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Full Length Research Paper

Antibacterial potential of crude leaf extracts of *Eucalyptus camaldulensis* against some pathogenic bacteria

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The antibacterial activity of the crude leaf extracts of *Eucalyptus camaldulensis* were determined using the agar well diffusion method against clinical isolates of *Escherichia coli*, *Staphylococcus aureus, Salmonella typhi, Proteus mirabilis* and *Klebsiella pneumoniae.* At an extract concentration of 50 mg/ml, the growths of all the pathogenic bacteria were arrested, though to varying degrees. The least activity in terms of zones of growth inhibition was shown by aqueous extract against *E. coli* (7 mm), *K. pneumoniae* (9 mm), *P. mirabilis* (13 mm), *S. typhi* (12 mm) and *S.aureus* (12 mm) while the highest was demonstrated by the acetone extract, with a recorded zone diameter for *E. coli (*12 mm), *K. pneumoniae* (13 mm), *S typhyi* (14 mm), *P. mirabilis* (15 mm) and *S. aureus* (14 mm). The effects of pH and temperature on the efficacy of the crude drug extracts were also determined. The effectiveness of the extracts were more pronounced under alkaline conditions and lower temperatures. Minimum inhibitory concentration (MIC) values ranged from 6.25 - 50 mg/ml and minimum bactericidal concentration (MBC) values ranged from 6.25 - 100 mg/ml. The ability of the crude extracts to inhibit the growth of the bacteria used in this study is an indication that the leaves of *E. camaldulensis* can be used as a source for the development and formulation of antibacterial drugs, thus justifying the use of the leaves in herbal medicines to treat a variety of infectious conditions.

Key words: *Eucalyptus camaldulensis*, maceration, agar well diffusion, efficacy, alkaline, antimicrobial activity.

INTRODUCTION

Presently, there is a wide range of antimicrobial drugs derived from microbial and synthetic sources available for the treatment of infectious conditions, at least for those in developed countries and the urban elites of developing countries. In resource poor communities, ignorance to good hygienic practices, poverty coupled with high cost of synthetic drugs and the circulation of drugs of questionable qualities and counterfeit pharmaceuticals combine to worsen the plight of the less privileged, forcing many to seek for the medicines of their ancestors. Herbs have been used as sources of food and medicinal purposes for centuries and these knowledge have been passed on from generation to generation (Adedapo et al., 2005). Even today, a significant proportion of the populace, particularly in the developing world depends on herbal medicines. This is particularly evident in the rural areas where infectious diseases are endemic and modern health care facilities are few and far between and where the people nurse their ailments back to health using local herbs. In Nigeria like in other parts of the African continent, practitioners of traditional system of medicine are still being consulted as a first choice before visiting western type health centre. This is partly due to the fact that traditional medicine blends perfectly into the socio-cultural life of the people, safer and easily available at minimal cost (Ananil et al., 2000). To overcome this problem, governments have actively encouraged the integration of modern and traditional systems of medicines and the scientific community is not left behind. Plants have been reported to contain many biologically active compounds which have potential for development as medicinal agents. Phytochemical progress has been aided by the development of rapid and accurate methods of screening plants for particular chemicals (Banso, 2009).

There are now many reports in the literature describing studies of higher plants with the aim of knowing their phytoconstituents and using same for the treatment of

various disorders as possible alternatives to synthetic drugs to which many microorganisms have developed resistance. The screening of higher plants for medicinal purposes represents a serious effort to discover newer, safer and possibly more effective drugs with the potential of fighting pathogenic bacteria and fungi.

Eucalyptus camaldulensis Dehnh Linn. is one of such medicinal plants belonging to the family *Myrtaceae* which is frequently seen occupying open waste spaces and grasslands, road sides, along river banks and wetlands. Of the more than 700 species that comprise this genus, most are native of Australia, though they are also widely cultivated throughout the tropics, especially in Asia and Central America as well as Africa (Jacobs, 1955; Stone and Bacon, 1994; Brooker et al., 2002). The wood has been used for heavy construction, railway sleepers, flooring, framing, fencing, plywood and veneer manufacture, wood turning, firewood and charcoal production (Boland, 1984). In Australia, it is also used as sources of wild honey, providing bees with good quality pollens and heavy yields of nectar (Boland, 1984). The medicinal usefulness of the redgum tree has been the subject of numerous studies. Some of the reported phytoconstituents of the tree included essential oils, sterols, alkaloids, glycosides, flavonoids, tannins and phenols. The tree is widely used in traditional medicine to treat a variety of diseased conditions including colds, asthma, coughs, diarrhea and dysentery, hemorrhage, laryngalgia, laryngitis, sore throat, spasm, trachagia and vermifuge (Duke and Wain, 1981). Traditional Aboriginal society in Australia used a wide range of Eucalyptus species in medicines to treat gastrointestinal symptoms, arrest bleeding, open wounds and cuts as well as the drinking of the decoctions for the relief of aches and pains in muscles, joints and even tooth. In some cases, the leaves are burnt and the smokes inhaled to treat fever. Commonly called “zaity” in Nigeria, the resinous exudates from the trunk is taken orally to cure bladder infections (Lassack and MacCarthy, 2006) and a decoction of the plant is used to treat enteric infections including diarrhea and dysentery, constipations and other stomach problems, asthma, oral thrush, boils, sores, skin and wound infections, asthma, bronchitis, eczema and athletes foot (Low et al., 2002; Bala, 2006; Duke and Ayensu, 1985). There is still little evidence on the antimicrobial properties of the plant under investigation against majority of the economically significant bacteria that cause infections. The objective of this study was to determine the antibacterial activity of the plant and ascertain the rationale for its use in traditional medicine. The effects of pH and temperature on the efficacy of the crude extracts were also investigated.

MATERIALS AND METHODS

Collection of plant material

The fresh leaves of the plant *E. camaldulensis* were collected during the flowering stage around the surroundings of Wuro-Dole village, Mbamba ward of Yola town, Yola South Local Government Area, Adamawa State, Nigeria. The taxonomical identification of the plant was confirmed by Mr. Bristones Bariri of the Department of Biological Sciences, School of Pure and Applied Science, Federal University of Technology Yola, Nigeria.

Preparation of plant material

The fresh leaves were harvested, rinsed with tap water and air dried under shade for fourteen days and reduced to coarse powder using pestle and mortar and then micronized to fine powder using the Kenwood electric blender (Kenwood LTD, Harvant, United Kingdom). The powder was stored in an airtight bottle until required.

Preparation of the extracts

The preparation of the leaves extracts were performed following the methods described by Emeruwa (1982) and Trease and Evans (1996). One hundred grams of the powdered leaves were extracted with 250 ml of solvent (water, ethanol and acetone respectively) contained in a 500 ml sterile conical flask and covered with cotton wool plug and wrapped with aluminum foil.

Extraction was allowed to proceed for 48 h in a shaker water bath maintained at 40°C. The extract was filtered using a clean muslin cloth and then Whatman No. 1 filter paper. The filtrate was then evaporated to dryness using a rotary evaporatoy attached to a vacuum pump (Model type 349/2, Corning Ltd). The yield for water was 39%; methanol 18% and hexane 13%. The percentage extract yield was estimated according to Parekh and Chanda (2007) as:

Dry weight / Dry material weight × 100

For the preparation of dilutions of crude extracts for antibacterial assay, the extracts was reconstituted by redissolving in the respective extracting solvents and further diluted to obtain 200, 100, 50, 25, 12.5, 6.25, 3.085 and 1.03 mg/ml.

Microorganisms

The microorganisms were clinical isolates obtained from the Microbiology Department of the Specialist Hospital, Yola, Nigeria. The organisms were collected in peptone water with the help of the laboratory staff and immediately transported to the Microbiology Department of the Federal University of Technology, Yola. The microorganisms were isolated and identified as *Escherichia coli*, *Staphylococcus aureus, Salmonella typhi, Proteus mirabilis* and *Klebsiella pneumoniae* following standard procedures as described by Cowan and Steel (1974) and Cheesborough (2002). The organisms were maintained in a refrigerator at a temperature between 4°C.

Standardization of microorganisms

Culture was standardized according to the methods described by Baker and Thomsberg (1983) and the National Committee for Clinical Laboratory Standards (NCCLS, 1990). Briefly, 0.2 ml of an 18 h culture of each organism was suspended into sterile universal bottles containing 20 ml nutrient broth and incubated for 5 h at 37°C.

Normal saline was gradually added so as to compare its turbidity to Mcfarland Standard of 0.5 which corresponds to approximately 1.0×10^8 cfu/ml. All the media used were supplied by Oxoid Ltd unless otherwise specified.

Screening for antimicrobial activity

The agar well diffusion method was used to test for antimicrobial activity of the plant extracts. Briefly, 1 ml of 18 h culture of bacteria adjusted to 1.0×10^8 cfu/ ml was spread onto a sterile plate so as to achieve a confluent growth. Three Petri dishes containing a particular bacterium was used. Then 19 ml of Mueller Hinton agar at 45°C was poured to each plate and the plates were rocked for few seconds for even spread and proper mixing of bacteria and agar. The contents of the plates were allowed to solidify and wells approximately equidistant to each other were bored on the surfaces of the agar medium using a sterile 6 mm cork borer. Then 0.5 ml of the reconstituted extract at a concentration of 50 mg/ml was pipetted into one of the holes. 0.5 ml of pure solvent was pippeted into another hole as negative control while an aqueous solution of 30 ∝g chloramphenicol was used as positive control. The plates were allowed to stand for 1 h for prediffusion of the extract to occur and then incubated at 37°C for 24 h and the zones of inhibition were measured to the nearest mm. The mean of triplicate results were taken.

Determination of MIC and MBC

Determination of the minimum inhibitory concentration (MIC) was carried out using the Broth dilution method (Sahm and Washington, 1990; Oyeleke et al., 2008). Briefly, 1.0 ml of the reconstituted extract solution at a concentration of 200 mg/ml was added to another test tube containing 1 ml of sterile broth so as to obtain a concentration of 100 mg/ml. 1 ml of this dilution was transferred to another test tube till the 7^{th} test tube was reached. The 8^{th} test tube did not contain any extract, but a solution of pure solvent served as negative control. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 10^8 cfu/1ml was put into each tube and thoroughly votexed. The tubes were incubated at 37°C for 24 h and observed for growth in form of turbidity. The test tube with the lowest dilution with no detectable growth by visual inspection was considered the MIC.

The MBC values were determined by removing 100 ∝l of bacterial suspension from the MIC tubes that did not show any growth and subcultured onto Mueller Hinton agar plates and incubated at 37°C for 24 h. After incubation, the concentration at which no visible growth was seen was recorded as the MBC.

Effects of pH

This was carried out as previously described by El-Mahmood et al. (2008). Briefly, the extracts were reconstituted into three separate test tubes each containing 100 mg/ml of extract in 4 ml test tubes. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 10^8 cfu/1 ml was put into each tube and thoroughly votexed. The first tube was treated with 1N hydrochloric acid by adding it drop wise until a pH of 2 was obtained. The second tube was treated with 1 M sodium hydroxide by adding it drop wise until a pH of 10 was reached. The test tubes were left to stand for 1 h and then neutralized by acid or alkali treatment as the case might be. The third test tube was not treated and served as control. The test tubes were incubated at 37°C for 24 h. Antibacterial activity was determined as previously described.

Effect of temperature

The effects of temperature on the efficacy of the crude extracts was determined by reconstituting the powdered extracts to obtain a concentration of 100 mg/ml in three separate test tubes of 4 ml each. Then 1 ml of an 18 h old culture of each of the bacteria earlier adjusted at 10^8 cfu/1ml was put into each tube and thoroughly mixed on a votex mixer. The first test tube was treated at a temperature of 10°C in a refrigerator for 1 h and the second test tube was treated at a temperature of 100°C in a water bath, also for 1 h, after which both test tubes were removed and left to acclimatize at room temperature. The third test tube was not subjected to either cold or heat treatment and served as control.

RESULTS AND DISCUSSIONS

The phytochemical components of the leaves of *E. camaldulensis* have been described in previous studies (Ghalem and Mohammed, 2008; Babayi et al., 2004). They were reported to contain amongst other compounds essential oils particularly cineol, cuminal, phellandrene, aromadendral, valerylaldehyde, geralniol, cymene, catechol, tannins, terpenes and isoprenoids, phenolics, cardiac glycosides, sterols, saponins and flavonoids. Several authors have linked the presence of these bioactive compounds to the antimicrobial properties of crude plant extracts (Sahm and Washington, 1990; Adesokan et al., 2007; Ogbolie et al., 2007; Owolabi et al., 2007; Oyeleke et al., 2008). Effectiveness of plants as antimicrobial agents is hinged on their mode of action in the body. Generally, plant products have been demonstrated to have tropism for specific organs or systems in the body with resultant multiple effects on the body (Okigbo and Mmeka, 2006; Putheti and Okigbo, 2008). Putheti and Okigbo (2008) quoting from Murray (1995), reported that the actions of plants often go beyond symptomatic treatment of diseases and gave an example that *Hydrastis Canadensis* do not only exhibit some antimicrobial properties, but also increase blood supply to the spleen, thus promoting optimal activity of the spleen to release mediating compounds. Some of these bioactive compounds which are synthesized as secondary metabolites as the plant grows, also serve to protect the plant against microbial attacks and predation by animals (El-Mahmood et al., 2008). Without doubt, several studies have been conducted in the past five decades that focused on the phytoconstituents and antimicrobial properties of herbs, higher plants, spices and their derivatives such as essential oils, extracts and decoctions (Hsieh et al., 2001; Shittu et al., 2006; El-Mahmood and Amey, 2007; El-Mahmood et al., 2008; El-Mahmood, 2009). As a result of these, several drugs have been developed, including quinine, emetin, belladonna, amongst others. Several workers including Dean and Svocoda (1990) and Shittu et al. (2006) have suggested the existence of a direct relationship between the chemical structure of a bioactive compound in plant extracts or essential oils and antimicrobial activity. The antimicrobial activity of *E. camaldulensis* were evaluated in this study by measuring the diameters of zones of growth inhibition on some pathogenic strains of *E. coli, S. aureus, P. mirabilis* and *S. typhi* using the agar well diffusion method as detailed in Figure 1. The growth of the entire test organisms were inhibited by the crude leaf extracts though to varying

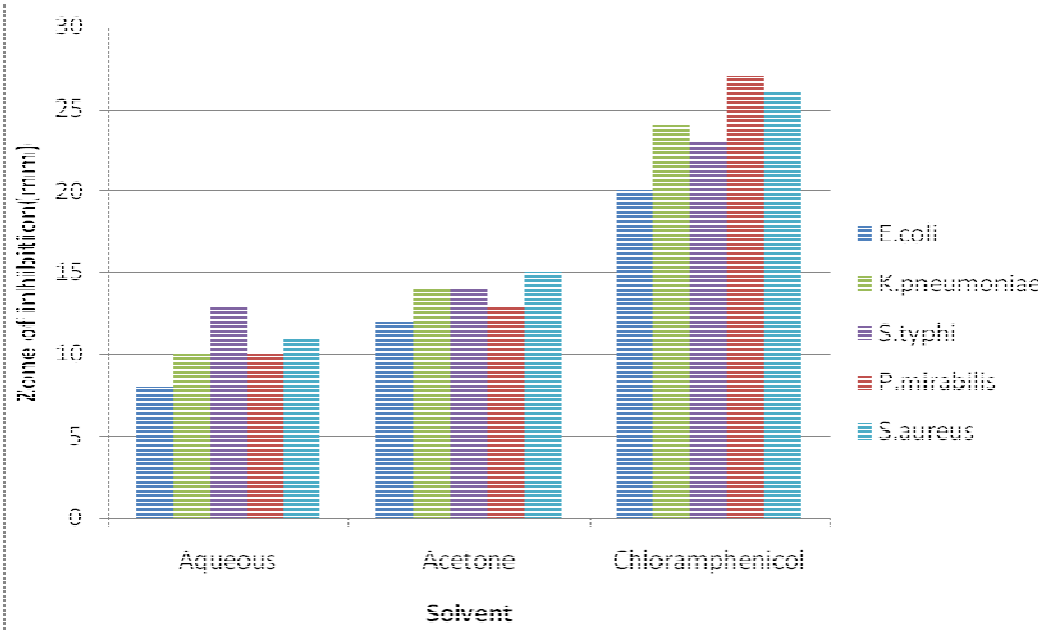

Figure 1. Antibacterial activity of crude leaf extracts of *Eucalyptus camaldulensis*.

degrees, depending on the extracting solvent and the bacterial species. Other workers have also shown that extracts of some plants inhibited the growth of various microorganisms at different concentrations (Esimone et al., 1998; Ogbolie et al., 2007; El-Mahmood and Amey, 2007; El-Mahmood et al., 2008). All these workers attributed the observed antimicrobial activities to the presence of some bioactive compounds like alkaloids, tannins, saponins, terpenes, essential oils and amongst others which were earlier reported to be present in abundance in *E. camaldulensis* (Jacobs, 1955; Stone and Bacon, 1994; Brooker et al., 2002). Karou et al. (2006) reported that the susceptibility of bacteria to plant extracts, on the basis of inhibition zone diameters varied according to strains and species, similar to the data obtained in this study. The least activity in terms of zones of growth inhibition was shown by aqueous extract against *E. coli* (7 mm), *K. pneumoniae* (9 mm), *P. mirabilis* (13 mm), *S. typhi* (12 mm) and *S. aureus* (12 mm) while the highest was demonstrated by the acetone, with a recorded zone diameter for *E. coli (*12 mm), *K. pneumoniae* (13 mm), *S. typhyi* (14 mm), *P. mirabilis* (15 mm) and *S. aureus* (14 mm). The ability of the crude extracts to inhibit the growth of recalcitrant bacteria as those used in this study is in agreement with previous reports of the antibacterial activities of other *Eucalyptus* species (Babayi et al., 2004; Sartorelli et al., 2007; Cook, 2009). These bacteria are associated with a number of infections including, but not limited to UTIs, lower and upper respiratory tract infections (*E. coli*, *K. pneumoniae*, *P.mirabilis* and *S.aureus*) and typhoid fever (*S. typhi*). Moreover, these pathogenic bacteria are capable of elaborating several virulent factors including the formation of biofilms on colonized surfaces (Indrayan et al., 2002). The large zones of growth inhibition exhibited by the crude extracts against the pathogenic bacteria used in this study justify the use of *E. camaldulensis* in tradi-tional medicine to treat open wounds, boils and a variety of enteric diseases. *E. camaldulensis* is reported to be very rich in essential oils (Moleyar and Narasimham, 1986; Singh et al., 2000; Batista-Pereira et al., 2006) and these oils were reported to exhibit some antibacterial acti-vity against a wide range of bacteria that were resistant to commonly used antimicrobial agents (Ghalem and Mohammed, 2008). Based on zone sizes, the water extracts were less effective than ethanol extracts, which in turn was less effective than acetone extracts. The large zone sizes produced by the plant extract against the test bacteria, especially the acetone extracts is an indication of the potency of the bioactive components of the plant against all the test bacteria. The antibacterial activity demonstrated by the extracts against the pathogens is an indication of the potential of *E. camaldulensis* as a source for antibacterial substances for the development and formulation of antibiotics and preservatives with broad spectrum of activity. Of recent, there has been renewed interests in plants as sources of antimicrobial agents due to their use historically and the fact that a good portion of the worlds population, particularly in developing countries, rely on plants for the treatment of infectious and non-infectious diseases (Ayoola et al., 2008). Chloramphenicol which served as positive control and at lower concentrations, produced larger zone diameters than the crude extracts. This is not unexpected because the antibiotic is produced by means of a reproducible manufacturing techniques and procedures, herbal

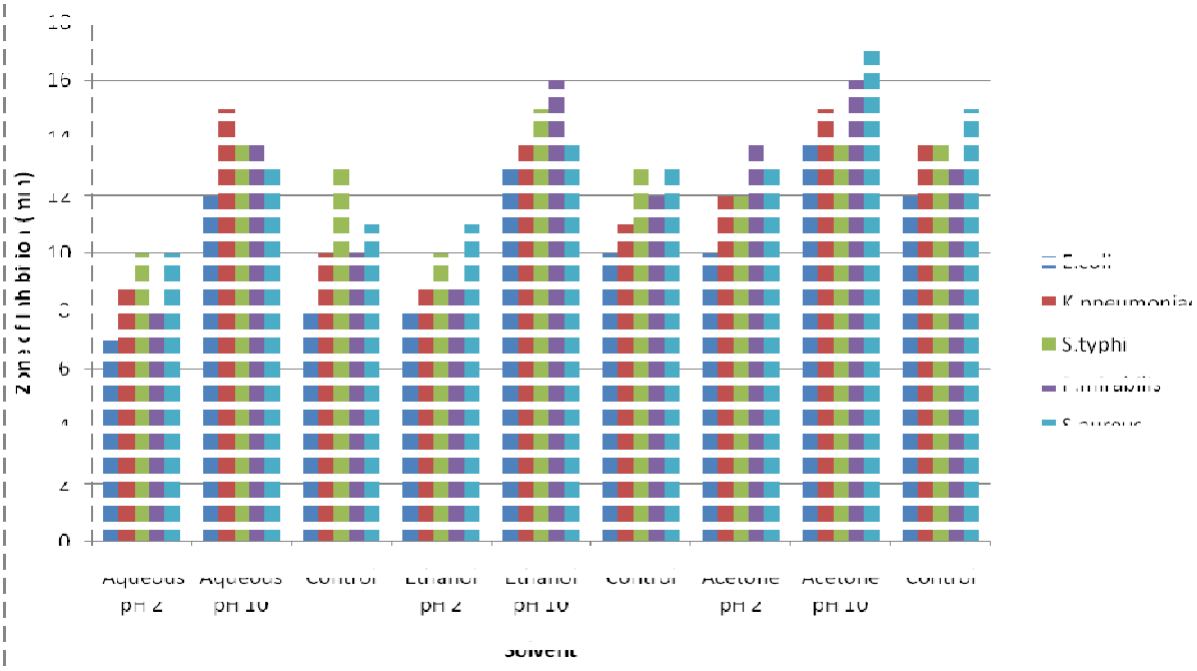

Figure 2. Effect of pH on the antibacterial activity of crude leaf extracts of *Eucalyptus camaldulensis*.

medicines and plant based medicines in general are prepared from crude materials, most of the time subject to contamination and degradation (El-Mahmood and Amey, 2007). Also, chloramphenicol is a drug of choice for the treatment of variety of infections including wound and enteric infections, to which this plant is traditionally used.

The effects of pH on the activity of the extracts are given in Figure 2. The antimicrobial activities of the crude leaf extracts of *E. camaldulensis* were evaluated at pH 2 and 10. For aqueous extracts, the zone diameters were: 7 mm at pH 2 and 12 mm at pH 10 for *E.coli*; 8 mm at pH 2 and 14 mm at pH 10 for *P. mirabilis*; 10 mm at pH 2 and 14 mm at pH 10 for *S. typhi*; 11 mm at pH 2 and 13 mm at pH 10 for *S. aureus* and 9 mm at pH 2 and 15 mm at pH 10 for *K. pneumoniae*. A similar pattern of growth inhibition was exhibited by the ethanol and acetone extracts, though with higher zone diameters. For all the extracts, activity was less under acidic than alkaline conditions. Similar observations were made by Doughari et al. (2008) and El-Mahmood et al. (2008). Acid stability is an important property of drugs, because it means that the plant components can be formulated to be taken orally and will not be inactivated under the acidic conditions of the stomach and the gastrointestinal tract. The results in this study indicated that the crude drugs prepared from this plant would not be stable under the acidic conditions of the stomach and the gastro-intestinal tract if taken orally. As observed in this study, the pH of compounds in dilution has an impact on the results, particularly when tannins, terpenes and phenols compounds are present. The effects of temperature on the efficacy of the extracts are given in Figure 3. For aqueous extract, *E. coli* gave a zone diameter of 6 mm at 100°C and 11 mm at 10°C, *P. mirabilis* gave a zone diameter of 7 mm at 100°C and 13 mm at 10°C; *S. typhi* gave a zone diameter of 9 mm at 100°C and 14 mm at 10°C, the diameter of zone of growth inhibition for *S.aureus* was 8 mm at 100°C and 12 mm at 10°C, while *K. pneumoniae* gave a zone diameter of 8 mm at 100°C and 13 mm at 10°C. The diameters of zones of inhibition of acetone extract for the test organisms were as follows: 9 mm for *E. coli* at 100°C and 14 mm at 10°C, 11 mm at 100°C and 14 mm at 10°C for *P. mirabilis*, 11 mm at 100°C and 16 mm at 10C for *S. typhi,* 10 mm at 100°C and 17 mm at 10°C for *S.aureus* and 10 mm at 100°C and 14 mm at 10°C for *K. pneumoniae*. As the temperature was increased, the antibacterial activity decreased, contrary to the data presented by Doughari et al. (2008) and El-Mahmood et al. (2008). The data obtained in this study showed that the crude extracts were more effective under lower temperatures irrespective of solvent used, probably as a result of the presence of volatile oils and therefore did not support the boiling of the plant parts as being practiced by some herbalists. It is evident from the results that organic extracts have some significantly high anti-bacterial activity, suggesting that the active principles are more soluble in organic solvents than water and that water is not the appropriate solvent for the extraction of the bioactive principles present in the plant, similar to the reports of Banso and Mann (2006) but contrary to that of Falodun et al. (2006) and El-Mahmood and Amey (2007). In their own studies, Alabashi et al. (1999) reported that the acetone and water extracts of *Euphorbia fruticosa*

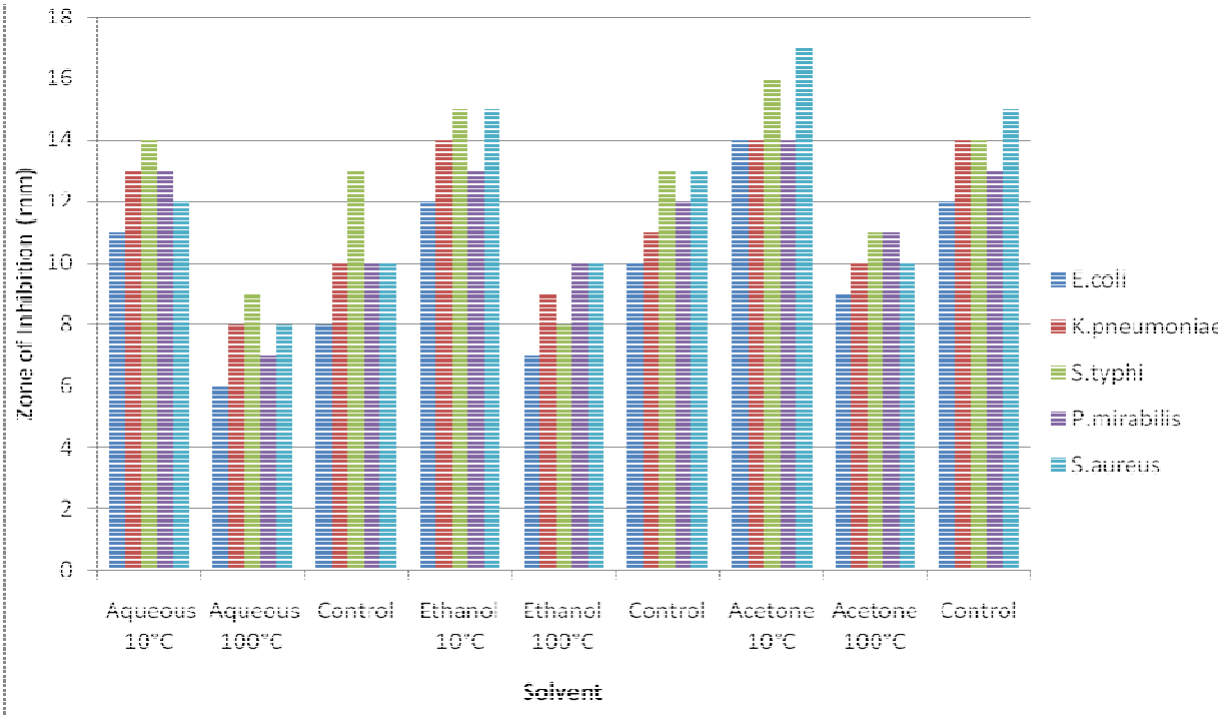

Figure 3. Effect of temperature on the antibacterial activity of crude leaf extracts of *Eucalyptus camaldulensis*.

showed significant antibacterial activity as well as the methanol extracts of *Euphorbia macroclada* studied by Darwish et al. (2002). In this study, the crude extracts of *Eucalyptus camaldulensis* inhibited the growth of such recalcitrant pathogenic bacteria that cause majority of diarrhoeal diseases and which usually display above average resistance to most antibiotics and non-antibiotics antibacterial agents. The efficacy of the extracts, probably due to the effects of the secondary metabolites, confirm its use as an antibacterial agent in folkloric medicine and may thus be useful in the treatment of enteric infections. The plant can be used to source for antibacterial drugs that can treat infections caused by susceptible bacteria.

One of the measures of assaying the effectiveness of antimicrobial agents is to determine their MIC and MBC values, which are predictive of therapeutic outcomes. Agents with low activity against a particular organism usually gives high MIC and MBC values, while a highly reactive agent gives low MIC and MBC values. The MIC and MBC values of the crude leaf extracts of *E. camaldulensis* is depicted in Figure 4. For the aqueous extracts, MIC for *E. coli* was 50 mg/ml, for *P. mirabilis* 25 mg/ml, for *S. typhi* 50 mg/ml, for *S.aureus* 50 mg/ml) and for *K. pneumoniae* 25 mg/ml. The MIC values for ethanol and acetone extracts followed similar pattern though with lower values. The aqueous extract gave an MBC of 100 mg/ml for *E. coli,* 50 mg/ml *P. mirabilis*, *K. pneumoniae* and *S. typhi* and for *S.aureus*, respectively. In the case of ethanolic extract, the MBC recorded was 50 mg/ml for *E. coli,* 25 mg/ml for *P. mirabilis*, 50 mg/ml for *K. pneumoniae* and *S. typhi* and *S.aureus*, respectively. For the acetone extract, the MBC values were 50 mg/ml for *E. coli,* 12.5 mg/ml for *P. mirabilis*, 25 mg/ml for *K. pneumoniae,* 25 mg/ml for *S. typhi*, and 25 mg/ml for *S.aureus*. The effects of the crude extracts correlate with reports that microorganisms varied widely in their degree of susceptibility (Emeruwa, 1982; Banso and Mann, 2006). The MIC and MBC techniques are used to evaluate the efficacies of antimicrobial agents and in this study, the MIC and MBC values tend to support the results obtained in the antibacterial screening above showing clearly that the aqueous extracts were less potent than either ethanol or acetone extracts.

One of the problems usually encountered in the use of medicinal plants is the quantity of extract required to enhance effective cure. In most cases of traditional usage of native plants, the quantity of extract taken is unknown. This however, does not pose a serious problem since plant based products are relatively safer than either microbial or synthetic based drugs. The MIC values obtained for the entire test bacteria were high ranging from 12.5 to 50 mg/ml, when compared to the MIC values of 0.01 - 10 µg/ml frequently recorded for conventional antibiotics. George et al. (2002) explained that the observed differences to be due to the fact that while synthetic antibiotics are in a pure form, crude plant extracts contains some impure substances that may be inert and do not have any antibacterial activities. Hugo and Russell (1984) have reported that the MBC values

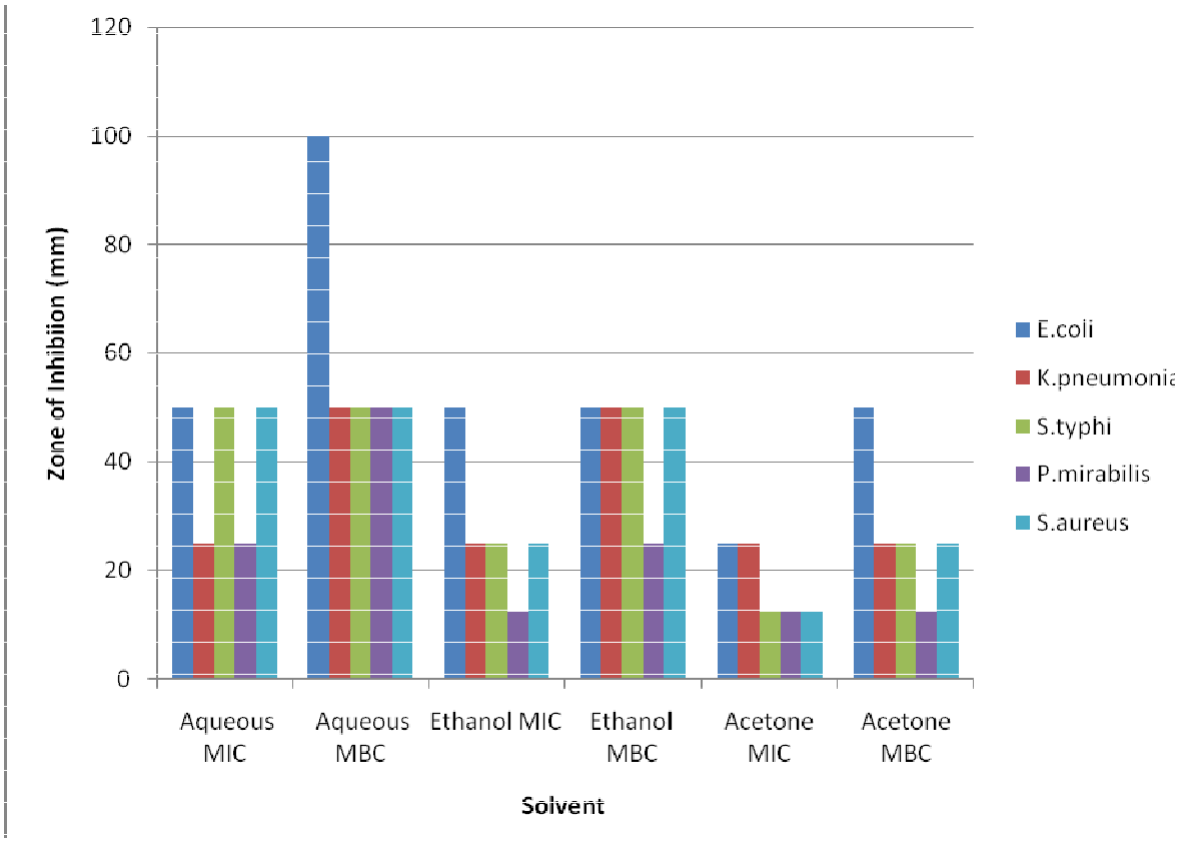

Figure 4. MIC and MBC values of the crude leaf extracts of *Eucalyptus camaldulensis*.

can either be the same or higher than the MIC values. In this study, the MIC values were either the same or slightly lower than the MBC values, similar to the results of Karou et al. (2006). The MIC and MBC values are predictive of the efficacy of agents *in-vivo*. However, the MBC values which are obtained after plating various dilutions of the extracts, is more reliable than the MIC values, obtained using turbidity as an index of growth (Junaid et al., 2006).

Although *E. camaldulensis* was found to exert pronounced antibacterial activities, further Pharmacological studies will be needed and also the evaluation of the antimicrobial activities against a wide range of microbial pathogens.

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