substituted in non-severe cases of infectious diseases (Shah, 2005).

Nature has been the source of medicinal agents of all times. Antimicrobial agents are the most important therapeutic discoveries of the 20th century (Peterson and Dalhoff, 2004). Modern drugs have been taken up from natural sources. About 80% of the world population is dependent mainly on plant-based traditional medicines (Owolabi *et al.,* 2007). According to WHO reports, the use of traditional medicine in the third world countries is at its peak (Ahmad *et al.,* 1998). Antibiotics is the main key therapy for microbial (both bacterial and fungal) infections. Although antibiotics play a fundamental role in the treatment of various diseases, as they are synthetic compounds so they also have many side effects. Antibiotics may fail over time due to their appearance of multi-drug resistant pathogens and the spread of new infections (Abdulla, 2011). Antibiotics are commonly used for the treatment against serious infections caused by aerobic gram-negative bacteria (Tumah, 2005). *Acacia cyanophylla* Lindl. {syn. *A. saligna* (Labill.) H.L Wendl.} is an Australian weed species which was introduced in 1930 in Tunisia to

restore the land back to its original state. It is especially grown in semiarid zones (World Bank, 1995).

Acacia cyanophylla is located in the sub humid and the semi-arid bio-climating regions as this species is highly resistant to drought and salinity (National Academy of Sciences, 1980). Little information is available on the allelopathic potential of *A. cyanophylla.* This species acts against gastrointestinal parasitism in sheep (Akkari *et al.,* 2008). The growth of herbaceous species beneath and around *A. cyanophylla* trees is totally absent. This lack of ground vegetation is due to allelopathic potential possible by caused by fallen leaves (through decomposition of litter). As a result, the release of allelochemicals (organic substances) into the soil inhibits seed germination which in turn causes a great effect on agriculture productivity (Rice *et al.,* 1979). The most evident substances in *A. cyanophylla* are flavonoids such as quercetion and kaem. This plant is considered to have high protein content, high level of tannins and abundant biomass (Ben-Salem *et al.,* 1997).

The aim of this study was to test the plant extracts against a diverse range of organisms comprising gram-positive and gram-negative bacteria and to investigate the antimicrobial activities of a weed plant i.e. *A. cyanophylla* leaves.

MATERIALS AND METHODS

Plants Collection and Extraction

A. cyanophylla was collected from Pakistan Forest Institute, University of Peshawar. The plants were given voucher numbers and placed in Herbarium, Department of Botany, Islamia College University Peshawar.

The collected plant parts i.e stem bark and leaves were shade dried for three weeks at room temperature. The leaves of the plant were grinded in electric grinder for powder formation. About 600 g powder of each plant was soaked in commercial grade methanol (5L) for 15 days at room temperature with occasional shaking followed by filtration. Methanol soluble residue obtained was concentrated with rotary evaporator at 40^{o}C.

Antimicrobial activity bioassay

Antimicrobial activity of plant extracts against various microorganisms was evaluated by means of Disc Diffusion Method. For gram-positive bacteria Azithromycin (6µl and 12µl) was used as positive control while solvent media as negative control. For gram-negative bacteria ciprofloxacin (6µl and 12µl) was used as positive and solvent media as negative controls.

RESULTS AND DISCUSSION

Analysis of the data reveals that all the selected extracts possessed anti-microbial potential at both concentrations against all the tested microorganisms.

Effect of *A. cyanophylla* leaves' extract against *Staphylococcus aureus*

Fig. 1 represents the data on antimicrobial activity of *A. cyanophylla* leaves extracted against *Staphylococcus aureus*. The methanol extracted sample showed 9 and 8 mm zone of inhibition with concentration of 1 and 2 mg/disc (52 and 36%) respectively. The ethanolic sample showed zone of inhibition of 7 mm (41%) and 8 mm (36%) with concentration of 1 and 2 mg/disc respectively, while aqueous extracted sample showed 8 and 7 mm (47 and 31%) zone of inhibition against *Staphylococcus aureus*.

The extracts showed inhibitory action on the pathogens used in the present study. This finding correlates with reports of Dabur *et al.* (2007). Somchita *et al.* (2003) investigated the antimicrobial activity of aqueous extracted sample of *A. alata* (leaves and barks). They observed that aqueous extracted sample (leaves) showed significant activity 11-14 mm against *S.aureus*. Mattana *et al.* (2010) reportedthe antimicrobial activity of aqueous extracted of *A. aroma* against *Staphylococus* strains and observed that it showed little activity against the tested *Staphlococcus*. Mustsafa *et al.* (2010) determined the antimicrobial activity of aqueous extracted sample of *A. nilotica* (fruit) and observed significant inhibitory activity against gram positive *Cocci.* Bhawna and Bharti (2011) investigated the antibacterial activity of *A. catechu* and found maximum ZI of 20 mm against *S. aureus*.

Effects of *A. cyanophylla* leaves' extract against *Bacillus subtilis*

The antimicrobial activity of methanol, ethanol and aqueous extracted samples of *A. cyanophylla* (leaves) against Gram Positive bacteria *Bacillus subtilis* have been shown in Figure 2. The methanol extracted sample showed 9 mm zone of inhibition (ZI) in concentration of 1 mg/disc and 8 mm ZI in concentration of 2 mg/disc (33 and 30% respectively). The ethanol showed 7 mm ZI (25%) using concentration of 1 mg/disc and 8 mm ZI (26%) with 2 mg/disc while the aqueous fraction showed 8 and 9 mm ZI in concentration of 1 and 2 mg/disc (29 and 30%) respectively.

Naumanthri *et al.* (2012) investigated the antimicrobial activity of ethyl acetate sample of *A. nilotica* (leaves bark and pods) against gram positive bacteria *Bacillus subtilis* and observed that bark possesed lower zone of inhibition as compared to the leaves extracts. These results correlate with the studies of antimicrobial activity of

stem bark of *Acacia* spp. on various microorganisms by Banso *et al.,* (2009).

Effects of *A. cyanophylla* leaves' extract against *Pseudomonas aerugonisa*

Data regarding antimicrobial activity of different extracts from *A. cyanophylla* leaves against *Pseudomonas aerugonisa* have been shown in Fig. 3. The methanol extracted sample exhibited 8 mm each antimicrobial activity in concentrations of 1 and 2 mg/disc (24 and 22%). Compared to methanol extracted sample, the ethanol possessed 7 mm each antimicrobial activity in concentrations of 1 and 2 mg/disc (21 and 20% respectively) while the aqueous samples showed 7 and 8 mm antimicrobial activity in concentrations of 1 and 2 mg/disc (22 and 21%) respectively.

The results revealed that methanol extracted sample showed highest antimicrobial activity against the tested bacterial strains. Kavitha *et al.* (2013) investigated the antimicrobial activity of methanol extracted sample of *A. nilotica*. The alkaloids showed optimum zone of inhibition 6 mm against *Pseudomonas aeruginosa*, whereas flavonoids showed very well ZI of 19 mm against *Pseudomonas aeruginosa*. Khan *et al.* (2001) measured the antimicrobial potential of *A. alata* (leaves, flowers, stem and root barks). They found that the methanol extracted samples were significantly effective against tested microbes.

Effects of *A. cyanophylla* leaves' extract against *Xanthomonas*

Figure 4 represents antimicrobial activity of *A. cyanophylla* leaves against *Xanthomonas* a gram-negative bacterium. The methanol extracted sample showed 8 mm ZI in concentration of 1 mg $disc^{-1}$ and 9 mm ZI in concentration of 2 mg/disc (66 and 60% respectively). The ethanol extracted sample showed 7 mm ZI in concentration of 1 mg/disc and 9 mm ZI in concentration of 2 mg/disc (58 and 60% respectively) while the aqueous extracted sample showed 9 mm ZI using concentration of 1 mg/disc and 8 mm ZI in 2 mg/disc (75and 53%) respectively.

The systemic screening of plant extracts for antibacterial activity is a continuous effort to find new antibacterial compounds. Sumia Faitma *et al.* (2012) observed the antimicrobial activity of ethyl acetate extracted samples of leaf, bark and root against *Xanthomonas malvacearum*. The ethyl acetate showed activity with concentration of 500 Ug ml^{-1} more than the pure antibiotic.

Effect of *A. cyanophylla* leaves' extract against *Escherichia coli*

Antimicrobial activity of methanol, ethanol and aqueous extracted samples from *A. cyanophylla* (leaves) against *Escherichia coli* has been given in Figure 5. Methanol extracted samples showed 8 and 7 mm ZI in concentrations 1 and 2 mg/disc which are 57 and 46%

respectively. Ethanol extracted samples showed 7 mm (50 %) and 8 mm ZI (53%) in concentrations of 1 and 2 mg/disc respectively, while aqueous extracted sample resulted 9 and 10 mm ZI with concentration of 1 and 2 mg/disc (64 and 66% respectively).

Somavhita *et al.* (2003) recorded the inhibitory activity of aqueous sample of *A. alata* (leaves and bark) and observed significant activity against *E.coli*. Kavitha *et al.* (2013) investigated the antimicrobial activity of methanol extracted sample of *A. nilotica*. The alkaloids showed optimum zone of inhibition 1 mm against *E.coli* whereas flavonoids showed very well ZI 21 mm against *E.coli*.

Effect of *A. cyanophylla* leaves' extract against *Citrobacte*r

The antimicrobial activity of methanol, ethanol and aqueous extracted samples from *A. cyanophylla* (leaves) against *Citrobacte*r is shown in Figure 6. All solvents showed good result against gram-negative bacterium. The methanol extracted sample inhibited the bacterial growth to 8 and 7.5 mm in concentrations of 1 and 2 mg/disc (50 and 44% respectively). Similarly, zone of inhibition of 7 and 8 mm was measured against *Citrobacter* by ethanol extracted sample in concentrations of 1 and 2 mg/disc (43 and 47%) respectively. While 56 and 47% inhibitory activity (9 and 8 mm) was determined by aqueous extracted sample in concentrations of 1 and 2 mg $disc^{-1}$ respectively.

Plants are important source for the development of new chemo therapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay and in the recent years several reports are available on the antibacterial activity of plant extracts on human pathogenic bacteria (Samy and Ignacimuthu, 2000; Palombo and Semple, 2001).

CONCLUSION

It is concluded that extracts of *A. cyanophylla* have strong antimicrobial potential. Further studies are required to isolate effective natural constituents from these extracts.

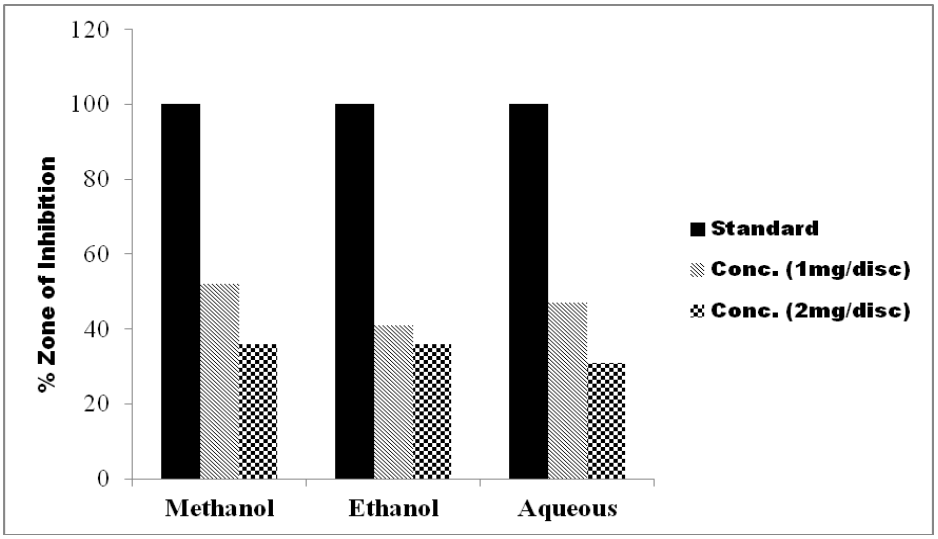

Figure 1: Antibacterial activity of different solvent extracted samples from *Acacia cyanophylla* against *Staphylococcus aureus.*

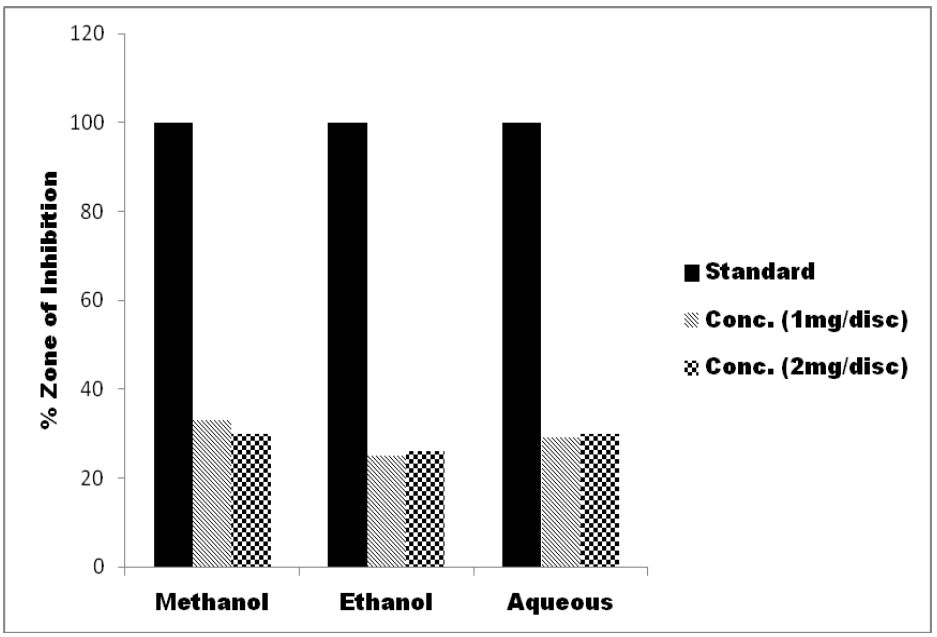

Figure 2: Antibacterial activity of different solvent extracted samples from *Acacia cyanophylla*against *Bacillus subtilis.*

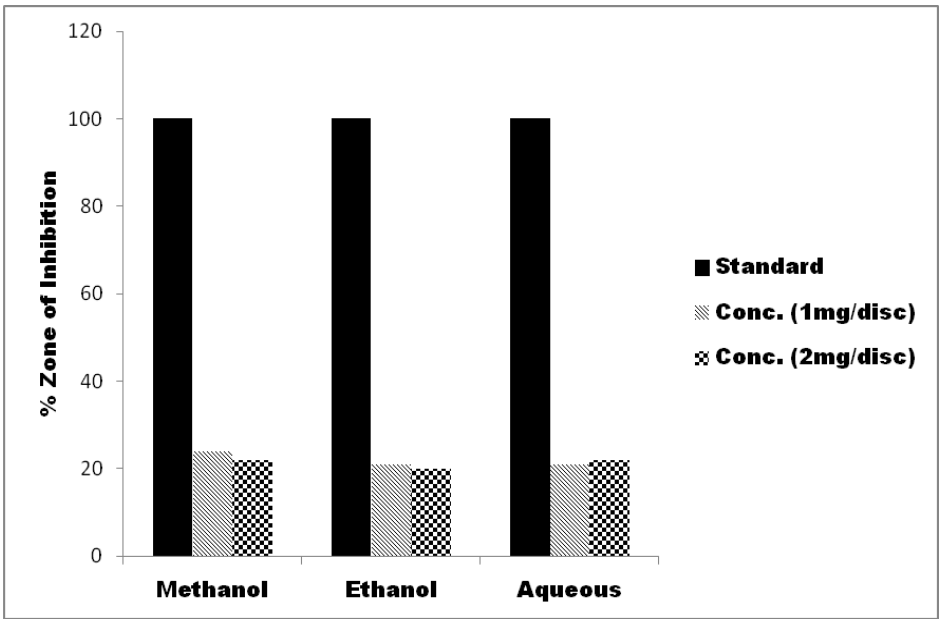

Figure 3: Antibacterial activity of different solvent extracted samples from *Acacia cyanophylla* against *Pseudomonas aeruginosa.*

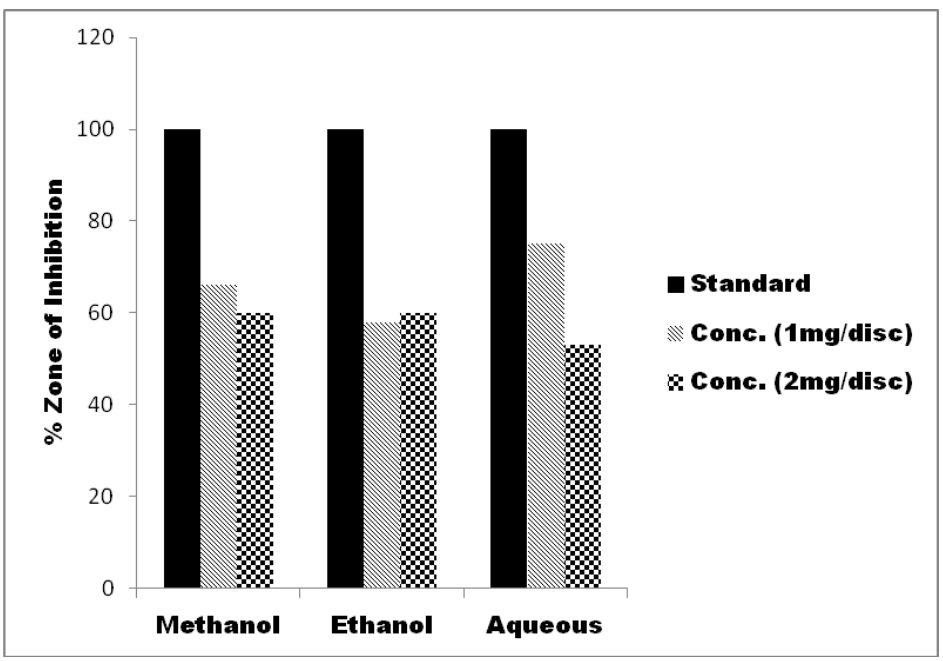

Figure 4: Antibacterial activity of different solvent extracted sample from *Acacia cyanophylla* against *Xanthomonas.*

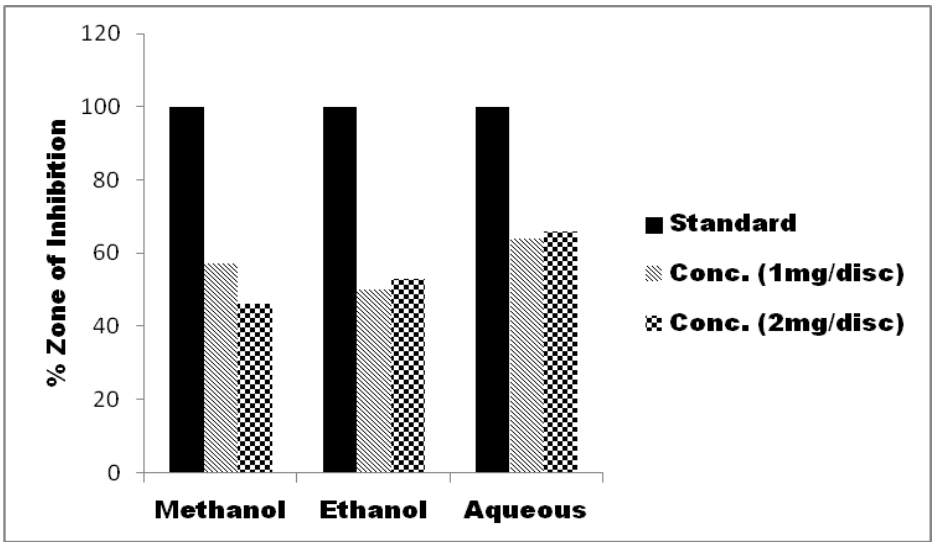

Figure 5: Antibacterial activity of different solvent extracted samples from *Acacia cyanophylla* against *Escherichia coli.*

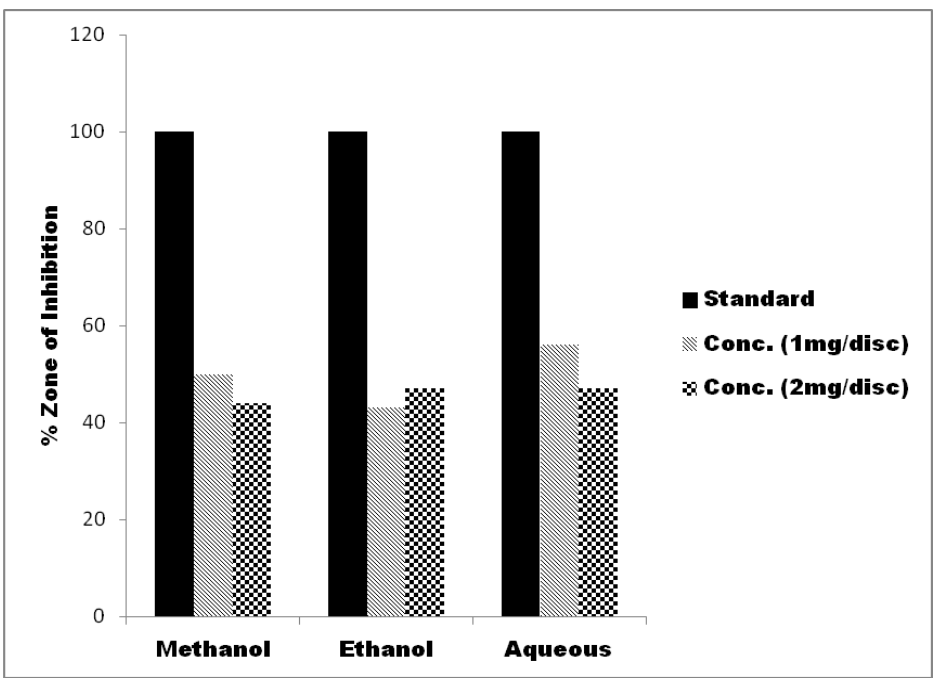

Figure 6: Antibacterial activity of different solvent extracted samples from *Acacia cyanophylla* against *Citrobacter*

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