ejpmr, 2019,6(3), 15-20

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

ANTIBACTERIAL ACTIVITY OF THE LEAF FRACTIONS OF ALCHORNEA CORDIFOLIA AGAINST ISOLATES FROM PATIENTS WITH RESPIRATORY TRACT INFECTION IN AHMADU BELLO UNIVERSITY TEACHING HOSPITAL ZARIA, NIGERIA

Yusuf I.[1]*, Tytler B. A.[1], Olayinka A. T.[2] and Adeshina G. O.[1]

[1]Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Medical Microbiology, Ahmadu Bello University Teaching Hospital, Zaria, Nigeria.

***Corresponding Author: Prof. Yusuf I.**

Department of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University, Zaria, Nigeria.

Article Received on 21/12/2018 **Article Revised on 12/01/2019** **Article Accepted on 04/02/2019**

ABSTRACT

Background: Recent years have witnessed a dramatic increase in antibacterial resistance and a decline in the development of novel antibiotics. This problem is prevalent among common etiologic pathogens associated with Respiratory Tract Infections. The aim of this study is to determine the antibacterial activity of the leaf fractions of *Alchornea cordifolia* against isolates from patients with respiratory tract infections. **Methodology:**The aqueous extract of *Alchornea cordifolia* leaf was fractionated using column chromatography. Thin layer Chromatography method was used to identify similar fractions. Agar-dilution method was employed to determine the Minimum Inhibitory Concentration(M.I.C) and Minimum Bactericidal Concentration (M.B.C) of fractions. Spread plate method was employed to determine the rate of kill of most active fraction. **Result:**The fractionation of the aqueous extract gave thirty five (35) fractions but after pooling together of similar ones, Seven (7) different fractions were obtained. Seventeen (17) bacteria species were isolated from samples of patients with respiratory tract infections and the isolates were identified as;*Staphylococcus aureus* (7), *Streptococcus* spp. (5), *Pseudomonas aeruginosa* (2), *Klebsiella pneumoniae* (2), *and Escherichia coli* (1). The M.I.C of the fractions showed that F2 had the lowest M.I.C values against all the isolates. The F2 fraction had M.I.C values that ranged between 2.5 – 5 mg/ml against *S. aureus* and 5 – 10 mg/ml against *Strep*. spp. The death/survival rate showed that at 1440 minutes, M.I.C concentration of 2.5 mg/ml of F2 had 100 % kill; there was reduction in surviving cells with both the Sub-M.I.C concentration of 1.25 mg/ml and amoxicillin clavulanic acid 30 µg/ml against *S. aureus* (T38) isolate. A total kill was observed at 240 minutes, with M.I.C concentration of 5 mg/ml and at 1440 minutes, with Sub-M.I.C concentration of 2.5 mg/ml against *Klebsiella pneumoniae* (S16). **Discussion:** The observed low M.I.C values from fraction F2 could be due to the fact that F2 contains the secondary metabolites responsible for the superior antibacterial activity of the aqueous extract. The lower M.I.C confirm the high activity of fraction (F2) at low concentration. High activity of antibacterial agent at low concentration is very essential for chemotherapeutic purposes because of their toxicity to patient system. **References:**Smith, R. P., Paxman, J. J., Scanlon, M. J., Heras, B. (2016). Targetting bacterial Dsb protein for the development of anti-virulence agent. *Molecules*. 21: 811 CLSI, (2006). Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Wayne, Pa, USA. Approved standard M7-A7.

BACKGROUND

Antimicrobial drug resistance is a global challenge for the 21st century with the emergence of resistant bacteria strains worldwide (Furin*et al.,* 2011).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)). In 2012, lower respiratory infections such as pneumonia and bronchitis were the second causes of mortality and morbidity in Sub-Saharan Africa, accounting for over 1 million or 11.5% of deaths in the region, while tuberculosis accounted for 2.4% (Siikamaki, 2015).Acute respiratory tract infection is a common cause of hospital admission in Nigeria, it was estimated that pneumonia accounted for 20% of deaths in children under age of 5 years (Akanbi *et al*., 2009). 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).

Medicinal properties of plants are hinged on the presence of bioactive principles such as alkaloids, phenols, tannins, glycosides and essential oils among others. This necessitates the continued screening of medicinal plants, not only to determine the scientific basis for their usage, but also to discover possible new active principles (Karou *et al.*, 2006). The primary benefits of using plantderived medicines are that they are relatively cheaper than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments.

Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional system of medicine relatively cheaper than modern medicine. Many works have been carried out with the aim of knowing the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of microbial infections as possible alternatives to antibiotics and other chemotherapeutic agents to which many infectious microorganisms have become resistant. *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.In Nigeria the local names are 'Bambami' in Hausa, 'Ubebe' in Igbo, 'epa' in Yoruba, 'Mbom' in Efik and commonly 'Christmas bush' in English. The plant is an important crude drug in the indigenous system of medicine for the management of pain, rheumatism, and arthritis, pile, toothache and some other inflammatory disease states (Osadebe and Okoye, 2003).

METHODOLOGY

Fractionation of Aqueous extractusing Column Chromatography: Forty grams (40g) of the aqueous extract was mixed with 40 grams of silica gel and 20 mls of Methanol and allowed to dry in open air. A column was mounted by first placing a cotton inside the column, then 150 grams of silica gel was introduced into the column, after which the extract was introduced. A cotton was finally placed. Two hundred (200) mls of different solvents with varying percentage were used to elute the column starting from the least polar solvent to the most polar solvents. i.e n- Hexane, Ethyl acetate and Methanol. After elution, various fractions were collected.

Thin Layer Chromatography (TLC) of fractions: The Thin Layer Chromatography was carried out by cutting equal sizes of silica gel plates, different fractions starting from the first fraction were spotted on the plate using a capillary at an interval of 0.5 cm. A mobile phase mixture of n-Hexane and Ethyl acetate at ratio of 4:1 was placed in a chromatographic tank for fractions 1-15 and the silica plate was placed in the tank vertically with the spotted portion facing down. The solvent move vertically upward by capillary movement. It was removed and allowed to dry before it was sprayed with 10% sulphuric acid in methanol and heated in an oven at 110 ^{o}C to bring out the bands clearly. This was carried out using mobile phase solvent system mixture of Ethyl acetate and Methanol at ratio of 3:2 for fractions 16-23. Similar bands of fractions were pooled together. The pooled fractions were evaporated to yield dry residues using rotary evaporator and water bath. The weight of the pooled fractions were determined using a weighing balance (Evans, 2002).

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. Sterilepunctured filterpaper discs (6mm) were aseptically placed on the solidified leaf extract-agar admixture plates. Using a micro pipette standardized innoculum ofthe isolates was immediately added to the discs in volumes of about 20µl. A 20 µl sterile distilledwater 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. Thelowest 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 asepticallytransferred into 5 ml sterile Nutrient broth using a pair of sterile forceps. This was incubated at 37°Cfor 24hrs. The Minimum BactericidalConcentration 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).

Determination of the Rate of Kill

The rate at which the most active fraction kills the bacterial isolates was determined using the method described by Adeshina *et al*., (2012).The M.I.C (5mg/ml and 2.5mg/ml) and sub- M.I.C (2.5mg/ml and 1.25mg/ml) of the fraction were prepared in 9mls of single strength sterile nutrient broth in bottles, after which 1.0ml of standardized overnight culture of *K. pneumoniae* (S16) and *S. aureus* (T38) were added to the bottles respectively. The reaction mixtures were shaken at 37^{o}C and at various time intervals that is; 0,30, 60, 120, 240, 360 and 1440 minutes, 1.0 ml of each mixture was taken using a micropipette,serially diluted in sterile normal saline containing 3% Tween 80, from this a 0.1ml aliquot was then plated on the surface of solidified sterile Mueller-hinton agar containing 3% Tween 80. It was allowed to stand and plates were incubated at 37^{o}C for 18 hours and the number of colonies was counted

using a colony counter, and recorded. A negative control was set containing nutrient broth and the test organism but without fraction (F2). The positive control was a mixture of the organism with amoxicillin-clavulanic acid (30ug/ml).

RESULTS

Fractionation of the aqueous extract

Fractionation of the aqueous extract gave a total of 35 fractions (Table 1).

Table. 1: Fractionation of aqueous extract of *A. cordifolia.*

Fraction No.	Ratio of Eluting Solvents		
	N-hexane	Ethyl acetate	Methanol
1	10	0	0
2	10	0	0
3	9	1	0
4	9	1	0
5	9	1	0
6	8	2	0
7	8	2	0
8	7	3	0
9	6	4	0
10	5	5	0
11	4	6	0
12	3	7	0
13	2	8	0
14	1	9	0
15	0	10	0
16	0	10	0
17	0	9	1
18	0	8	2
19	0	8	2
20	0	7	3
21	0	7	3
22	0	7	3
23	0	6	4
24	0	6	4
25	0	5	5
26	0	5	5
27	0	5	5
28	0	4	6
29	0	3	7
30	0	2	8
31	0	1	9
32	0	10	10
33	0	10	10
34	0	10	10
35	0	10	10

Similar bands after TLC were pooled together and seven fractions were obtained. (Table 2.) Shows the combinations of fractions.

Table. 2: Number of fractions after TLC and the combination of pooled fractions.

Number of pooled Fractions	Combination of Fractions pooled.
F1	Combination of fractions 1-9.
F2	Combination of fractions 10-14.
F3	Combination of fractions 15-17.
F4	Combination of fractions 18-20.
F5	Combination of fractions 21-24.
F6	Combination of fractions 25-27.
F7	Combination of fractions 28-35.

Antibacterial Activity of Fractions: Fraction F1 had the highest M.I.C values against isolates from throat swabs specimens and fraction F2 had the lowest (Table 3).

Table. 3: The M.I.C of Fractions against Isolates from Throat swab specimens.

M.I.C (mg/ml)							
Isolates	F1	F2	F3	F4	F5	F6	F7
E.coli (T13)	>20	5	10	10	10	>20	10
S.aureus (T38)	>20	2.5	5	20	10	10	20
S.aureus (T44)	>20	2.5	10	>20	>20	>20	>20
S.aureus (T31)	>20	5	5	20	10	10	10
S.aureus (T20)	>20	2.5	5		20	>20	10
Strep. spp (T12)	>20	5	10	20	10	20	>20

As shown in (Table 4.) F2 fraction had the lowest M.I.C values followed by F3 fraction against isolates from ear swab specimens.

Table. 4: The M.I.C of Fractions against Isolates from Ear swab specimens.

M.I.C (mg/ml)							
Isolates	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
P.aeruginosa (E6)	>20	5	20	>20	>20	>20	>20
P.aeruginosa (E24)	>20	5	10	>20	>20	>20	>20
S.aureus (E27)	>20	2.5	5	>20	20	10	20
Strep. spp (E20)	>20	5	>20	>20	>20	>20	>20
Strep. spp (E22)	>20	10	>20	>20	>20	>20	>20

The M.I.C values of most of the fractions against isolates from sputum samples were high all having values >20. Only fraction F2 had the lowest M.I.C values followed by F3 (Table 5).

Table. 5: The M.I.C of Fractions against Isolates from Sputum specimens.

M.I.C (mg/ml)							
Isolates	F1	F2	F3	F4	F5	F6	F7
K. pneumoniae (S16)	>20	5	20	>20	>20	>20	>20
K.pneumoniae (S20)	>20	5	20	>20	>20	>20	>20
S.aureus (S10)	>20	2.5	20	>20	>20	>20	>20
S.aureus (S44)	>20	5	>20	>20	>20	>20	>20

M.I.C and M.B.C of fraction F2

The values of the M.I.C and M.B.C of the fraction F2 against *Strep*. spp isolated from throat swab were higher than those against *S. aureus* (Fig 1).

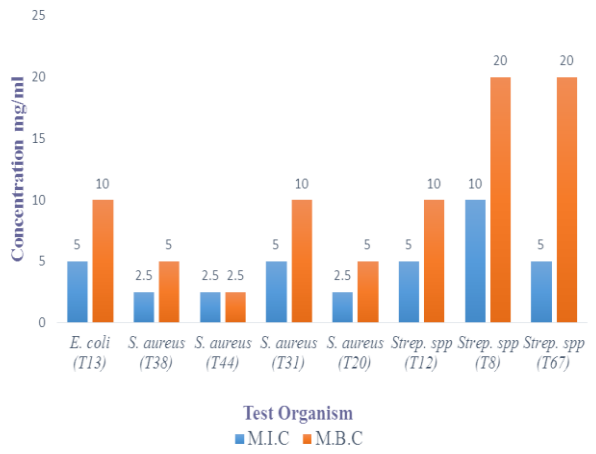

Fig. 1: M.I.C and M.B.C of the most active fraction (F2) against isolates from throat swab specimens.

The values of the M.I.C and M.B.C of the fraction F2 against *Strep*. spp and *P. aeruginosa* isolated from ear swab were higher than that against *S. aureus* (Fig 2).

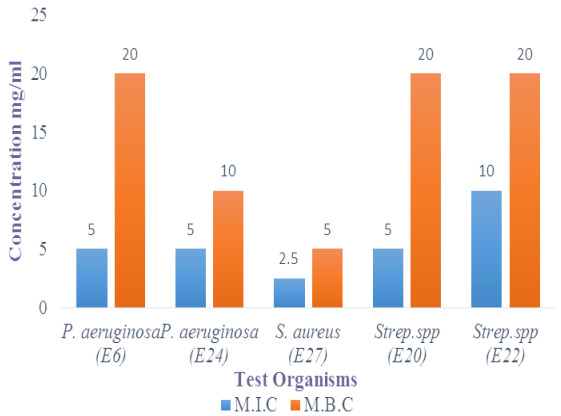

Fig. 2: M.I.C and M.B.C of the most active fraction (F2) against isolates from ear swab specimens.

Fraction F2 M.I.C and M.B.C values against *S. aureus* (S10) isolated from sputum sample was the lowest. While the M.I.C and M.B.C values of fraction F2 against *K. pneumoniae* isolates and *S. aureus* (S44) were higher (Fig 3).

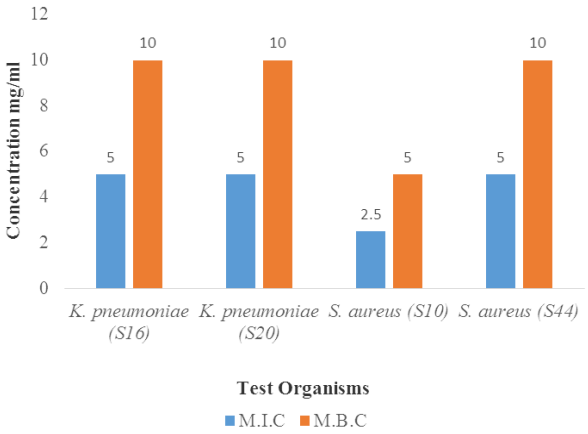

Fig 3: M.I.C and M.B.C of the most active fraction (F2) against isolates from Sputum specimens.

Death/Survival Rate of most active Fraction (F2).

The Death/Survival Rate of *S. aureus* (T38) in Fig 4.5 showed that as contact time increases the number of surviving cells decreases with no cells recovered at 1440 minutes at M.I.C concentration (Fig 4).

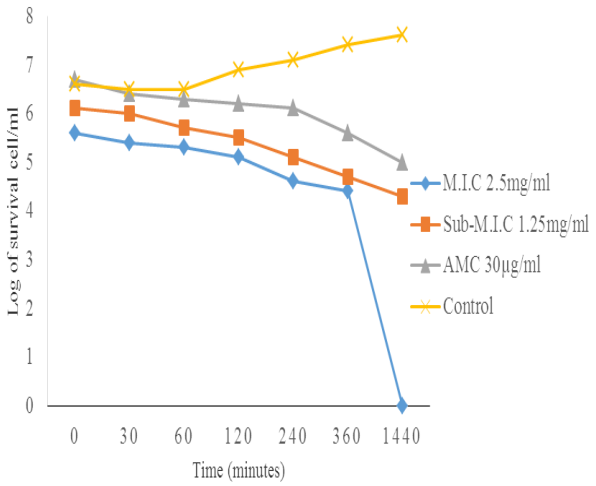

Fig. 4: Death/Survival rate of *S. aureus* (T38) on exposure to M.I.C and Sub-M.I.C of F2.

The Death/Survival Rate of *K. pneumoniae* (S16) is concentration dependent with no cells recovered at 240 minutes of the M.I.C concentration and 1440 minutes at Sub-M.I.C concentration (Fig 5).

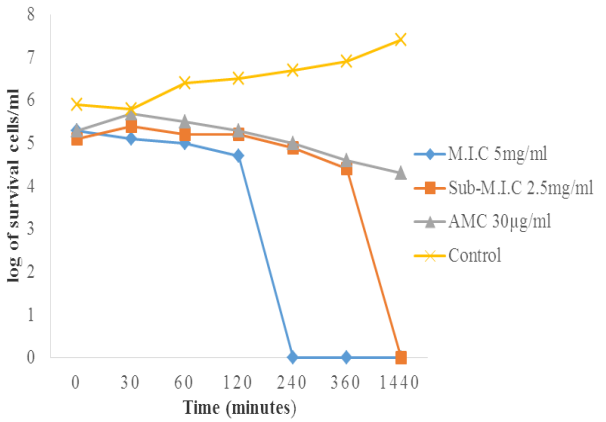

Fig. 5: Death/Survival rate of *K. pneumoniae* (S16) on exposure to M.I.C and Sub-M.I.C of F2.

DISCUSSION

The lower M.I.C confirm the high activity of the fraction (F2) at low concentration. High activity of antibacterial agent at low concentration is very essential for chemotherapeutic purposes because of their toxicity to patient system. The observed low M.I.C values from fraction F2 could be due to the fact that F2 contains the secondary metabolites responsible for the antibacterial activity of the aqueous extract. The M.I.C and M.B.C values of F2 against the isolates was lower compared to the crude extract, this could be due to the fact that the crude extract contained a lot of constituent that play little or no role in the antibacterial activity of the extract (Adewunmi *et al.*, 2001).

There was increase in the number of surviving cells in all the negative controls. The general bactericidal activity of F2 was rapid from onset and generally concentration dependent. Killing of cells occur chiefly as a function of time within a range of concentrations and these possibly explain the increase lethal activity of the F2 with increase concentration above the Sub – M.I.C.

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

REFERENCES

1. Aboaba, O. O., Smith, S. I., Olude, F. O. Antibacterial effect of Edible Plant on Escherichia coli015:H.Pakistan Journal of Nutrition, 2006; 5(4): 325-327.
2. Adeshina, G.O., Kunle, O. F.,Onaolapo, J.A., Ehinmidu J.O., Odama L.E. Antibacterial Activity of Aqueous and Ethyl Acetate sub-fractions of

Alchornea cordifolia leaf. European Journal of Medicinal plants, 2012; 2(1): 31-41.
3. Adewunmi, C. O., Agbedahunsi, J. M., Adebajo, A. C., Aladesanmi, A. J., Murphy, N. and Wando, J. Ethno-veterinary medicine: Screening of Nigerian medicinal plants for trypanocidal properties. Journal of Ethnopharmacology, 2001; 77: 19-14.
4. Akanbi, M. O., Ukoli, C. O., Erhabor, G. E., Akanbi, F. O., Gordon, S. B. The burden of respiratory disease in Nigeria. The African Journal of Respiratory Medicine, 2009; 17.
5. CLSI, (2006). Method for dilution antimicrobial susceptibility test for bacteria that grow aerobically. Wayne, Pa, USA. Approved standard M7-A7.
6. CLSI, Performance Standard for Antimicrobial disk Susceptibility Test, Approved StandardM02-A10.10th Edition. CLSI, Wayne, PA, 2009; 29: 1.
7. Evans, W. C. Trease and Evans Pharmacognosy, (Fifteenth Edition), Saunders, W. B. Company limited, 2002; (137-139, 230-240).
8. Furin, J., Gegia, M., Mitnick, C. (2011). Eliminating the category II retreatment regime from National tuberculosis progamme: The Georgian experience Bulletin of the World Health Organiztion. Article ID: BLT.11.092320.
9. Jafari, N. J., Ranjbar, R., Haghi-Ashtiani, M. T., Abedini, M., Izadi, M. The study of prevalence and antimicrobial susceptibility of tracheal bacterial strains isolated from paediatric patients. Pakistan Journal of Biomedical sciences, 2009; 12(5): 455-458.
10. Karou, D., Savadogo, A., Canini, A., Yameogo, S., Montesano, C., Simoire, L., Colizzi, V., Traore, A.S. Antibacterial activity of alkaloids from Sida acuta. African Journal of Biotechnology, 2006; 5(2): 195-200.
11. Siikamaki, H. (2015). Health problems of finish travelers focus on infections.
12. Zafar, A., Hussain, Z., Lomama, E., Sibliie, S., Irfan, S., Khan, E. Antibiotic susceptibility of pathogens isolated from patients with community-acquired respiratory tract infections in Pakistan- the active study. Journal of Ayub Medical College, Abbottabad, 2008; 20: 7-9.
13. Zy, E.A., Area, A., Aam, K. Antimicobial activity of some medicinal plant extracts in Palestine. Pakistan Journal of Medical Science, 2005; 21: 187-193.