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Full Length Research Paper

Phytochemical and antimicrobial studies of the ethyl acetate extract of *Alchornea cordifolia* leaf found in Abuja, Nigeria

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The phytochemical screening of the ethyl acetate extract revealed the presence of tannins, flavonoids, glycosides, resins and carbohydrates. Seventy-eight fractions were elucidated from the extract using the bioassay-guided fractionation by employing the accelerated gradient chromatography (AGC) technique. All the fractions showed antimicrobial activity against the test organisms. Antimicrobial activities of the ethyl acetate extract of *Alchornea cordifolia* leaf against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli* and *Candida albicans* (clinical isolates); *Ps. aeruginosa* ATCC 10145*, S. aureus* ATCC 12600*, E. coli* ATCC 11775 *and C. albicans* ATCC 18804 using agar well diffusion and agar diffusion methods showed that the extract possess broad spectrum of activity against the test Gram-negative and Gram-positive bacteria and the fungi/yeast with diameter of zones of inhibition ranging from 10.0 - 35.0 mm. The minimum inhibitory concentration (MIC.) and the minimum bactericidal concentration (MBC) of the extract against the test organisms were found to fall between 0.625 - 10 mg/ml. The results of the rates of kill revealed that ethyl acetate extract produced 100% kill of *Ps. aeruginosa, E. coli* and *S. aureus* at low concentrations after 2 h. The results have therefore proved the claims of traditional healers in the use of the plant leaf.

Key words: *Alchornea cordifolia,* ethyl acetate extract, phytochemical, antimicrobial activity, fractionation.

INTRODUCTION

Herbal medicines are one type of complementary and alternative medicine whose use has dramatically increased over the past decade, growing from 2.5 - 12% in community survey (Roy-Byrne et al., 2005). The most recent figure from a 2002 community survey is 14% (Kaufman et al., 2002). A variety of plants or materials derived from plants have been used for the prevention and treatment of diseases virtually in all cultures. Herbs have been used as sources of food and medicinal purposes for centuries and this knowledge have been passed from one generation to another (Adedapo et al., 2005). Medicinal plants also represent a rich source from which antimicrobial agents can be obtained (Kubmarawa et al., 2007). Many pharmaceuticals currently available to physicians have a long history of use as herbal remedies, including opium, aspirin, digitalis and quinine (WHO, 2008). Pravin, (2006) reported that about 70% of the human population is dependent (wholly or partially) on plant-based medicines and the World Health Organisation (WHO) estimates that 80% of the world population presently uses herbal medicine for some aspect of primary health care (WHO, 2008). The potential of herbal medicines and medicinal plant research results in health care is no longer in doubt, having gained recognition in several nations of the world and the World Health Organisation (WHO).

Infections due to pathogenic bacteria and fungi represent a critical problem to human health (Eswarappa, 2009). Despite the extensive use of antibiotics and vaccines

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programs, infectious diseases continue to be a leading cause of morbidity and mortality worldwide (Bloom, 2000). Widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones and the lack of effective new therapeutics exacerbate the problems (Bloom, 2000). Microbial infections have been reported to be the major cause of inflammation (Du-Shieng et al., 2005).

Alchornea cordifolia Muell. Arg. belongs to the family Euphorbiaceae and is distributed in secondary forests usually near water, moist or marshy places. It grows to a considerable height but is always of a shrubby or scrambling habit. It has long stalked cordate leaves and flowers in hanging racemes about one foot long. It is a small tree of many stemmed, almost climbing shrub up to 5m high. Stems are armed with blunt spines; leaves are long- petiolate; broadly ovate, cordate at base, the apex is short acuminate, entire or slightly dentate margin, finely stellate-puberulous or slightly glabrescent beneath with glands in axils of basal nerves. Flowers are greenish while in lax pendulous spikes or raceme, styles long and permanent on mature fruits. Fruits are 2-celled, small, stellate pubscent (Dalziel, 1956).

The antimicrobial properties of crude extracts prepared from plants have been reported (Hassan et al., 2006; Kubmarawa et al., 2007). *A. cordifolia* leaf extracts have been reportedly used in various African countries such as Senegal in the treatment of venereal diseases, conjunctivitis, dermatoses, stomach ulcers, bronchitis, cough, toothache (Le Grand and Wondergem, 1987; Le Grand, 1989) and Zaire in the treatment of urinary tract infection, infected wound, diarrhoea, cough, dental caries, chest pain and anaemia (Kambu et al., 1990; Muanza et al., 1994). In Sierra Leone it was used for diarrhoea and piles (Dalziel, 1956; Macfoy and Sama, 1990) and in Nigeria for gonorrhoea, yaws, rheumatic pain and cough (Gbile and Adeshina, 1986; Ogungbamila and Samuelson, 1990). Extracts from leaves of *A. cordifolia* have been reported to inhibit the growth of bacteria such as *Staphylococcus aureus, S. albus, Escherichia coli, Bacillus sp* and *Pseudomonas aeruginosa* (Ogunlana and Ramastad, 1975; Ebi, 2001). Anti-inflammatory activities of *A. cordifolia* have also been reported (Osadebe and Okoye, 2003; Manga et al., 2004; Mavar-Manga et al., 2008).

Many plants synthesize substances that are useful to the maintenance of health in humans and animals (Lai and Roy, 2004). Many of these substances are secondary metabolites of which at least 12,000 have been isolated from different parts of plants, a number estimated to be less than 10% of the total *(*Tapsell et al., 2006), which constitute an important source of the pharmaceutical drugs. Some of these compounds have been reported to be present in *A. cordifolia* such as flavonoids (Ogungbamila and Samuelson, 1990), alkaloids and tannins (GHP, 1992), inulin and alchornine (Abdullahi et al., 2003). Not much has been reported about the presence of compounds such as, glycosides in the leaf of this plant especially the species found in Northern, Nigeria.

A. cordifolia has been documented to be in abundant supply in Nigeria; therefore, this work aims at investigating its antimicrobial properties and identifying the active phytochemical constituents of the leaf extract.

MATERIALS AND METHODS

Collection of plant

A. cordifolia was collected from the side of a flowing stream in Abuja, Nigeria in the early hours of the day, at different intervals between the months of June and October. The plant was identified in the herbarium of the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja-Nigeria. There is a deposit of the plant specimen with the number NIPRD herbarium 4334 in the Institute for future reference.

Extraction of the plant material

The leaves of *A. cordifolia* were air-dried and reduced to coarse powder using wooden mortar and pestle and then grinded to fine powder using electric blender (Kenwood Limited, Harvant, United Kingdom). Using Soxhlet extractor (QUICK FIT- England), 1.0 kg of the powdered leaf was extracted successively and exhaustively each time with each of the various solvents starting from the less polar solvent to the more polar solvent. The solvents used were hexane, ethyl acetate and methanol respectively. After each extraction, the extract was concentrated, dried and weighed.

Phytochemical screening of the leaf powder

The phytochemical constituents were tested for by using standard methods of Trease and Evans (1996).

Test for tannins

a. Lead sub-acetate test: A few drops of lead sub-acetate were added to 1.0 ml of the extract. The colour change was noted.
b. Bromine water test: Few drops of bromine water were added to 1.0 ml of extract in a fume chamber. The colour change was observed.
c. Ferric Chloride test: Four milliliter (4 ml) of distilled water was added to 2.0 g of the extract. Few drops of Ferric chloride were added to the mixture. The colour reaction noted.
d. Ferric Ammonium Chloride test: Ferric ammonium citrate solution (0.25%) was added to 1.0 ml of the extract. Sufficient solid sodium acetate was added to the mixture to adjust the solution to pH 8 using pH indicator paper. This was boiled on a water bath and filtered. The colour reaction noted.

Test for flavonoids

a. Magnesium Chips or Shinoda Test:

The powdered leaf (0.5 gm) was extracted in ethanol by boiling on a water bath for 5 min, filtered and cooled. A small quantity of Magnesium chips was added to the filtrate and few drops of concentrated HCl were added down the side of the test-tube. The colour change was noted.

b. Zinc Chips Test:

A small quantity of zinc chips was added to the extract and few

drops of concentrated HCl were added down the side of the test-tube. The colour change was noted.

Test for glycoside

a.) Five milliliters of the extract was placed in a test tube; 2.5 ml of dilute sulphuric acid was added and boiled for 15 min. It was cooled and neutralized with 10% sodium hydroxide solution. 5.0 ml of a mixture of Fehling's solutions A and B were added. The colour reaction noted.
b.) Test for Cyanogenic glycosides:

The powdered leaf extract was evaporated to dryness. 0.5 g of the extract was placed in a clean test-tube and moistened with sufficient water. A moist sodium picrate paper was suspended in the neck of the test tube using a cork to trap it. The closed tube was placed in a warm water bath 45°C for 1 h. The colour change was noted.

Test for resins

a. Fifteen milliliters (15.0 ml) of petroleum ether extract were prepared using 0.1 gm of the powdered leaf and filtered into a test-tube. An equal volume of copper acetate solution was added and shaken vigorously then allowed to separate. The colour change was noted.
b. The powdered leaf (0.5 g) was dissolved in acetic anhydride and one drop of concentrated sulphuric acid was added. The colour change was noted.

Test for carbohydrates (Molisch's test)

A few drops of Molisch's reagent were added to 2.0 ml of the extracts, and then a small quantity of concentrated sulphuric acid was added and allowed to form a lower layer. A purple ring at the interface of the liquids indicated the presence of carbohydrates. The mixture was then shaken, allowed to stand for 2 min and then diluted with 5.0 ml of water. The colour reaction noted.

The secondary metabolites present in the leaf were extracted with different organic solvents and quantified (Marcek, 1972; Brain and Turner, 1975).

Chromatographic analyses of ethyl acetate extract (EAE)

Ethyl acetate extract was analyzed for chemical composition using the bioassay-guided fractionation by employing the Accelerated Gradient Chromatography (AGC) technique. Silical gel G (E-Merck, Germany) was used as an absorbent. Gradient elution was effected using hexane and ethyl acetate sequentially with increasing polarity. The fractions were eluted with hexane (100%), hexane-ethyl acetate (98:2, 95:5, 90:10, 70:30, 50, 20:80%) and ethyl acetate (100%). A total of 78 fractions were collected. The thin layer chromatography (TLC) analyses of the fractions were carried out using Whatman TLC plates of size 10 x 20 cm precoated with K5 silical gel 150A (Whatman Limited Maidstone, England). The chromatograms were developed using solvent mixture specific for separating alkaloid compounds especially hexane and ethyl acetate, 3:1. After development, the chromatograms were dried and detection was made using Ultra-violet light at both wavelength 254 nm and 365 nm. Similar fractions were combined and concentrated giving 30 fractions.

Preparation of the fractions for antimicrobial analysis

Antimicrobial properties of the fractions were analyzed using agar dilution methods. Five millilitres (5.0 ml) of distilled water was added to 0.1 g of the fraction to give a concentration of 20 mg/ml. One milliliter (1.0 ml) of the mixture was added to 19.0 ml of sterile molten nutrient agar, kept at 45°C in a water bath and poured into sterile petri dishes. The plates were allowed to set and the sterility tested by incubating them overnight at 30°C. A loopful of 18 h broth culture, which was further diluted to give about 10^6cfu/ml using MacFarland 0.5 standard, of each of the test organisms was then plated out on the prepared plates. A control for each of the organisms was also included using plain nutrient agar, which contained no extract. The plates were then incubated for 18 hours at 37°C and then examined for growth.

The above experiment was carried out three times on all the fractions using both the clinical isolates and the type cultures. Sabourauds' dextrose agar was used for *C. albicans*. The antimicrobial activities of the secondary metabolites (tannins, flavonoid, and glycoside) were also tested.

Test organisms

Clinical isolates of *S. aureus, P. aeruginosa, E. coli and Candida albicans* collected in peptone water from the National Institute for Pharmaceutical Research and Development Diagnostic Centre, Idu-Abuja, Nigeria. The organisms were isolated and identified using standard procedures (Cheesbough, 2002) and maintained at a temperature between 2 - 8°C. Type organisms of *S. aureus* ATCC 12600, *P. aeruginosa* ATCC 10145, *E. coli* ATCC 11775, *C. albicans* ATCC 18804 were collected from the Department of Pharmaceutical Microbiology, University of Benin, Benin, Nigeria.

Inoculum preparation

Eighteen-hour broth culture of the test organism was suspended into sterile nutrient broth. It was standardized according to National Committee for Clinical Laboratory Standards (NCCLS, 2002) by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately 1.0 x 10^6 cfu/ml.

Susceptibility of the organisms to the ethyl acetate extract

Overnight broth cultures were diluted appropriately using McFarland scale (0.5 McFarland which is about10^6 cfu/ml). The molten sterile nutrient agar (20 ml) was poured into sterile petri dish and allowed to set. The sterile nutrient agar plates were flooded with 1.0 ml of the standardized inoculum and the excess was drained off. A sterile cork borer (No. 4) was used to bore equidistant cups into the agar plate. One drop of the molten agar was used to seal the bottom of the bored hole, so that the extract will not sip beneath the agar. 0.5ml of the different concentrations (20 - 0.625 mg/ml) of the extracts was added to fill the bored holes. Negative control was prepared by putting 0.5 ml of pure solvent in one of bored hole and aqueous solution of 2 µg of gentamicin (for Gram positive organism) and 4 µg of gentamicin (for Gram negative organism) in another bored hole which served as positive control. One hour pre-diffusion time was allowed, after which the plates were incubated at 37°C for 18 h. The zones of inhibition were then measured in millimeter. The above method was carried out in triplicates and the mean of the triplicate results were taken. This method was also used to determine the susceptibility of the organisms to the secondary metabolites from the extract. Sabouraud's Dextrose Broth and Agar were for *Candida albicans*.

Determination of the minimum inhibitory concentrations (M.I.Cs.) and minimum bacteriocidal concentrations (M.B.Cs.)

Graded concentrations of the leaf extracts ranging from 20 - 0.625

mg/ml were used. These concentrations in sterile melted nutrient agar plates were prepared using double dilution method. The solidified leaf extract-agar admixture plates were inoculated with 20 µl of standardized 18 h culture test organism. The inocula were allowed to diffuse into the test agar plates for 30 min. The test agar plates were then incubated at 37°C for 18 h and the lowest concentration of the extract in the test agar plates that showed no growth was considered as the M.I.C. of the extract against the test organism.

The concentration of the extract in the test agar plates showing no visible growth was inoculated into sterile nutrient agar containing inactivating agents 3% $^{v}/_{v}$ Tween 80 plates. These plates were then incubated at 37°C for 24 h after which they were examined for presence or absence of growth. The plates that yielded less than six colonies were taken as the M.B.C. (Ehinmidu, 2005).

Determination of the rate of kill

Five flasks labeled 1, 2, 3, 4, and 5 containing 30.0 ml of sterile nutrient broth were inoculated with a previously standardized culture in a water shaker bath maintained at 37°C. Flask 1 contained sterile nutrient broth and the organism thus acting as the control for the experiment. One milliliter of the EAE in the 30.0 ml volume contains 10, 5 and 2.5 mg in flask 2, 3 and 4 respectively. Flask 5 contained an antibiotic (gentamicin) for comparative purposes. Gentamicin 2 µg/ml for Gram-positive organisms and 4 µg/ml for Gram-negative organisms were used (Sweetman, 2005). The water shaker bath was set at a speed of 80 oscillations per minute (80 rpm). 0.5 ml of the mixture in the flask was aseptically withdrawn, properly diluted in sterile normal saline containing 3% $^{v}/_{v}$ Tween 80 and inoculated into sterile nutrient agar containing 3% $^{v}/_{v}$ Tween 80. Subsequent inoculations into the sterile agar plates were carried out at a timed interval. All the plates were incubated at 37°C for 18 h, after which the colonies that developed were counted and compared with control experiments. Same experiments were carried out for *C. albicans* using Sabouraud's dextrose broth and agar.

RESULTS

The result of the phytochemical screening indicated the presence of tannins, flavonoids, glycosides, resins and carbohydrates in the ethyl acetate extract. The extraction of the secondary metabolites showed that tannin was the highest (9.8%) followed by flavonoids (9.1%) and glycosides (5.1%).

A total of 78 fractions were collect from the elucidation of the ethyl acetate extract. Identical fractions were combined giving 30 fractions altogether as follows:

B_1 - Fraction 1
B_2 - Fraction 2
B_3 - Combination of fractions 3 - 9
B_4 - Fraction 10
B_5 - Fraction 11
B_6 - Fraction 12
B_7 - Fraction 13
B_8 - Combination of fractions 14 - 17
B_9 - Combination of fractions18 - 23
B_{10} - Combination of fractions 24 - 30
B_{11} - Combination of fractions 31 - 42
B_{12} - Combination of fractions 43 - 44 and 46
B_{13} - Fraction 45
B_{14} - Combination of fractions 47 - 56
B_{15} - Combination of fractions 57 - 63
B_{16} - Fraction 64
B_{17} - Fraction 65
B_{18} - Fraction 66
B_{19} - Fraction 67
B_{20} - Fraction 68
B_{21} - Fraction 69
B_{22} - Fraction 70
B_{23} - Fraction 71
B_{24} - Fraction 72
B_{25} - Fraction 73
B_{26} - Fraction 74
B_{27} - Fraction 75
B_{28} - Fraction 76
B_{29} - Fraction 77
B_{30} - Fraction 78

The susceptibility of the organisms to the secondary metabolites of the plant leaf extracts showed that tannins and flavonoids were more active than glycoside with tannin showing the highest activity against the test organisms. Furthermore, the secondary metabolites were more active against the bacterial isolates than the fungal isolate. The gram positive bacteria, *S. aureus* was the more susceptible to the metabolites than the gram negative bacterial isolates, *P. aeruginosa* and *E. coli.* The type organisms were also observed to be less susceptible to the secondary metabolites than the other isolates except in the case of *C. albicans* (Table 1).

The antimicrobial activity of the fractions of ethyl acetate extract was eluent-dependent. Fractions B_{15} and B_{25}-B_{30} were found to have bactericidal and fungicidal activities against all the organisms, fractions $B_{14,}$ B_{21}-B_{23} also showed both activities against *S. aureus, E. coli* and *C. albicans* but showed inhibitory effect against *P. aeruginosa* while fractions $B_{1,}$ $B_{2,}$ $B_{4,}$ $B_{5,}$ $B_{6,}$ B_{10}, B_{11}, B_{13}, B_{16} and B_{17} were not active against any of the organisms. Fraction B_3 had inhibitory effect against *S. aureus, E. coli* and *C. albicans*, B_{19} showed bactericidal and fungicidal activities against these same organisms, but both fractions had no activity against *P. aeruginosa*. Fraction B_8 showed bactericidal effect against *E. coli*, but had no activity against the other organisms while B_9 had bactericidal and fungicidal effects against *E. coli* and *C. albicans* but showed no activity against *P. aeruginosa* and *S. aureus*. Fraction B_{12} showed bacteristatic effect against *P. aeruginosa* and bactericidal effect against *E. coli*, B_{18} showed bactericidal effect against *S. aureus*, B_{20} had only inhibitory effect against C. *albicans* and lastly, B_{24} only had inhibitory effect against *P. aeruginosa* (Table 2).

The result of the sensitivity test of the organisms to the ethyl acetate extract and Gentamicin showed that the bacterial isolates were more susceptible to the test

Table 1. Susceptibility of the test and type organisms to the secondary metabolites from *A. cordifolia* ethyl acetate leaf extract.

Test organisms	Zones of inhibition (mm)		
	Tannin	Flavonoids	Glycosides
P. aeruginosa	28	27	23
P. aeruginosa ATCC 10145	25	25	19
S. aureus	30	28	23
S. aureus ATCC12600	26	27	21
E. coli	23	22	19
E. coli ATCC11775	19	19	15
C. albicans	14	13	12
C. albicans ATCC18804	17	15	13

agents than *C. albicans*. The extract was more active against the test bacterial isolates than the type bacterial isolates while gentamicin showed more activity against the type bacterial isolates than their test counterparts. *C. albicans* ATCC 18804 was more susceptible to the extract than the test C. *albicans* (Table 3).

Generally, the results of the M.I.C. and M.B.C. revealed that ethyl acetate extract had more antimicrobial activity against the bacterial isolates than the fungal isolate. The M.I.C. and M.B.C. values of the extract range between 0.625 to 10.0 mg/ml (Table 4).

Generally, there is reduction in the cell population with time on exposure to different concentrations of the extract. Also a general trend of cell reduction with time was observed when compared with the growth control. The result also showed some marked difference when compared with the standard organisms and drug.

In the first 2 h, the inhibition/death rate was slow but after then there was drastic reduction of the cell population. EAE had bacteriocidal effect on *P. aeruginosa* (test and type), *S. aureus* (test and type) and *E. coli* (test) at 1.0 mg/ml after 6 h and at 0.5 mg/ml after 2 h, gentamicin showed the same effect against *Ps. aeruginosa* (test and type), *S. aureus* (test and type) after 2 h. *P. aeruginosa* (test and type), *S. aureus* (test and type) and *E. coli* (test) were totally killed at 0.5 mg/ml after six hours. The extract showed inhibitory effect against the organisms at 0.25 mg/ml for six hours after which increase in the cell population was observed, also the extract and gentamicin could not achieve a total kill of *E. coli* (test and type) and *C. albicans* (test and type) but inhibited their growth (Figures 1 - 4).

DISCUSSION

The result of this work revealed the presence of tannins, glycosides, flavonoids, resins and carbohydrates in the extract. This result agrees with the findings of Agbor et al. (2004) and Palombo (2006) who reported the presence of tannins and flavonoids in the leaf extract of *A. cordifolia.* These secondary metabolites have been found to be present in plants at various parts and levels of growth (Adeshina et al., 2007). All these secondary metabolites were active against the tested organisms. The active compounds isolated from this extract has been reported to have antimicrobial properties (Lewis and Ausubel, 2006; Adeshokan et al., 2007; Oyeleke et al., 2008; Udobi et al., 2008). Tannins have been reported to hasten the healing of wounds, inflamed mucous membrane and to arrest bleeding (Shivananda et al., 2007; Manjunatha et al., 2007). Plants containing tannin are astringent in nature and are used in the treatment of intestinal disorders such as diarrhoea and dysentery therefore showing antibacterial activity (Akinpelu and Onakoya, 2006). The presence of tannins and flavonoids in *A. cordifolia* leaf extract may account for the increased colonic water and electrolyte reabsorption, a mechanism suggested for the antidiarrhoeal activity of the plant (Agbor et al., 2004; Palombo, 2006). Glycosides and flavonoids are known to inhibit tumor growth and also protect against gastrointestinal infections (El-Mahmood, 2009). The combined effect of tannins and flavonoids present in the EAE can account for its high activity.

The fraction eluted with 100% ethyl acetate had more antimicrobial activity than the ones done with 100% hexane and the mixture of hexane: ethyl acetate. This is an indication that ethyl acetate was able to elute more of the active ingredient than hexane. The activity increased as the polarity of the eluents increased.

The antimicrobial activities of the various chromatography fractions of the ethyl acetate extract showed that different chemical substances are present in the extract depending on the type of eluent used. With further emphasis on this area, the active ingredient of the extract can be obtained.

The extract had reduced activity against *C. albicans*; this may be due to the fact that fungi have different cell structure from bacteria. *S. aureus* was found to be more susceptible to the extract at a low concentration of 0.625 mg/ml. This is of great importance, as it has been reported that this organism has developed resistance to many antibiotics which sometimes makes its clinical management difficult (Willey et al., 2008). *P. aeruginosa*

Table 2. Antimicrobial activities of the various fractions of ethyl acetate extract.

Fraction	Organism			
	P. aeruginosa	***S. aureus***	***E. coli***	***C. albicans***
B_1	NA	NA	NA	NA
B_2	NA	NA	NA	NA
B_3	NA	IN	IN	IN
B_4	NA	NA	NA	NA
B_5	NA	NA	NA	NA
B_6	NA	NA	NA	NA
B_7	NA	NA	NA	NA
B_8	NA	NA	+	NA
B_9	NA	NA	+	+
B_{10}	NA	NA	NA	NA
B_{11}	NA	NA	NA	NA
B_{12}	IN	NA	+	NA
B_{13}	NA	NA	NA	NA
B_{14}	IN	+	+	+
B_{15}	+	+	+	+
B_{16}	NA	NA	NA	+
B_{17}	NA	NA	NA	NA
B_{18}	NA	+	NA	NA
B_{19}	NA	+	+	+
B_{20}	NA	NA	NA	IN
B_{21}	IN	+	+	+
B_{22}	IN	+	+	+
B_{23}	IN	+	+	+
B_{24}	IN	+	+	+
B_{25}	+	+	+	+
B_{26}	+	+	+	+
B_{27}	+	+	+	+
B_{28}	+	+	+	+
B_{29}	+	+	+	+
B_{30}	+	+	+	+

KEY: + = Activity (bactericidal or fungicidal).
IN = Inhibitory or bacteristatic.
NA = No activity.

also was susceptible to the EAE at 1.25 mg/ml. This organism has been reported to have gained a reputation as the most resistant of the gram-negative organisms (Gorman and Scott, 2004) and is long been a troublesome cause of secondary infection of wounds especially burns (Finch, 2004).

Generally, the result of this work also showed that the extract showed activity against both Gram-positive and Gram-negative bacteria, indicating that the extract has a broad spectrum of activity. Extracts from *A. cordifolia* have been reported to have a wide spectrum of antibacterial activity (Pesewu et al., 2008). The Gram-positive bacteria showed more susceptibility to the extract than the Gram-negative bacteria. Gram-negative bacteria are known to be resistant to the action of many antimicrobial therapeutic agents including plant based extracts (Kambezi and Afolayan, 2008; El-Mahmood, 2009). The large zones of inhibition produced by the EAE against the test organisms confirm the potency of the active components of the plant against all the test organisms.

The activity of the extract also confirm the works of Tona et al. (1999), Manga et al., (2004) who showed that ethyl acetate extract of this plant leaf were active against *E. coli*, *P. aeruginosa* and *S. aureus*. The M.I.C. values of ethyl acetate extract against *P. aeruginosa*, *E. coli* and *S. aureus* were 1.25 mg/ml, 1.25 and 0.625 mg/ml respectively which were lower than 100.0 mg/ml reported earlier by Tona et al. (1999).

The low M.I.C. values of the extract confirm the high

Table 3. Susceptibility of the test and type organisms to ethyl acetate extract and gentamicin

Test organisms	Zones of inhibitions (mm)					
	20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml	*Gentamicin
P. aeruginosa	30	29	22	18	12	31
" " ATCC 10145	23	20	18	13	NA	32
S. aureus	35	30	25	18	15	23
" " ATCC 12600	26	23	19	16	10	25
E. coli	30	28	24	18	17	20
" " ATCC11775	22	20	19	16	10	22
C. albicans	15	13	11	NA	NA	11
" " ATCC18804	20	18	12	10	NA	10

KEY: *Gentamicin 2 µg/ml for Gram positive bacteria and *C. albicans*; 4 µg/ml for Gram negative bacteria.
KEY: NA = No activity.

Table 4. The minimum inhibitory concentrations (M.I.C.) and minimum bactericidal concentrations (M.B.C.) of the ethyl acetate extracts against the test and type organisms.

Test organism	M.I.C. (mg/ml)	M.B.C. (mg/ml)
P. aeruginosa	1.25	2.5
" " ATCC 10145	2.5	5
S. aureus	0.625	1.25
"" ""ATCC 12600	1.25	2.5
E. coli	1.25	2.5
" " ATCC 11775	1.25	2.5
C. albicans	5	10
" " ATCC18804	5	10

KEY: NA = No activity.

activity of the extract at low concentrations. High activity of antimicrobial agent at low concentration is very essential for chemotherapeutic purposes because of their toxicity to the patient's system.

Generally, the action of EAE can be compared with that of gentamicin from the results of the rate of kill; this can be due to the combined action of the secondary metabolites present in the extract. The results of the rate of kill confirm the bacteriocidal effects of the EAE on some of the micro-organisms, which mean this extract, can be a prospective agent, to be formulated into various dosage forms. The results also showed that the extract had fungistatic effect against *C. albicans,* this suggests that the extract can be a good antibacterial agent but not good antifungal.

Conclusion

The activity of the plant leaf ethyl acetate extract has been attributed to the presence of tannins, flavonoids and glocosides in it. The extract was active against Gram-positive and Gram-negative bacteria; it also showed antimicrobial activity against fungi/yeast. So it could therefore, be suggested that the extract contains broad-spectrum antimicrobial agents. The extract was also found to be active at low concentration, which is very essential for antimicrobial agents. The ethyl acetate extract compares favourably with the standard drug gentamicin) judging from the results of the zones of inhibition and the rate of kill. This study has justified and authenticated the use of *A. cordifolia* in the treatment of

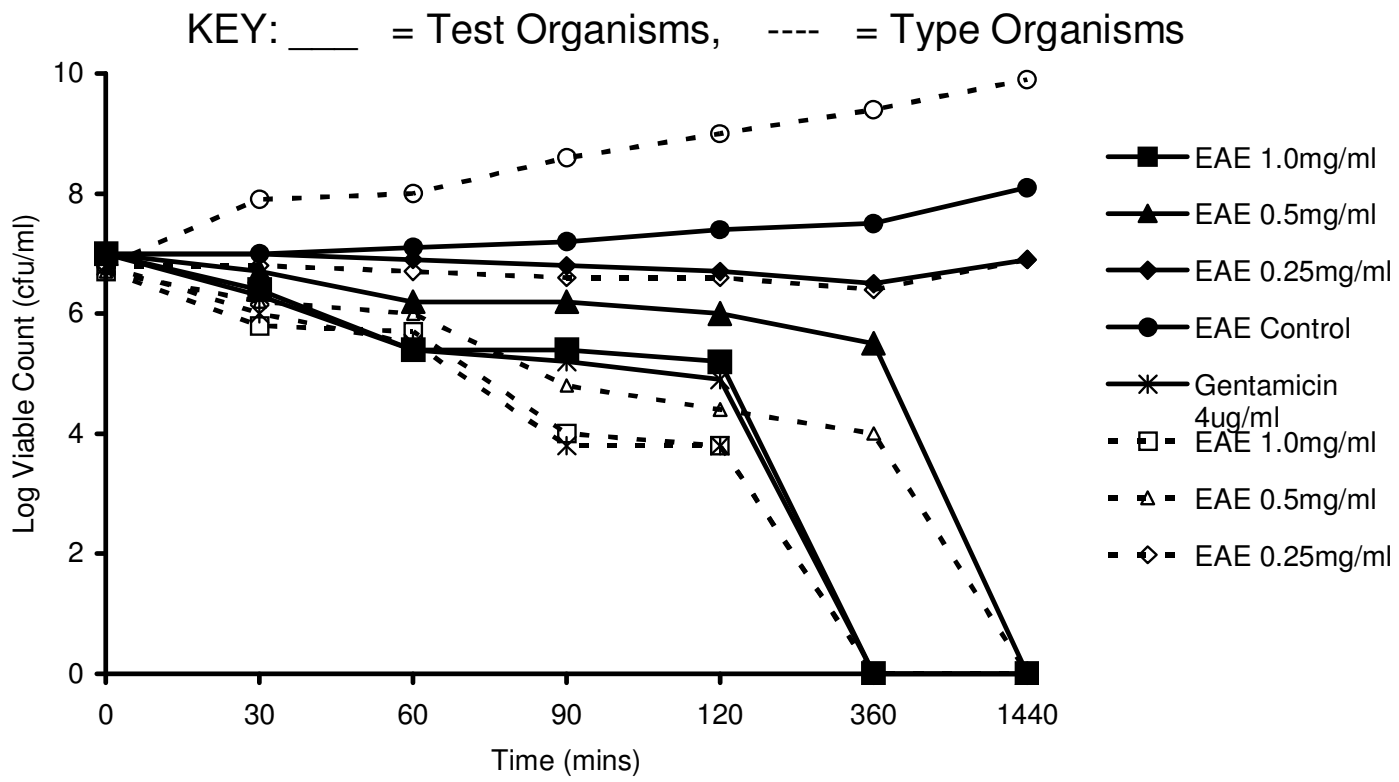

Figure 1. Death/survival rate of *P. aeruginosa* (test and ATCC 10145) on exposure to different concentrations of ethyl acetate extract (EAE) and gentamicin.

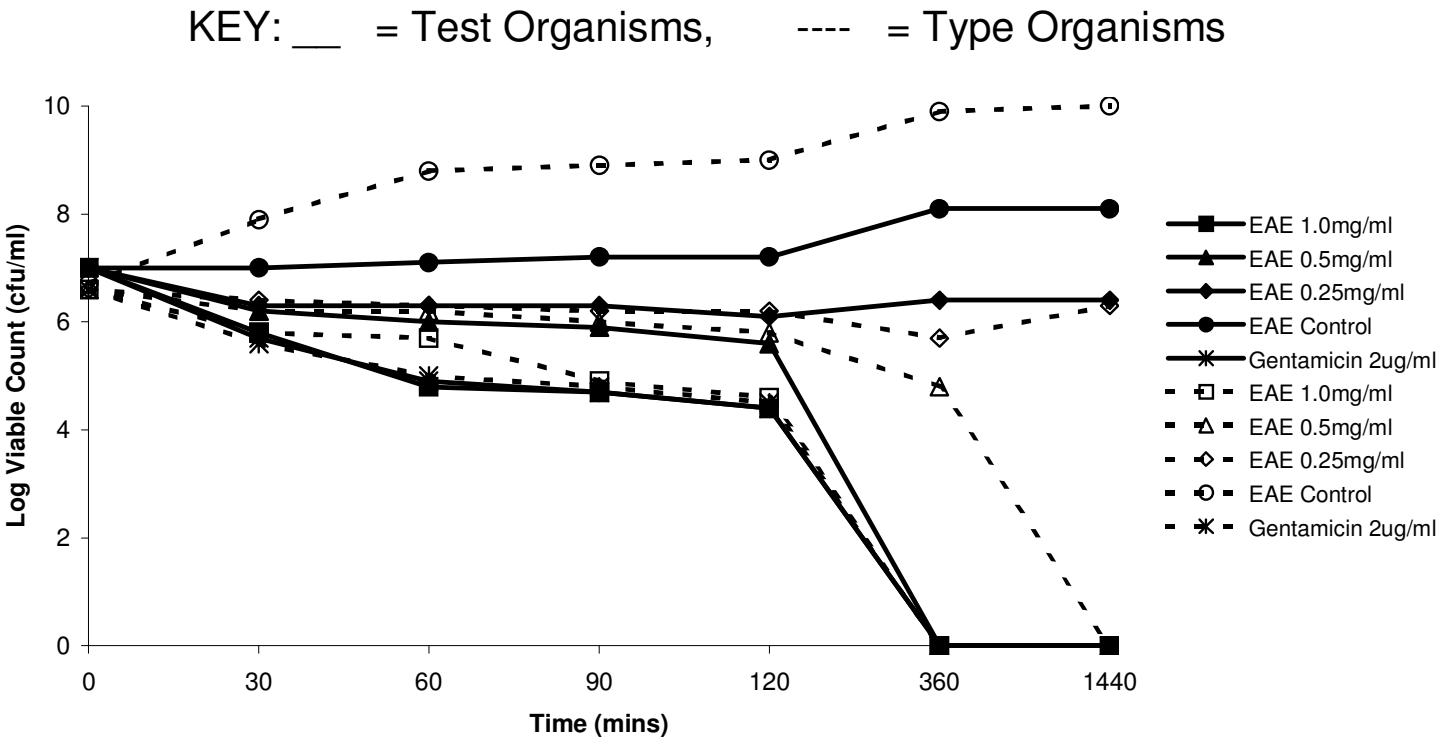

Figure 2. Death/ survival rate of *S. aureus* (test and ATCC12600) on Exposure to different concentrations of ethyl acetate (EAE) and gentamicin.

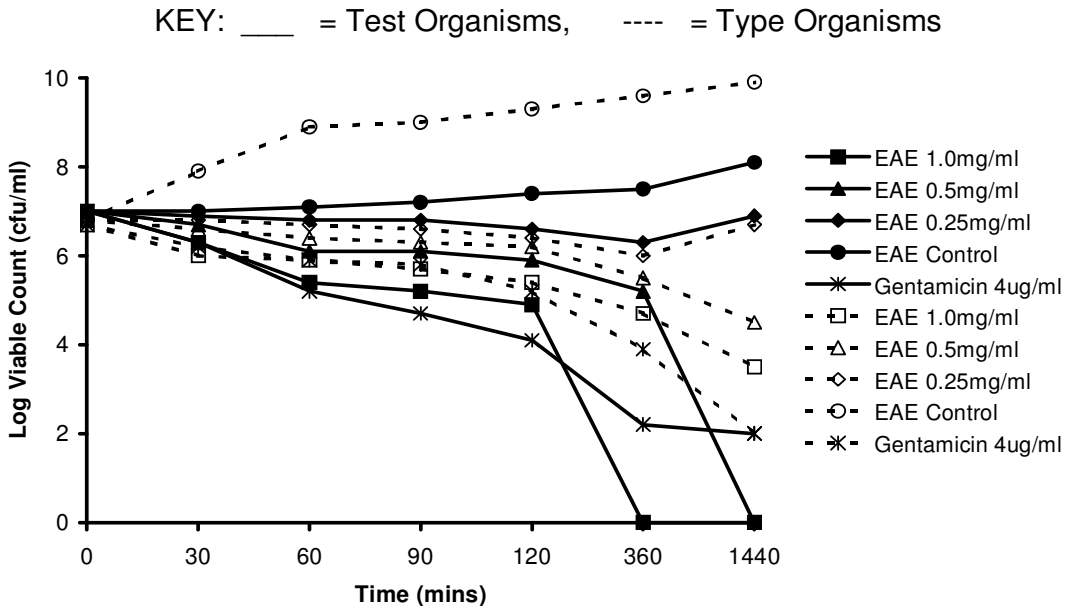

Figure 3. Death/ survival rate of *E. coli* (test and ATCC11775) on exposure to different concentrations of ethyl acetate extract (EAE) and gentamicin.

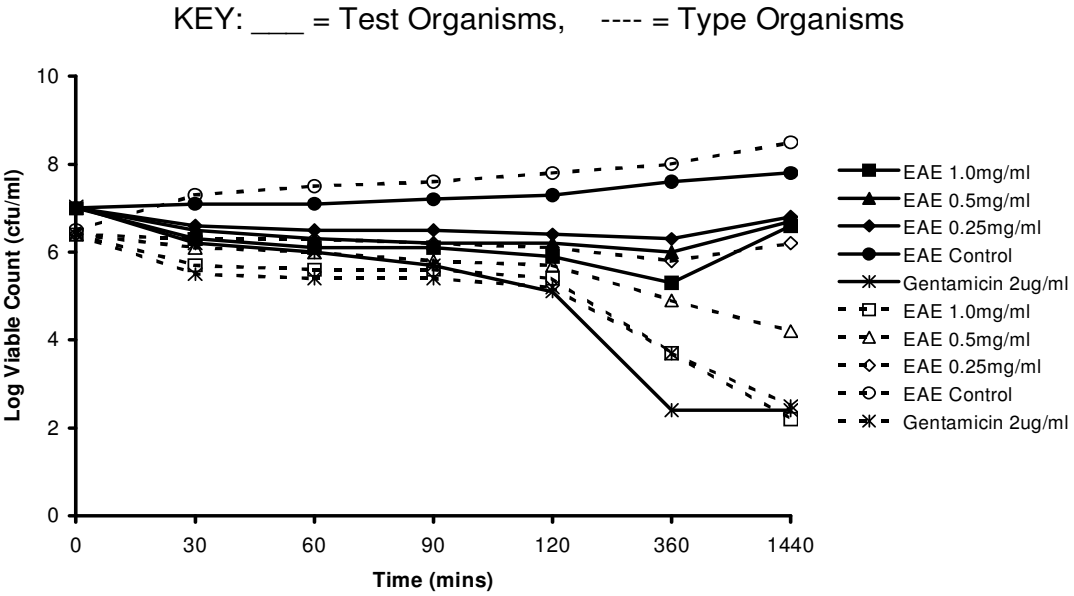

Figure 4. Death/survival rate of *C. albicans* (test and ATCC 18804) on exposure to different concentrations of ethyl acetate extract (EAE) and gentamicin.

some microbial diseases as established in herbal medicine.

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