In-vitro Antibacterial Activity of the Aqueous and Ethanolic Leaf Extracts of *Alchornea cordifolia* against Isolates from Throat Swabs, Ear Swabs and Sputum Samples

Yusuf. I[1*]., Tytler. B. A.[2]., Olayinka. A. T.[3]., Aroh .K. E[1]., Adeshina. G. O.[2]

1. Department of Microbiology, Alex Ekwueme Federal University Ndufu Alike, Ebonyi State, Nigeria.
2. Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.
3. Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

Corresponding author: wamya20@gmail.com; Tel: 07038266298

Abstract: Many bacteria species have been reported to develop resistance to antibiotics commonly prescribed for respiratory tract infections. This study aims to search for natural products for remedy of this problem and also to validate the claim by locals in the use of *Achornea cordifolia* in the treatment of respiratory tract infections. Isolation and identification of bacteria isolates were carried out using standard microbiological methods and MicroGen identification kits. . Cold maceration extraction method was employed for the extraction of ethanol and aqueous extracts of *Alchornea cordifolia* leaves. Agar well diffusion and agar dilution methods were employed to determine the zone of inhibition, minimum inhibitory concentration and minimum bactericidal concentration of the extracts respectively. The result showed that out of 180 samples from throat (68), ear swabs (57) and sputum (55) collected from patients with respiratory tract infection in Ahmadu Bello University Teaching Hospital Zaria, Nigeria, 208 isolates were obtained. Seventeen (17) bacteria species were identified as; *Staphylococcus aureus* (7), *Streptococcus* spp. (5), *Pseudomonas aeruginosa* (2), *Klebsiella pneumoniae* (2), *and Escherichia coli* (1). The two extracts showed broad spectrum activity but the aqueous extract had larger zones of inhibition ranging from 11.5mm - 32. 5mm and lower M.I.C and M.B.C values ranging from 5 mg/ml – 20 mg/ml. The aqueous and ethanol leaf extracts of *Alchornea cordifolia* was found to possess antibacterial activity against isolates from patients with respiratory tract infection in Ahmadu Bello University Teaching Hospital Zaria, Nigeria.

INTRODUCTION

Respiratory tract infections continue to be the most frequent and important cause of short term illnesses that compel an individual to seek medical attention not only in the developing world, but also in the developed world (Zafar *et al*., 2008). Respiratory tract infections impose a serious economic burden on society, ranging from reduced output in workplaces to frequent prescription by physicians of antibiotics, even when the causative agents of infection is not bacteria (Jafari *et al.,* 2009). Respiratory tract infections are amongst the most wide spread and serious infection, accounting for over 50 million deaths globally each year (Zafar *et al*., 2008)). The increasing prevalence of multi-drug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raised the specter of 'untreatable' bacterial infections and adds urgency to the search for new infection-fighting strategies (Zy *et al.,*2005; Rojas *et al.*, 2006).

Due to resistance to antibiotics by pathogens, recent research has been directed towards the use of traditional medicine/natural products for treatment and control of infections. *Alchornea cordifolia* (Euphorbiaceae) is a medium-sized shrubby treefound along the coastal regions of West Africa. Widespread in secondary forest and riverine forest, especially in marshy areas but sometimes in drier sites. It belongs to the subfamily Acalypholdeae and family Euphorbiaceae or Spurge family. The leaves are mostly used, but the stem bark, stem pith, leafy stems, root bark, roots and fruits are also used in local medicine.

In Nigeria the local names are 'Bambami' in Hausa, 'Ubebe' in Igbo, 'epa' in Yoruba, 'Mbom' in Efik and commonly 'Christmas bush' in English.

It is widely distributed throughout Africa where it is used extensively in traditional medicine (Adeshina *et al*., 2012).

The aim of this study is: to evaluate the antibacterial activity of ethanol and aqueous extracts of *Alchornea cordifolia* leaf against some bacterial isolates from throat swabs, ear swabs and sputum clinical samples.

Objectives of this study are to:

Isolate and identify bacteria species associated with respiratory tract infection from throat swabs, ear swabs and sputum clinical samples.

Prepare ethanol and water extracts from dried powdered leaves of *A. cordifolia* using cold maceration extraction.

Determine the antibacterial activity (zone of inhibition, MIC and MBC) of the two extracts against the identified bacterial isolates.

MATERIALS AND METHODS

Collection, Identification and Preparation of Plant leaf

Alchornea cordifolia leaves were collected from Chaza area of Suleja in Niger State, Nigeria. The plant was authenticated in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen number of 1868 was kept for future reference. The leaves were air-dried at room temperature and reduced to powder using mortar and pestle.

Ethical Approval

Ethical clearance with the number ABUTH/HREC/CL/05 was obtained from the ethical committee of Ahmadu Bello University Teaching Hospital for all the sample collection.

Collection of Clinical Specimen

Specimen collection commenced immediately the Ethical Committee approved the research proposal. The consent of patients that presented with upper and lower respiratory tract infections were sought before taking the specimens. One hundred and eighty (180) consecutive, non-duplicate specimens made up of Throat swabs (68), Ear swabs (57) and Sputum (55) were collected in the General out Patient (GOP) clinic of the Department of Family Medicine, Ahmadu Bello University Teaching Hospital Zaria, over a period of six months.

Isolation and Characterization of Bacteria Species

The specimens were cultured on Blood agar, Chocolate agar and Mac-Conkey agar plates at 37°C for 24 h. Discrete colonies were picked based on their morphology and further sub-cultured to obtain pure strains. The isolated colonies were Gram stained and based on their Gram reactions were inoculated on different selective media; Mannitol Salt agar, Cetrimide agar, Eosin Methylene blue agar. Different biochemical tests were conducted (Catalase test, Coagulase test, Oxidase test), after which MicroGen Identification Kits were used to identify different species with Staph. ID kits for S. *aureus,* MicroGen A for enterobacteracea and MicroGen A+B for oxidase positive organisms. All the isolates were then placed on nutrient agar and chocolate agar slants and maintained in a refrigerator at 4°C.

Aqueous and Ethanol Extraction of Plant Material

Seven hundred grams (700g) each of powdered leaf extract were weighed. To one portion 2.5litres of ethanol was added, covered to prevent evaporation and allowed to macerate for 2hrs; it was filtered and excess ethanol evaporated to dryness using a rotary evaporator at 35^{o}C. The dried extract was then stored in a desiccator till required. The second portion was extracted in water at 60^{o}C for one hour and filtered. The filtered extract was then concentrated on a water bath at 70^{o}C (Evans, 2002).

Susceptibility Testing

Each of the overnight cultures of organism was standardized to a 0.5 MacFarland density. Sterile molten Mueller Hinton agar (20 ml) was poured into sterile Petri dishes and allowed to set. The sterile Mueller Hinton agar plates were flooded with 1.0 ml each of the standardized test organism and the excess is drained off and dried.

A sterile cork-borer 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 seep beneath the agar. Serial dilutions of the stock solution of the extracts were made to obtain concentration between 20 – 1.25 mg/ml. One hundred microlitres of the extracts of different concentrations (1.25 – 20.0 mg/ml) was added to fill the bored holes. Negative control was prepared by putting 0.1 ml of sterile distilled water in one of the bored holes for each plate and amoxicillin-clavulanic acid antibiotic disc (30µg) served as a positive control. The plates were left to stand for one hour to allow for diffusion, after which the plates were incubated at 37°C for 18 h. The zones of inhibition were measured in millimetres. The above method was carried out in duplicates and the mean of the duplicate results was taken. For all isolates and both extracts (CLSI, 2009).

Determination of Minimum Inhibitory Concentration (M.I.C)

The MIC was determined by agar-dilution method according to CLSI, (2006) with some modifications (Aboaba *et al*., 2006). Serial dilution of the stock solution of the extracts/fractions was made to obtain concentration between 20 – 1.25 mg/ml. A 10 ml portion of each dilution containing double concentration of extract/fraction was incorporated into 10 mls double strength Mueller Hinton Agar and poured into sterile Petri dishes. Sterile punctured filter paper discs (6mm) were aseptically placed on the solidified leaf extract-agar admixture plates. Using a micro pipette standardized inoculum of the isolates was immediately added to the discs in volumes of about 20µl. A 20 µl sterile distilled water was added to the sterile paper disc as a negative control. The plates were left at ambient temperature for 30 minutes for pre-diffusion prior to incubation at 37°C for 24 hrs. The lowest concentration of the extract/fraction in each of the test agar plates that showed no growth when compared to the control was considered as the M.I.C. of the extract against the test organism.

Determination of Minimum Bactericidal Concentration (M.B.C)

The filter paper discs that did not show any visible growth from the M.I.C plates were aseptically transferred into 5 ml sterile Nutrient broth using a pair of sterile forceps. This was incubated at 37°Cfor 24hrs. The Minimum Bactericidal Concentration was considered as the minimum concentration of those nutrient broth bottles in which no turbidity was observed (CLSI, 2006) as modified by (Aboaba et al., 2006).

RESULTS

Out of the 180 specimens collected, 208 bacteria were isolated. Seventeen (17) isolates were identified and confirmed using MicroGen identification kits. (Table 1.) shows the distribution of bacterial isolates from clinical specimens. *S. aureus* has the highest number with seven isolates while *E. coli* has just one isolate.

Table 1. Distribution of bacterial isolates from clinical specimens

Organism	Number of isolates (Throat swab)	Number of isolates (Ear swabs)	Number of isolates (Sputum)
S. aureus	4	1	2
Strep.spp	3	2	-
K.pneumoniae	-	-	2
P.aeruginosa	-	2	-
E. coli	1	-	-
Total	**8**	**5**	**4**

The aqueous extract of *A. cordifolia* showed activity against isolates from throat swab specimens with highest activity recorded against *E. coli*(T13) and least zone of inhibition against *Strep. spp* (T67) (Table 2).

Table 2. Zone of inhibition values of the aqueous extract of *A.cordifolia* against isolates from throat swabs

Isolates	Zone of Inhibition (mm) for aqueous extract						
	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	Amc30µg	C
E. coli (T13)	25.5±0.7	22.5±1.4	17.0±1.4	15.5±0.7	14.5±0.7	27.5±0.7	NI
S. aureus (T20)	20.0±0.0	18.5±0.7	16.5±0.7	12.5±0.7	11.5±0.7	26.5±0.7	NI
S. aureus (T31)	22.5±0.7	19.0±0.0	17.5±0.7	11.5±0.7	NI	25.0±1.4	NI
S. aureus (T44)	20.5±0.7	17.5±0.7	14.0±0.0	12.5±0.7	NI	26.5±0.7	NI
S. aureus (T38)	20.0±0.0	17.5±0.7	16.0±0.0	14.5±0.7	11.5±0.7	28.5±0.7	NI
Strep.spp (T12)	22.5±0.7	20.5±0.7	17.5±0.7	12.5±0.7	NI	27.0±1.4	NI
Strep.spp (T8)	20.5±0.7	19.5±0.7	15.5±0.7	12.0±0.0	NI	25.0±1.4	NI
Strep.spp (T67)	16.5±0.7	13.0±0.0	11.5±0.7	NI	NI	24.5±0.7	NI

KEY:± Standard deviation, NI = No Inhibition, AMC= amoxicillin /clavulanic acid, C =Control (Sterile distilled water)

The ethanol extract showed activity against the isolates from throat swab specimens but with smaller zones of inhibition compared to the aqueous extract. The larger zone of inhibition is seen in *E. coli* (T13) and the lowest in *Strep.* spp (T67) (Table 3).

Table 3. Zone of inhibition values of the ethanol extract of *A.cordifolia* against isolates from throat swabs.

Isolates	Zone of Inhibition (mm) for ethanol extract						
.	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	Amc30µg	C
E.coli (T13)	23.5±0.7	20.5±0.7	17.5±0.7	12.5±0.7	NI	29.5±0.7	NI
S.aureus (T20)	18.0±0.0	16.5±0.7	14.0±1.4	11.5±0.7	NI	24.0±0.0	NI
S.aureus (T31)	18.5±0.7	14.5±0.7	12.0±0.0	NI	NI	24.5±2.1	NI
S.aureus (T44)	17.5±0.7	15.5±0.7	12.5±0.7	NI	NI	25.5±0.7	NI
S.aureus (T38)	18.5±0.7	16.0±0.0	12.5±0.7	11.0±0.0	NI	24.5±0.7	NI
Strep.spp (T12)	19.5±0.7	17.0±0.0	12.5±0.7	NI	NI	25.5±0.7	NI
Strep.spp (T8)	19.5±0.7	16.5±0.7	13.5±0.7	NI	NI	23.5±0.7	NI
Strep.spp (T64)	16.0±0.0	12.5±0.7	NI	NI	NI	23.0±1.4	NI

KEY: ± Standard deviation, NI = No Inhibition, AMC= amoxicillin /clavulanic acid, C =Control (Sterile distilled water)

The aqueous extract showed higher activity against isolates from ear swab specimens with the highest diameter zones of inhibition recorded against *P. aeruginosa*. (Table 4.)

Table 4. Zone of inhibition values of aqueous extract of *A.cordifolia* against isolates from ear swabs.

Isolates	Zone of Inhibition (mm) for aqueous extract						
	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	Amc30µg	C
P.aeruginosa (E6)	32.5±0.7	30.0±1.4	27.5±0.7	24.5±0.7	20.0±1.4	29.0±1.4	NI
P.aeruginosa (E24)	30.5±0.7	26.0±1.4	23.5±0.7	21.5±0.7	18.0±1.4	26.0±0.7	NI
S.aureus (E27)	23.5±0.7	21.5±0.7	19.0±0.0	15.5±0.7	NI	25.5±0.7	NI
Strep.spp (E20)	18.5±0.7	16.0±0.0	14.5±0.7	12.0±0.0	NI	24.0±0.0	NI
Strep.spp (E22)	20.5±0.7	16.5±0.7	14.5±0.7	11.5±0.7	NI	25.0±0.0	NI

KEY: ± Standard deviation, NI = No Inhibition, AMC= amoxicillin /clavulanic acid, C =Control (Sterile distilled water)

Smaller zones of inhibition have been recorded in ethanol extract against isolates from ear swab specimens compared to the aqueous extract. At 1.25mg/ml concentration there was no zone of inhibition recorded. (Table 5).

Table 5. Zone of inhibition values of ethanol extract of *A.cordifolia* against isolates from ear swabs

Isolates	Zone of Inhibition (mm) for ethanol extract						
	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	Amc30µg	C
P.aeruginosa (E6)	21.0±1.4	17.5±0.7	15.5±0.7	12.5±0.7	NI	26.5±2.1	NI
P.aeruginosa (E24)	19.0±0.0	15.5±0.7	13.5±0.7	NI	NI	26.5±2.1	NI
S.aureus (E27)	20.5±0.7	18.5±0.7	15.0±0.0	12.5±0.7	NI	24.5±0.7	NI
*Strep.*spp (E20)	17.5±0.7	15.5±0.7	12.5±0.7	NI	NI	24.0±1.4	NI
*Strep.*spp (E22)	16.5±0.7	14.5±0.7	11.5±0.7	NI	NI	24.0±0.0	NI

KEY: ± Standard deviation, NI = No Inhibition, AMC= amoxicillin /clavulanic acid, C =Control (Sterile distilled water)

The aqueous extract had clear activity against isolates from sputum specimens with *S. aureus* (S44) having the least zone of inhibition values. At the concentration of 1.25mg/ml only *K. pneumoniae* (S16) had a zone of inhibition value of 12.5 mm. (Table 6).

Table 6. Zone of inhibition values of aqueous extract of *A.cordifolia* against isolates from sputum specimens.

Isolates	Zone of Inhibition (mm) for aqueous extract						
	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	Amc30µg	C
K.pneumoniae (S16)	27.5±0.7	24.5±0.7	22.0±1.4	16.5±0.7	12.5±0.7	27.5±2.1	NI
K.pneumoniae (S20)	25.5±0.7	19.5±0.7	17.0±0.0	12.5±0.7	NI	27.0±2.1	NI
S.aureus (S44)	20.5±0.7	18.5±0.7	16.0±0.0	13.5±0.7	NI	26.0±0.7	NI
S,aureus (S10)	25.5±0.7	20.5±0.7	17.5±0.7	12.5±0.7	NI	23.5±0.7	NI

KEY: ± Standard deviation, NI = No Inhibition, AMC= amoxicillin /clavulanic acid, C =Control (Sterile distilled water)

The ethanol extract showed smaller zones of inhibition against isolates from sputum specimens. With *K. pneumonia* (S20), *S. aureus* (S44) and *S. aureus* (S10) having no zones of inhibition at 2.5 mg/ml concentration. (Table 7).

Table 7. Zone of inhibition values of ethanol extract of *A.cordifolia* against isolates from sputum specimens.

Isolates	Zone of Inhibition (mm) for ethanol extract						
	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	1.25mg/ml	Amc30µg	C
K.pneumoniae (S16)	19.5±0.7	17.5±0.7	15.5±0.7	14.0±0.7	11.5±0.7	26.5±0.7	NI
K.pneumoniae (S20)	19.0±1.4	15.5±0.7	12.5±0.7	NI	NI	29.5±0.7	NI
S.aureus (S44)	18.5±0.7	16.0±0.0	12.5±0.7	NI	NI	26.5±0.7	NI
S,aureus (S10)	20.5±0.7	18.0±0.0	14.5±0.7	NI	NI	25.0±1.4	NI

KEY: ± Standard deviation, NI = No Inhibition, AMC= Amoxicillin /clavulanic acid, C =Control (Sterile distilled water)

The aqueous extract showed lower values of M.I.C and M.B.C with *S. aureus* (T31) having the lowest values. While *Strep*. spp (T67) having the highest values. (Table 8).

Table 8. M.I.C and M.B.C of aqueous and ethanol extracts against isolates from throat swab specimens.

Isolates	Aqueous extract		Ethanol extract	
	M.I.C (mg/ml)	M.B.C (mg/ml)	M.I.C (mg/ml)	M.B.C (mg/ml)
E.coli (T13)	20	>20	20	>20
S.aureus (T38)	5	5	10	20
S.aureus (T44)	5	20	10	20
S.aureus (T31)	5	5	10	10
S.aureus (T20)	5	10	10	20
*Strep.*spp (T12)	10	20	20	20
*Strep.*spp (T8)	20	>20	20	>20
*Strep.*spp (T67)	20	>20	20	>20

The Ethanol extract has higher M.I.C and M.B.C values than the aqueous extract with *P. aeruginosa* (E27) and (E6) having lower M.I.C values. (Table 9).

Table 9.The M.I.C and M.B.C values of aqueous and ethanol extracts against isolates from ear swab specimens.

Isolates	Aqueous extract		Ethanol extract	
	M.I.C (mg/ml)	M.B.C (mg/ml)	M.I.C (mg/ml)	M.B.C (mg/ml)
P.aeruginosa (E6)	5	10	10	20
P.aeruginosa (E24)	5	20	10	20
S.aureus (E27)	5	10	5	20
*Strep.*spp (E20)	10	20	20	>20
*Strep.*spp (E22)	20	>20	20	>20

Both the ethanol and aqueous extract M.I.C values against isolates from sputum specimens were higher and the same in the two *K. pneumonia* isolates and *S. aureus* (S44). *S. aureus* (S10) had lower M.I.C and M.B.C. (Table 10).

Table 10. The M.I.C and M.B.C values of aqueous and ethanol extracts against isolates from sputum specimens.

Isolates	Aqueous extract		Ethanol extract	
	M.I.C (mg/ml)	M.B.C (mg/ml)	M.I.C (mg/ml)	M.B.C (mg/ml)
K.pneumoniae (S16)	20	>20	20	>20
K.pneumoniae (S20)	20	>20	20	>20
S.aureus (S10)	5	10	10	20
S.aureus (S44)	20	>20	20	>20

DISCUSSION

A total of 180 specimens were collected from patients having RTIs. The bacteria isolated from the specimens collected included; *S. aureus, K. pneumoniae, E. coli, P. aeruginosa* and *Strep.* spp. These isolates clearly represented clinically significant pathogens and are known to cause majority of community and hospital acquired infections and are capable of elaborating severe virulence factors. This result is similar with the work of Kumari *et al*., (2007) in India, EL – Mahmood *et al*., (2010) and Okesola and Oni, (2009) in Nigeria who isolated similar pathogens from patients with respiratory tract infection. It is also in line with the work of Taura *et al*., (2013) that isolated *Klebsiella pneumoniae* and *Staphylococccus aureus* from sputum samples in Aminu Kano Teaching Hospital in Kano State, Nigeria.

S. aureus was isolated in all the specimens with throat swabs (4), ear swabs, (1) and sputum (2). While *K. pneumoniae* was isolated in only sputum specimens (2), *P. aeruginosa* was isolated in only ear specimens (2) and *Strep*. spp in throat swab (3) and ear swab (2) specimens. This is in line with the work of Adedeji *et al*., (2007) in Osun State, Nigeria and the work of Somia *et al*., (2014) in Pakistan who showed that *P. aeruginosa* was the commonest organism isolated from ear infections followed by *S. aureus*. *P. aeruginosa* infections such as Otitis media and externa are often chronic infections. In this study, *S. aureus* has the highest number of isolates and is also isolated in all the three specimen sources, this could be as a result of the availability of *Staphylococcus aureus* as a normal flora of the nares, mouth and some non-sterile sites. The presence of *K. pneumoniae* from sputum could be as a result of *K. pneumoniae* being one of the causes of broncho-pneumonia. It colonizes the lower respiratory tract and common in hospital patients receiving antibiotics, it sometimes causes chronic destructive lesions and multiple abscess formation in the lungs (Friedländer's pneumonia), (Greenwood *et al*., 2007).

The diameter zones of inhibition, showed that the aqueous extract had more activity than the ethanol extract. The degree of activity varied with the isolates and the extracts. This variation of activity could be due to the differences in the solubility of the secondary metabolite in the different solvents used and also the structural or morphological variability of the tested isolates thus, larger zones of inhibition were produced by the susceptible organisms than the resistant ones. It could also be due to the polarity of the solvents, water been more polar dissolve more of the secondary metabolites. This result is different from the work of Adeshina *et al*., (2012) which showed that the ethyl acetate fraction (non-polar solvent) of methanol extract of *A. cordifolia* leaf was relatively more active than the aqueous fraction (polar solvent) against type isolates of *E. coli*, *S. aureus*, *P. aeruginosa* and *Candida albican*. The observed differences may be as a result of variation of plants location and method of extraction. The result is similar to the findings of Mohammed *et al.,* (2012) who reported that water extract of *Alchornea cordifolia* exerted highest activity against *S. aureus* isolated from wound samples in Aminu Kano Teaching Hospital in Kano, Nigeria more than the ethanol extract. The work of Gatsing *et al.,* (2010) in Cameroon who showed that the aqueous leaf extract of *A. cordifolia* was more active than the methanol and ethanol extracts against *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *S. aureus*. The result is also in line with the work of Osumah *et al*., (2012) that showed that the aqueous root extract of *A. cordifolia* had more activity than the ethanol extract against *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhi* isolates from fecal material and wounds

The diameters zone of inhibition showed a concentration dependent result and the result also showed that the zone of inhibition values of the extracts was far lesser than that of the positive control amoxicillin/clavulanic acid.

This may be attributed to the fact that conventional antibiotics are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, while herbal medicinal plants products are still crude, prepared from plant and animal origins and are subjected to contamination and deterioration most of the time (EL – Mahmood and Ameh, 2007).

The M.I.C and M.B.C values were generally lower for the aqueous extract against the test isolates compared to those of the ethanol extract. *S. aureus* was more susceptible to the extracts especially the aqueous extract which showed lowest M.I.C and M.B.C values of 5 mg/ml – 10 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 (Adewunmi *et al*., 2001).

CONCLUSION

The aqueous and ethanol leaf extracts of *Alchornea cordifolia* obtained from Chaza, Niger State, Nigeria was found to possess antibacterial activity against *S. aureus*, *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *Strep*. spp isolated from throat swabs, ear swabs and sputum specimens of patients with respiratory tract infection in Ahmadu Bello University Teaching Hospital Zaria, Nigeria. The leaf extracts of *A. cordifolia* have shown broad spectrum of activity and a consistent and concentration dependent inhibition of bacterial isolates. The aqueous extract had more yield than the ethanol extract, and have shown to have higher antibacterial activity than the ethanol extract with zones of inhibition ranging from 32.5 mm – 11.5 mm and lower M.I.C and M.B.C values ranging from 5 mg/ml – 20 mg/ml.

This study has justified the use of *Alchornea cordifolia* in the treatment of some bacterial diseases in folkloric herbal medicine.

REFERENCES

Aboaba, O. O., Smith, S. I., Olude, F. O.(2006). Antibacterial effect of Edible Plant on *Escherichia coli*015:H. *Pakistan Journal of Nutrition* 5 (4): 325-327.

Adedeji, G. B., Fagade, O. E., Oyelade, A. A. (2007). The prevalence of *Pseudomonas aeruginosa* in clinical samples and its sensitivity to citrus extract. *African Journal of Biomedical Research*. 10: 183-187.

Adeshina, G.O., Kunle, O. F.,Onaolapo, J.A., Ehinmidu J.O., Odama L.E. (2012) Antibacterial Activity of Aqueous and Ethyl Acetate sub-fractions of *Alchornea cordifolia* leaf. *European Journal of Medicinal plants*. 2 (1): 31-41.

Adewunmi, C. O., Agbedahunsi, J. M., Adebajo, A. C., Aladesanmi, A. J., Murphy, N. and Wando, J. (2001). Ethno-veterinary medicine: Screening of Nigerian medicinal plants for trypanocidal properties. *Journal of Ethnopharmacology*. 77: 19-14.

CLSI, (2006). Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Wayne, Pa, USA. Approved standard M7-A7.

CLSI, (2009). Performance Standard for Antimicrobial disk Susceptibility Test, Approved StandardM02-A10.10th Edition. CLSI, Wayne, PA, 29: 1.

EL-Mahmood, A., Ameh, J. M. (2007). In-vitro antibacterial activity of *Parkia biglobosa* (Jacq) root, bark extract against some microorganisms associated with Urinary tract infections. *African Journal of Biotechnology*. 6, 195-200.

El-Mahmood A. M., Isa. A., Tirmidhi, S.B. (2010). Antimicrobial susceptibility of some respiratory tract pathogens to commonly used antibiotics at the Specialist Hospital, Yola, Adamawa State, Nigeria. *Journal of Clinical*

*Medicine and Research.*2(8), pp. 135-142.

Evans, W. C. (2002). Trease and Evans Pharmacognosy, (Fifteenth Edition), Saunders, W. B. Company limited. Pp: (137-139, 230-240).

Gatsing, D., Nkeugouap, F. N. C., Nji-Nkah, F. B., Kuiate, J. R., Tchouanguep, M. F. (2010). Antibacterial activity, bioavailability and acute toxicity evaluation of the leaf extracts Of *Alchornea cordifolia* (Euphorbiaceae). *International Journal of Pharmacology*. 1811 – 7775.

Greenwood, D., Slack, R., Peutherer, J. and Barer, M. (2007). A guide to microbial infections: Pathogenesis, Immunity, Laboratory diagnosis and control. Medical microbiology, 17th Edition. Elsevier limited. Pp: 286-287.

Jafari, N. J., Ranjbar, R., Haghi-Ashtiani, M. T., Abedini, M., Izadi, M. (2009). The study of prevalence and antimicrobial susceptibility of tracheal bacterial strains isolated from paediatric patients. *Pakistan Journal of Biomedical sciences.* 12 (5): 455 - 458.

Kumari, H.B.V., Nagarathna. S., Chandramuki, A. (2007). Antimicrobial resistance pattern among aerobic gram negative bacilli of lower respiratory tract specimens of intensive care unit patients in a neurocentre. *Indian Journal of Chest. Diseases Allied. Science*. 49: 19-22.Org. 86, 408-416.

Mohammed, A., Adeshina, G. O., Ibrahim, Y. K. E. (2012). Incidence of wound infections in a teaching hospital in Kano and susceptibility of the bacteria isolates to *Alchornea cordifolia* leaf extracts. M.sc Research Dissertation (Unpublished).Department of Pharmaceutics/ Pharmaceutical Microbiology Library. Ahmadu Bello University, Zaria

Okesola, A. O., Oni. A. A. (2009). Antimicrobial resistance among common bacterial pathogens in South Western Nigeria. *Journal of Environmental Sciences*. 5(3): 327-330.

Osadebe, P., Okoye, F. B. C. (2003). Anti inflammatory effects of crude methanolic extract and fractions of *Alchornea cordifolia* leaves. *Journal of Ethnopharmacology.* 89:19-24.

Osumah, R. O., Adeshina, G. O., Ehinmidu, J. O. (2012). Phytochemical and antibacterial studies of the stem bark and root of *Alchornea cordifolia* (Euphorbiaceae). M.sc Research Dissertation (Unpublished).Department of Pharmaceutics/ Pharmaceutical Microbiology Library. Ahmadu Bello University, Zaria. Pp: 58-66.

Somia, G., Asma, E., Zehre, A. (2014). Pseudomonas: A common causative agent of Ear infections in South Asian Children. *International Journal of Current Microbiology and Applied Sciences*. 3(6): 156-160.

Taura, D. W., Adamu, S., Koki, Y. A., Musa, M. A., and Muhammed, B. B. (2013). Mycotic Infections associated with pulmonary symptoms in patients attending infectious diseases hospital, Kano. *Greener Journal of Microbiology and Antimicrobials*. 2(1): 015-020.

Zafar, A., Hussain, Z., Lomama, E., Sibliie, S., Irfan, S., Khan, E. (2008). Antibiotic susceptibility of pathogens isolated from patients with community-acquired respiratory tract infections in Pakistan- the active study. *Journal of Ayub Medical College, Abbottabad*. 20: 7-9.

Zy, E.A., Area, A., Aam, K. (2005). Antimicobial activity of some medicinal plant extracts in Palestine. *Pakistan Journal of Medical Science*. 21: 187-193.