Antimicrobial activity of *Anogeissus leiocarpus* stems bark extracts and an isolate from the plant against some microbes

Ali, E.O.[1]*, Tor-Anyiin, T.A.[1], Igoli, J.O.[1], Anyam, J.V[1] and Hammuel, C.[2]

[1]Chemistry Department, University of Agriculture, Makurdi, Benue, Nigeria
[2]National Research Institute for chemical Technology, Basawa Zaria, Kaduna, Nigeria
Corresponding author's E-mail: ochowechie@gmail.com

ABSTRACT

The challenge of resistance of microbes to antimicrobial substances is a global issue. This has led the researchers all over the world to search for new antibiotics that will be alternative in the treatment of diseases caused by the resistant pathogens. In this study, *Anogeissus leiocarpus* which is a plant that is widely used in Northern Nigeria as medicine was used to determine its antimicrobial potentials. The extract was obtained using microwave. The zones of clearance of the microbes by the extracts from this plant range from 18-30 mm. None of extracts had effect on methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant enterococci (VRE), *Streptococcos pyogenes*, *Shigella dysenteriae*, *Proteus mirabilis*, *candida krusei*, and *Candida tropicalis*. The MIC ranges from 5-20 mg/mL and MBC from 10-40 mg/mL . The isolate was obtained using vacuum chromatography (VLC) and thin layer chromatography (TLC). The isolate from the plant was potential antimicrobial agent with zone of clearance range from 23-34 mm against the pathogens including MRSA and VRE. The MIC of the isolate ranges from 12.5-25 µg/mL and MBC from 25-100 µg/mL . Thus the plant is a potential candidate for drug development for the treatment of diseases caused by these pathogens.

KEYWORDS: *Anogeissus leiocarpus*, zone of inhibition, MIC, MBC, pathogens and the isolate.

{**Citation:** Ali, E.O., Tor-Anyin, T.A., Igoli, J.O., Anyam, J.V., Hammuel, C. Antimicrobial activity of *Anogeissus leiocarpus* stems bark extracts and an isolate from the plant against some microbes. American Journal of Research Communication, 2017, 5(8): 9-25} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

The resistance of microorganisms to one of more of antimicrobial agents is a global health challenge which led scientists all over the world to come up chemotherapeutic agents for treatment of diseases caused by the pathogens (Hammuel *et al*., 2011). *Streptococcus pyogenes, Stephylococcus aureus* and *Streptococcus pneumoniae*, the organisms that causes respiratory and cutaneous infection, as well as *pseudomonas* and members of *Enterobacteriaceae*, causing diarrhoea and urinary tract infections, and sepsis, are now resistant to virtually all of the older antibiotics. This resistance is largely due to indiscriminate use of antimicrobial drugs commonly used on the treatment of these infectious diseases (Afolayan and Aleiro, 2006).

Plants have great potential uses, especially as traditional medicine and pharmaceutical drugs. A large proportion of the world's population depends on traditional medicine because of the scarcity and high costs of orthodox medicine (Tagboto and Townson, 2001; Hudaib *et al*., 2008). Natural products play a dominant role in the development of novel drug leads, for the treatment and prevention of diseases (Newman *et al*., 2003; Gilani and Rahman, 2005).

Plants are remarkable factories of chemical compounds referred to as phytochemicals. Every plant synthesizes a diverse array of phytochemicals. These compounds partake in a variety of roles in the plant life including maintenance of physiological functions and act as defence against pathogens such as bacteria, fungi and also insects and animals. In addition, bioactive plant compounds have served as templates for several synthetic drugs and as precursors. They are used in the production of semi synthetic drugs (NewMan *et al.*, 2003). The need to screen plants for pharmaceuticals is particularly urgent in the light of rapid deforestation and the concurrent loss of biodiversity throughout the world (Ayo, 2010). There is serious need to develop new antimicrobial agents that are very effective in the treatment of diseases cause by the pathogens and other ailments.

Anogeissus leiocarpus is a plant that is widely used in Northern Nigeria ethnomedicine. It belongs to the phylum, Tracheophyta; Order; Myrtales and Family: Combretaceae (combretoideae). It is commonly called Axle-wood tree, and in Nigeria, it is referred to as Marke (Hausa), Maaki (Tiv), Otra (Idoma), Kojoli (Fulani), Annum (Kanuri), Ayin or Orin-odan Ainy (Yoruba), Atara (Igbo) and Kukunchi (Nupe). It is a very graceful tropical tree which grows up to 28m and occurs in the most of the savannah areas from the driest regions to the borders of the forest zone. In Africa, its occurrence extends from Senegal in West Africa to Sudan and Ethiopia in East Africa. Those growing in the driest area tend to have smaller leaves and more hairy flowers than those growing under wetter conditions, but

both differences are not sufficiently marked to create distinct varieties (Abdullahi, *et al*., 2003).

The decoction and maceration of the stem bark are used against anorexia, constipation, malaria, jaundice, fatigue, itching, eczema, psoriasis, carbuncles, wounds, sores, boils, eradication of cysts of parasites in host and various forms of diseases e.g. helmintosis, schistosomiasis, leprosy, amaebic dysentery, trypanososmiasis, tuberculosis and sexual transmitted infections (Mann *et al*., 2014). Research has revealed that *A. leicarpus* has many pharmacological activities, for example the bark, fruit, and leaves possess antimicrobial activities (Mann *et al*., 2014). It is one of the Nigerian chewing sticks that possess antimicrobial activity against oral flora such as *Staphylococcus aureus*.

MATERIALS AND METHODS

Collection and preparation of plant materials

The plant materials (bark) was collected from Enumgba Agatu, Agatu local government area of Benue state. It was identified and authenticated at the department of forestry and wild life Management University of agriculture Makurdi. The bark of *Anogeissus leiocarpus* collected was dried under shade for one month and subsequently pulverized with wooden mortar and pestle to fine powder. The powdered plant material (bark) was stored in a polythene bag until needed for analysis.

Extraction Procedure

Microwave assisted extraction method:

The powdered bark of *A. leiocarpus* (2 kg) was sequentially extracted with n-hexane 3300 cm^3, then ethyl acetate 3300 cm^3 and methanol 3300 cm^3 my maceration using microwave assisted extraction (MAE) method (Abbas *et al*, 2012).

The powdered bark (*A.leiocarpus*) was distributed into three digestion flasks, to each flask 1000 cm^3 of a solvent was added, the flask was agitated for proper mixing of the solvent with the powdered bark. A flask of the mixture was placed in a microwave oven for a period of 3 minutes, after which it was brought out to cool, and then returned into the oven for another 3 minutes. This process was repeated until a combined time of 30 minutes for each of the three

flasks. After desorption, the extracts were decanted, the flasks rinsed with 300 cm^3 of the solvents (hexane, ethyl acetate and methanol) then filtered using filter paper. The resulting filtrate was then concentrated using a rotary evaporator at 40 ^oC, the final product was stored in universal bottles for further analysis.

Vacuum liquid chromatography (VLC)

Crude extracts (hexane, ethyl acetate and methanol) were subjected to vacuum liquid chromatography. Each extract was pre-adsorbed on celite (diatomaceous earth) and air dried into fine flowing powder. The sintered funnel (porosity 3) used for the VLC was loaded with silica gel (as the stationary phase) under vacuum to ensure that it was compacted and uniformly spread (Hostettmann *et al.*, 1998, Leopold, 1982).

Hexane was run through the column under vacuum, after which each pre-adsorbed extract (starting with the hexane extract) was loaded on the column. Suction was applied to compress the extract to the silica gel and a piece of whatman filter paper was used to cover the surface to prevent disturbance by introduction of solvents. Gradient elution was used under vacuum; the column was eluted stepwise under vacuum with solvents of increasing polarity: ranging from 100% hexane, slowly increasing concentration of a more polar ethyl acetate solvent to finally 100% ethyl acetate. This was done with a concomitant decrease in hexane levels. The column was also eluted ethyl acetate/ methanol (100 % ethyl acetate to increasing concentrations of methanol, to finally 100 % methanol). The collected fractions were taken for antimicrobial screening.

The antimicrobial screening

The antimicrobial activities of plant extract was determined using some pathogenic microbes, the microbes were obtained from the department of medical microbiology ABU teaching Hospital Zaria. The concentration of the extract was prepared by dissolving 0.4 g of the weighed extract in 10 ml of DMSO to obtained concentration of 40 mg/mL as initial concentration. And 0.0001mg of the isolate was weighed and dissolved in 10mL of DMSO to obtain a concentration of 100µg/mL. This was the initial concentration used for the extract. Diffusion method was the method used for screening the extract. Mueller-Hinton agar was the medium used as the growth medium for the microbe. The medium was prepared according to the manufacturer's instruction, sterilized at 121^oC for 15min poured into sterile Petri dishes and was allowed to cool and solidify. The medium was with 0.1mL of the

standard inoculums of the test microbe, the inoculums were spread evenly over the surface of medium by the use of a sterile swab. By the use of a sterile cork borer of 6 mm in diameter, a well was cut at the centre of each inoculated medium. Into each well on the inoculated medium 0.1mL of the solution of the extract of the concentration of 40 mg/mL was then introduced. This was done the same for the isolate with initial concentration. Incubation of the inoculated medium was made at 37^{o}C for 24hrs after which the medium was observed for the zone of inhibition of growth, the zone was measured with a transparent ruler and the result recorded in millimetre.

Minimum inhibition concentration

The minimum inhibition concentration (MIC) of the extract was determined using the broth dilution method. Mueller Clinton broth was prepared, 10mls was dispensed into test tubes and was sterilized at 121^{o}C for 15 mins, and the broth was allowed to cool. Mc-Farland's, turbidity standard scale number 0.5 was prepared to give turbid solution. Normal saline was prepared, 10mls was dispensed into sterile test tube and the microbe was inoculated and incubated at 37^{o}C for 6hrs. Dilution of the test microbe was done in the normal saline into the turbidity marched that of the Mac-Farland's scale by visual comparison at this point the test microbe has a concentration of about 1.5 x 10^{8}cfu/mL. Two –fold serial dilution of the extract in the sterile broth was made to obtain the concentrations of 40, 20, 10, 5 and 2. 5 mg/mL. Dilution was also carried on isolate to obtain concentration of 100, 50, 25, 12.5 and 6.25 µg/mL. Having obtained the difference concentration of the extract in the sterile broth, 0.1mL of the test microbes in the normal saline was then inoculated into the different concentrations, inoculation was made at 37^{o}C for 24hrs, after which the test tube of the broth were observed for turbidity (growth). The lowest concentration of the extract in the sterile broth, which shows no turbidity, was recorded as the minimum inhibition concentration.

Minimum bactericidal concentration/minimum fungicidal concentration

Minimum bactericidal concentration/minimum fungicidal concentration (MBC/MFC) was then carried out to determine whether the test microbes were killed or only their growth was inhibited. Mueller-Hinton agar was prepared, sterilized at 121^{o}C for 15 min poured into sterile Petri dish and was allowed to cool and solidify. The content of the MIC in the serial dilution was then sub-cultured onto the prepared medium incubation was made at 37 ^{o}C for 24hrs after which the plate of the medium was observed for colony growth, the MBC/MFC

were the plates with lowest concentration of the extract and the isolate without colony growth.

RESULTS AND DISCUSSION

Table 1 shows the activity of the three extracts on the selected microbes. The extracts have the effect of inhibition against the microbes. All the extracts could not have effect on methicillin resistant staphylococcus aureus (MRSA), vancomycin resistant enterococci (VRE), *Streptococcos pyogenes*, *Shigella dysenteriae*, *Proteus mirabilis*, *candida krusei*., and *Candida tropicalis* i.e. these pathogens were resistant to all the extracts. Other pathogens such as *Staphylococcus aureus*, *Streptococcus faecalis*, *Coryenbacterium ulcerans*, *Bacillus subtilis*, *Escherichia coli*, *Kleibsella pneumonia Salmonella typhi*, *Pseudomonas aeruginosa Candida albicans* and *Candida stellatoidea*. Mann *et al*. (2015), Elsidding *et al*. (2015), Timothy *et al*. (2015) and Alhassan *et al*. (2016) have also reported the potential effect of *Anogeissus leiocarpus* against pathogenic microorganisms such as *S. aureus*, *Klebseilla* species, *C. albicans*, *E. coli*, *S. dysenteriae*, *Aspergillus niger* and *P. aeruginosa*. Mann (2012) reported wide range of activity of this plant sample against *S. pyogenes* and *B. subtilis*

Table 1: Antimicrobial activities of *A. leiocarpus* bark extract

Test organism	Ethyl acetate extract	Hexane extract	Methanolic extract
MRSA	R	R	R
VRE	R	R	R
Staphylococcus aureus	S	S	S
Streptococcus pyogenes	R	R	R
Streptococcus faecalis	S	S	S
Corynebacterium ulcerans	S	S	S
Bacillus subtilis	S	S	S
Escherichia coli	S	S	S
Klebsiella pneumoniae	S	S	S
Salmonella typhi	S	S	S
Shigella dysenteriae	R	R	R

Proteus mirabilis	R	R	R
Pseudomonas aeruginosa	S	S	S
Candida albicans	S	S	S
Candida krusei	R	R	R
Candida tropicalis	R	R	R
Candida stellatoidea	S	S	S

KEY: S=Sensitive, R= Resistant

Table 2: Zone of inhibition of the extracts against the test microorganism (in mm)

Test organism	Ethylacetate extract	Hexane extract	Methanolic extract
MRSA	0	0	0
VRE	0	0	0
Staphylococcus aureus	27	22	25
Streptococcus pyogenes	0	0	0
Streptococcus feacalis	24	18	22
Corynebacterium ulcerans	29	21	24
Bacillus subtilis	30	24	27
Escherichia coli	25	20	21
Klebsiella pneumoniae	29	21	24
Salmonella typhi	23	18	20
Shigella dysenteriae	0	0	0
Proteus mirabilis	0	0	0
Pseudomonas aeruginosa	24	18	21
Candida albicans	26	20	20
Candida krusei	0	0	0
Candida tropicalis	0	0	0
Candida stellatoidea	27	21	23

Table 3: Minimum inhibition concentrations (MICs) of the extracts against the test microbes

Test organism	Ethyl acetate extract					n-hexane extract					Methanolic extract					
	40mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	40mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	40mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml
MRSA															
VRE															
Staphylococcus aureus	-	-	-	ox	+	-	-	ox	+	++	-	-	ox	+	++
Streptococcus pyogenes															
Streptococcus feacalis	-	-	ox	+	++	-	ox	+	++	+++	-	-	ox	+	++
Corynebacterium ulcerans	-	-	-	ox	+	-	-	ox	+	++	-	-	ox	+	++
Bacillus subtilis	-	-	-	ox	+	-	-	ox	+	++	-	-	-	ox	+
Escherichia coli	-	-	ox	+	++	-	-	ox	+	++	-	-	ox	+	++
Klebsiella pneumoniae	-	-	-	ox	+	-	-	ox	+	++	-	-	ox	+	++
Salmonella typhi	-	-	ox	+	++	-	ox	+	++	+++	-	-	ox	+	++
Shigella dysenteriae															
Proteus mirabilis															
Pseudomonas aeruginosa	-	-	ox	+	++	-	ox	+	++	+++	-	-	ox	+	++
Candida albicans	-	-	ox	+	++	-	-	ox	+	++	-	-	ox	+	++
Candida krusei															
Candida tropicalis															
Candida stellatoidea	-	-	-	ox	+	-	-	ox	+	++	-	-	ox	+	++

KEY: - = No growth, ox = MIC, + = Scanty growth, MRSA = Methicillin Resistant *Staphylococcus aureus*, Vancomycin Resistant Enterococci,

Table 4: Minimum bactericidal/fungicidal concentration of the extracts against the test microbes

Test organism	Ethyl acetate extract					n-hexane extract					Methanolic extract				
	40mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	40mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml	40mg/ml	20mg/ml	10mg/ml	5mg/ml	2.5mg/ml
MRSA															
VRE															
Staphylococcus aureus	-	ox	+	++	+++	ox	+	++	+++	++++	-	ox	+	++	+++
Streptococcus pyogenes															
Streptococcus feacalis	ox	+	++	+++	++++	ox	+	++	+++	++++	ox	+	++	+++	++++
Corynebacterium ulcerans	-	-	ox	+	++	ox	+	++	+++	+++	ox	+	++	+++	++++
Bacillus subtilis	-	-	ox	+	++	ox	+	++	+++	+++	-	ox	+	++	+++
Escherichia coli	-	ox	+	++	+++	ox	+	++	+++	++++	ox	+	++	+++	+++
Klebsiella pneumonia	-	ox	+	++	+++	ox	+	++	+++	+++	ox	+	++	+++	+++
Salmonella typhi	ox	+	++	+++	+++	ox	+	++	+++	++++	ox	+	++	+++	++++
Shigella dysenteriae															
Proteus mirabilis															
Pseudomonas aeruginosa	ox	+	++	+++	++++	ox	+	++	+++	++++	ox	+	++	+++	++++
Candida albicans	-	ox	+	++	+++	ox	+	++	+++	++++	ox	+	++	+++	++++
Candida krusei															
Candida tropicalis															
Candida stellatoidea	-	ox	+	++	+++	ox	+	++	+++	++++	ox	+	++	+++	++++

KEY: - = No growth, ox = MBC, + = Scanty growth, ++ = Moderate growth, +++ and ++++ = Dense growth, MRSA = Methicillin Resistant *Staphylococcus aureus*, Vancomycin Resistant Enterococci.

Table 2 shows the zones of inhibition of the extracts against the susceptible isolates (microbes). The zones of inhibition range from 18-30 mm which indicates strong zone of clearance by the extracts against the microbes. This indicates that the stem bark of this plant can be used in the management of infections caused by the test pathogens in this research as reported by Alhassan *et al*. (2016). This result has confirmed the claims of other researchers that the plant possesses antimicrobial activity against many pathogens.

Table 5: The antimicrobial activities of plant isolate

Test organism	SM
MRSA	S
VRE	S
Staphylococcus aureus	S
Streptococcus pyogenes	S
Streptococcus feacalis	R
Corynebacterium ulcerans	R
Bacillus subtilis	S
Escherichia coli	S
Klebsiella pneumoniae	R
Salmonella typhi	S
Shigella dysenteriae	S
Proteus mirabilis	R
Pseudomonas aeruginosa	S
Candida albicans	S
Candida krusei	R
Candida tropicalis	S
Candida stellatoidea	S

KEY : SM= Sample, S= Sensitive, R= Resistant

Table 6: Zone of inhibition of the isolate against the test microbe (in mm)

Test organism	SM
MRSA	27
VRE	24
Staphylococcus aureus	34
Streptococcus pyogenes	25
Streptococcus feacalis	0
Corynebacterium ulcerans	0
Bacillus subtilis	29
Escherichia coli	26
Klebsiella pneumoniae	0
Salmonella typhi	24
Shigella dysenteriae	29
Proteus mirabilis	0
Pseudomonas aeruginosa	23
Candida albicans	27
Candida krusei	0
Candida tropicalis	25
Candida stellatoidea	23

KEY: SM= Sample

Table 7: Minimum inhibition concentration of the isolate against the microbe

Test organism	100 ug/mL	50 ug/mL	25 ug/mL	12.5 ug/mL	6.2 ug/mL
MRSA	-	-	-	ox	+
VRE	-	-	ox	+	++
Staphylococcus aureus	-	-	-	ox	+
Streptococcus pyogenes	-	-	ox	+	++
Streptococcus feacalis					
Corynebacterium ulcerans					
Bacillus subtilis	-	-	-	ox	+
Escherichia coli	-	-	ox	+	++
Klebsiella pneumoniae					
Salmonella typhi	-	-	ox	+	++
Shigella dysenteriae	-	-	-	ox	+
Proteus mirabilis					
Pseudomonas aeruginosa	-	-	ox	+	++
Candida albicans	-	-	-	ox	+
Candida krusei					
Candida tropicalis	-	-	ox	+	++
Candida stellatoidea	-	-	ox	+	++

KEY: ox = MIC, - =No turbidity, + = Turbid (light growth), ++ = moderate turbidity

Table 3 shows minimum inhibition concentrations (MICs) of the extracts against the test microbes. The MIC range from 5-20 mg/mL and the MIC in this research is lower than the MIC reported by Mann (2012) and Timothy *et al*. (2015). Umar and Mohammad (2015) reported higher MIC of methanol extract of 90 mg/mL against *S. aureus* and *P. aeruginosa*. Ethyl acetate extract had the least MIC of 5mg/mL against *S. aureus*, *C. ulcerans*, *K. pneumoniae* and *C. stellatoidea* and higher concentration of n-hexane extract was needed to inhibit the pathogens at 10-20 mg/mL. The methanol extract at 10mg/ml inhibited more of the pathogens and only *B. subtilis* was inhibited at 5 mg/mL by the methanol extract. This could be as a result of adequate and inadequate presence of active compounds extracted by these solvents. The inhibition of the pathogens by these extracts shows the plant has the potential in the management of diseases caused by these pathogens.

Table 8: Minimum bactericidal/ fungicidal concentration of the isolate against the microbe

Test organism	100ug/mL	50ug/mL	25ug/mL	12.5ug/mL	6.25ug/mL
MRSA	-	ox	+	++	+++
VRE	-	ox	+	++	+++
Staphylococcus aureus	-	-	ox	+	++
Streptococcus pyogenes	-	ox	+	++	+++
Streptococcus feacalis					
Corynebacterium ulcerans					
Bacillus subtilis	-	-	ox	+	++
Escherichia coli	-	ox	+	++	+++
Klebsiella pneumoniae					
Salmonella typhi	ox	+	++	+++	+++
Shigella dysenteriae	-	-	ox	+	++
Proteus mirabilis					
Pseudomonas aeruginosa	ox	+	++	+++	+++

Candida albicans	-	ox	+	++	+++
Candida krusei					
Candida tropicalis	-	ox	+	++	+++
Candida stellatoidea	ox	+	++	+++	+++

KEY ox = MIC, - =No turbidity, + = Turbid (light growth), ++ = moderate turbidity, +++ = Heavy turbidity

Table 9: Controls against test organism

Test organism	Ciprofloxacin	Erythromycin	Fluconazole
MRSA	S	S	R
VRE	R	S	R
Staphylococcus aureus	S	R	R
Streptococcus pyogenes	S	S	R
Streptococcus feacalis	S	S	R
Corynebacterium ulcerans	R	S	R
Bacillus subtilis	S	S	R
Escherichia coli	S	S	R
Klebsiella pneumoniae	S	S	R
Salmonella typhi	S	S	R
Shigella dysenteriae	S	S	R
Proteus mirabilis	S	S	R
Pseudomonas aeruginosa	R	S	R
Candida albicans	R	R	S
Candida krusei	R	R	S
Candida tropicalis	R	R	S
Candida stellatoidea	R	R	S

KEY: R= Resistant, S= Sensitive

Table 10: zone of inhibition of the test drug against the test microorganism (in mm)

Test organism	Ciprofloxacin	Erythromycin	Fluconazole
MRSA	34	35	0
VRE	0	34	0
Staphylococcus aureus	37	0	0
Streptococcus pyogenes	35	37	0
Streptococcus feacalis	39	35	0
Corynebacterium ulcerans	0	32	0
Bacillus subtilis	37	39	0
Escherichia coli	40	35	0
Klebsiella pneumoniae	35	32	0
Salmonella typhi	42	34	0
Shigella dysenteriae	35	35	0
Proteus mirabilis	34	34	0
Pseudomonas aeruginosa	0	32	0
Candida albicans	0	0	34
Candida krusei	0	0	36
Candida tropicalis	0	0	32
Candida stellatoidea	0	0	35

The minimum bactericidal (MBC) and minimum fungicidal concentration (MFC) of the extracts against the test microbes range from 10-40 mg/mL. There was variation in the cidal effect of the three extracts as seen in table 4, the pathogens were killed at 40mg/mL by n-hexane extract, more were still killed at this concentration by ethyl acetate and methanol extracts. This could be as a result of the presence of bioactive compounds extracted by the polar solvents (i.e. the ethyl acetate and methanol).

Table 5 shows the activity of the isolate obtained from plant using VLC against the microbes selected for the study. Many of the microbes were susceptible to the isolates including MRSA and VRE. Therefore, the isolate is a candidate of potential antibiotic that will be one of the options use in the management of diseases caused by these pathogens. But *S. faecalis*, *C. ulcerans*, *K. pneumoniae*, *P. mirabilis* and *C. krusei* were resistant to the isolate. Then table 6 shows the zones of clearance by the isolate against the pathogens. The

isolates had good zone of clearance some of which were closed to the control set for this research. The MIC of the bioactive isolate against the selected isolates is presented in table 7.

CONCLUSION

The extracts of *A. leiocarpus* in this study demonstrated significant bioactive components against some of the test pathogenic organisms. The isolate from the plant had much more activity against the most of the selected pathogens. Therefore, the plant is a potential candidate for drug development for the treatment of diseases caused by these pathogens.

REFERENCE

Abbas, D. Lutfun, N. Sanaz, H and Satyajit, D. S. (2012). Microwave-assisted Extraction in natural products isolation, pp.89-93.

Afolayan, A.J. and Aliero A.A. (2006). Antimicrobial activity of solanum tomentosum. *African Journal of Biotechnology*, 5(4):369-372.

Alhassan, D.A., Uba, A.I., Muhammad, A.U. and Muhammad, Y.Y. (2016). Phytochemical Screening and Antimicrobial Activity of Crude Stem Bark Extracts of *Anogeissus leiocarpus*. *European Journal of Medicinal Plants*, 11(2):1-7.

Ayo, R.G (2010). Phytochemical constituents and bioactivities of the extracts of *cassia nigricans Vahl* : A Review. Journal of medicinal plants research vol. 4(14), pp.1339 1348.DOI: 10.5897/JMPR 10.10, ISSN1996-0875.

Elsiding, I.M.E., Muddather, A.K., Ali, H.A.R. and Ayoub, S.M.H. (2015). A comparative study of antimicrobial activity of the extracts from root, leaf and stem of *Anogeissus leiocarpous* growing in Sudan. *Journal of Pharmacognosy and Phytochemistry*, 4(4):107-113.

Gilani A.H., Rahman A.U (2005). Trends in ethnopharmacology. J. Ethnopharmacol., 100: 43-49.

Hammuel, C., Abdullahi, M.S., Mankilik, M., Anyim, B.P., Adesina, O.B., Inekwe, U.V., Udiba U.U. and Batari, M. L. (2011). The phytochemical and antimicrobial activities of oil from the seed of *thevetia peruviana* plant. *Journal of Applied Environmental and Biological Science*, 1(12):597-601

Hostettmann, K., Marston, A. and Hostettmann, M. (1998). Special column chromatography. *Preparative Chromatography Techniques.* Springer.

Hudaib M, Mohammad M, Bustanji Y, Tayyem R, Yousef M, Aburjaie M, Aburjai T (2008). Ethnopharmacological survey of medicinal plants in Jordan, Mujib nature reserve and surrounding area. *J. Ethnopharmacol*., 120: 63-71.

Leopold, E. J. (1982). Vacuum dry-column chromatography. *The Journal of Organic Chemistry,* 47**,** 4592-4594.

Mann, A. (2012). Evaluation of Antimicrobial Activity of *Anogeissus leiocarpus* and *Terminalia avicennioides* against Infectious Diseases Prevalent in Hospital Environments in Nigeria. *Journal of Microbiology Research*, 2(1):6-1.

Mann, A., Yusuf, A. and Daniyan, S. (2015). TLC Analysis and Bioactivity Screening of the Stem Bark Extract of *Anogeissus Leiocarpus* Against Multi-Resistant *Staphylococcus Aureus* and Quantification of Its Phytoconstituents. *A Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 5(2):187-203.

Newman DJ, Cragg GM, Snadder KM (2003). Natural products as sources of new drugs over the period, 1981 – 2002. J. Nat. Prod., 66(7): 1022 -1037.

Ndjonka, D., Agyare, C., Luersen, K., Hansel, A. and Liebau, E.(2010). *In vitro* Anti-leishmanial activity of traditional medicinal plants from Cameroon and Ghana. *International Journal of Pharmacology*, 6(6):863-871.

Tagboto S., Townson S (2001). Antiparasitic properties of medicinal plants and other naturally occurring products. Adv. Parasitol., 50: 199-295.

Timothy S.Y., Mashi F.I., Helga B.I., Galadima I.H., Midala, T.A.S. (2015). Phytochemical screening, antibacterial evaluation and in vitro spasmodic effect of the aqueous and ethanol leaf and bark extract of *anogeissus leiocarpus* (dc.) Guill. & perr. *Asian Journal of Pharmaceutical science & Technology,* 5(4):302-208.

Umar H.Y., Muhammad A.I. (2015). Antibacteria activities of leave extract of *Anogeissus leiocarpus* and *Vitex doniana* against some bacteria.The American journal of innovative Research and applied sciences. ISSN 2429-5396.