

JOPAT Vol 19(2), 429– 447, July-December, 2020 Edition

ISSN 2636 – 5448. <https://dx.doi.org/10.4314/jopat.v19i2.4>**Evaluation of the Antibacterial Activities of Isolated Bioactive Components from the plant *Adenodolichos paniculatus*****<sup>1</sup>Onwuliri, A. E, <sup>1</sup>Kyahar, I. F, <sup>2</sup>Ehinmidu, J. O and <sup>3</sup>Oladosu P. O.**

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**Abstract**

The three bioactive components isolated included: component A (major phytochemicals were Bis (2-ethylhexyl) phthalate (16.36 %), 9,12-Octadecadienoic acid, ethyl ether (14.77 %) and 9.cis., 11.trans.-octadecadienoic acid (14.77 %), component B (major phytochemicals were 9,12-Octadecadienal (Linoleic acid) (40.98 %), Octadecanoic acid (Stearic acid) (9.26 %), Undecanoic acid, 10-bromo- (10-bromodecanoic acid) (9.26 %) and n-Hexadecanoic acid (Palmitic acid) and component C (cis-9-octadecenoic acid (Oleic Acid) (30.45 %), Octadecanoic acid (Stearic acid) (17.33 %)). These components isolated from the chloroform fraction of *Adenodolichos paniculatus* are used by traditional medicinal practitioners for the management of mouth and throat infections. The antibacterial activities against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were evaluated using bioautography and agar-well diffusion methods. The bioautogram result showed that component A had inhibited spots against *S. pyogenes* (17.50 mm) and *P. aeruginosa* (16.00 mm), corresponding to the TLC spots with Rf values of 0.594, 0.55 and 0.26, respectively. Component B showed inhibition spots against *Streptococcus pyogenes* (36.50 mm), *Staphylococcus aureus* (16.00 mm) and *Pseudomonas aeruginosa* (11.00 mm), corresponding to the TLC spots with Rf values 0.891, 0.87, 0.85 and 0.25, respectively. Component C showed inhibition spots against *Streptococcus pyogenes* (16.50 mm), *Staphylococcus aureus* (15.00 mm) and *Pseudomonas aeruginosa* (10.50 mm), corresponding to the TLC spots Rf values of 0.938, 0.44, 0.21 and 0.90, respectively. For the agar-well diffusion method, component A at 1 mg/ml inhibited *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* growths with zones of inhibition 23.0, 19.5 and 17.50 mm, respectively. MIC and MBC of component A were 125, 250 and 250 and 250, 500 and 500 µg/ml, respectively. Component B at 1 mg/ml inhibited *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth with zones of inhibition 30.0, 28.0 and 18.5 mm, respectively. MIC and MBC of the compound B were 31, 62 and 125 and 62, 125 and 250 µg/ml, respectively. Component C at 1 mg/ml inhibited *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* growth with zones of inhibition 24.5, 20.5 and 17.0 mm, respectively. MIC and MBC of the component C were 62, 125 and 250 and 125, 250 and 500 µg/ml, respectively. This study confirmed that bioactive compounds of *A. paniculatus* root have antibacterial properties and support the use of this part of the plant as a traditional remedy for mouth and throat infections possibly caused by the test bacteria.

**Keywords:** *Adenodolichos paniculatus*, isolated compounds, antibacterial activity, pathogenic bacteria, bioautogram, minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), Inhibition zone diameter (IZD)

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## Introduction

For a long time, plants have been a valuable source of natural products for maintaining human health, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has increased in Nigeria. According to the World Health Organization (WHO, 2002), medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated for a better understanding of their properties, safety and efficiency (Ellof, J.N, 1998). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plants. These products are known by their active substances; for example, the phenolic compounds which are part of the essential oils as well as tannin (Jansen, Cheffer and Svendsen, 1987). Hence, more studies on the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic-resistant microbes.

*Adenodolichos paniculatus* (a shrub of 4 - 5 m high found in the savanna, the bush and the jungle, from Guinea to Northern Nigeria and across to Sudan) is also known in Nigeria by various ethnic names such as kpàrák (Berom), gargung (Mwaghavul), kilikainawa (Fulfulde) and waken wuta (Hausa) to mention but a few. It

is one of the important medicinal plants with widespread traditional uses in the remedy and management of mouth and throat infections in Pushit community of Plateau state. Other folkloric uses include dressing of burns, healing of dysentery, liver trouble, venereal diseases, dysentery, diarrhea, and blennorrhoea and also for pain relief (Burkill 1985a; Sani et al., 2010).

The scientific study of *A. paniculatus* has become imperative due to the alarming incidence of antibiotic resistance in bacteria of medical importance and the need to look for other sources of novel antibacterial agents particularly from medicinal plants, based on ethno-pharmacological information (Parekh, et al., 2005). Although pharmaceutical industries have produced several new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, M.L, 1992). Nascimento et al. (2000) documented a high incidence of resistant microorganisms in clinical microbiology in Brazil. This fact has also been verified in other clinics all over the world (Mulyanningih et al., 2010).

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem; for

example, to control the use of antibiotic, increase research to better understand the genetic mechanisms of resistance, and continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

There has been an effort to document both the phytochemicals present in methanolic leaf extract of *A. paniculatus* while investigating the analgesic properties of the plant (Sani *et al.*, 2010) in Nigeria. Similar efforts have been made to document the bioactive compounds present in ethyl acetate leaf extract of the plant while studying the isolation, characterization and antimicrobial activity of the plant (Isyaku, Bello and Ndukwe, 2017). To date, there was no literature on the antibacterial activities of isolated bioactive compounds of the root of *Adenodolichos paniculatus*. It was not also known which component of the root bioactive compounds has antibacterial activity, if any. This study was, therefore, conducted to evaluate the antibacterial susceptibility of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* to the bioactive compounds isolated from the chloroform fractionated components of the root of *A. paniculatus*.

## Materials and Methods

### Collection, Identification and Extraction of Plant Materials

The plant specimens were harvested from the wild plant between October 2018 and March

2019. A herbalist was consulted as his experience was used to determine the accurate location of the plant within the District of Pushit in Mangu LGA of Plateau state, Nigeria. The collected roots were washed with clean water, cut into bits and spread thinly and evenly over an old newspaper and air-dried at room temperature (25 °C). The dried samples were pulverized with a mechanical grinder, sieved with 2.5 mm sieve and packed into clean, dried bottles and covered tightly. The bottles were labeled with the plant name and date of collection and kept/stored at room temperature until ready for extraction. The plant was identified as *Adenodolichos paniculatus* on voucher number FHJ 205 and deposited at the Herbarium unit of Federal College of Forestry, Jos. The bioactive constituents of the plant were extracted with five solvent systems (n-hexane, ethyl acetate, chloroform, methanol and water). This was to ensure that compounds with a wide range of polarity could be extracted. The serial maceration extraction technique was employed and successive extraction was carried out with solvents of increasing polarity from non-polar to more polar solvents (Thakare, 2004).

One kilogram (1 kg) of the powdered sample was extracted in a flat bottom flask with 2.5 liters hexane by maceration for 24 hours with intermittent shaking with an Orbital flask shaker. The sample mixture was filtered twice with muslin cloth, then with vacuum pump filtration and the filtrate was collected. The

filtrate was concentrated using a vacuum rotary evaporator at 40 °C. The concentrated/dried extract was collected into a pre-weighed sterile universal bottle, allowed to dry to constant weight at room temperature (25 °C) and weighed. The contents were then weighed for their extract yields and recorded. The bottle was labeled accordingly and stored in a refrigerator for microbial assay. This process was repeated using chloroform, ethyl acetate and methanol after allowing the marc to air dry for 2 to 3 hours. After successive extraction with these solvents, the residue (marc) was collected, air dried (to remove any residue of the solvent) and then macerated with cold distilled water to obtain the aqueous (water) extract. The percentage extract yields of the plant samples were calculated as:

Percentage extract yield (%) =

$\frac{\text{Weight of dried extract}}{\text{Weight of dried powder}} \times 100$  (Obafemi et al., 2006).

Weight of dried powder

### Test Microorganism

The typed and clinical isolates used for the study included:

*Staphylococcus aureus*-ATCC6538, *Escherichia coli* -ATCC43888 and *Pseudomonas aeruginosa*-ATCC9027 were obtained from Bacteriology Unit-National Veterinary Research Institute (NVRI), Vom, Plateau state and *Streptococcus*

*pyogenes*- Clinical isolate obtained Department of Microbiology and Bacteriology, National Institute for Pharmaceutical Research and Development-Diagnostic Centre, Abuja-Nigeria.

The purity of the isolates was ascertained by plating on different selective agar media before carrying out biochemical tests. The purity of the test bacteria was confirmed by sub-culturing into nutrient broth and incubating at 37 °C for 18 hours. The 18-hour broth culture was streaked onto sterile nutrient agar plates and incubated at 37 °C for 18 hours. The colonies were observed under the light microscope after simple Gram staining. Isolation of specific bacteria was done by streaking on selective media. A loopful of inoculum from the 18-hour broth culture was streaked on selective agar and incubated at 37 °C for 18 hours. The cultural characteristics of *Escherichia coli* were streaked on MacConkey agar to differentiate coliform (pink colony) from non-coliform (non-lactose fermenters) and checked on Eosin Methylene blue agar. *Pseudomonas aeruginosa* was checked on Cetrimide agar, *Staphylococcus aureus* on mannitol salt agar and *Streptococcus pyogenes* on blood agar. The cultural characteristics were observed and compared to standard references (Murray et al, 2007). They were further subjected to various biochemical tests for confirmation.

### Standardization of Inocula

The inoculum was standardized by using the Clinical Laboratory Standard Institute (CLSI, 2000 a) as adapted by Adeshina et al. (2010).

Eighteen-hour broth culture of each test organism was standardized by gradually adding normal saline to compare its turbidity to McFarland standard of 0.5 which is approximately  $1.0 \times 10^6$  cfu/ml. The turbidity of the cell culture was matched with that of the 0.5 McFarland standard by holding the mixture and the standard in front of light against a white background with contrasting black lines through visual comparison with its density by the addition of normal saline.

#### **Standard McFarland preparation**

The solution of 0.5 ml of 0.048 M BaCl<sub>2</sub> was added to 99.5 ml of a 0.18M H<sub>2</sub>SO<sub>4</sub> solution and vortex for 2 minutes. A UV-Vis spectrophotometer was used to measure the absorbance of the solution at 625 nm. An absorbance of 0.1 was obtained, which was in the accepted range of 0.08 to 0.13. This standard (0.5) was used to make a visual comparison with the density of the suspension against a white background with black lines.

#### **Antimicrobial Susceptibility Test**

The agar-well diffusion method of CLSI (2009) as adapted by Sedighinia et al., (2012) was employed to screen the isolated components for antibacterial activity. Overnight broth cultures were diluted appropriately using the McFarland scale (0.5 McFarland which is approximately  $1.0 \times 10^6$  cfu/ml). The molten sterile Mueller-Hinton agar-MHA (20 ml) was poured into a sterile Petri dish and allowed to set. The sterile

MHA plates were flooded with 1.0 ml of standardized culture of each test organism and the excess drained off. Wells of 6 mm diameter were bored into the inoculated plates using sterile a cork borer (6 mm). One drop of the molten agar was used to seal the bottom of the bored hole so that the isolated compound would not seep beneath the agar. Ten milligrams (10 mg) of each isolated component was dissolved in 2 ml of 10 % dimethyl sulfoxide (DMSO) solution in water to obtain a stock solution of 5 mg/ml concentration. Using a micropipette, two hundred microliters (200  $\mu$ l) of the isolated component stock concentration was dispensed into each agar-well. Standard antibiotic (ofloxacin) was used as positive control while sterile distilled water and 10 % DMSO served as negative controls. They were allowed to diffuse into the agar at room temperature for one hour before incubation at 37 °C for 18 hours. The experiment was carried out in duplicates; the inhibition zone diameters were measured to the nearest millimeter (mm) and mean and standard deviation of the duplicates were recorded (Ali et al., 2011).

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

The agar-well dilution method (modified) according to CLSI (2012) as adapted by Campana et al. (2014) was employed to determine the minimum inhibitory concentration of isolated compounds. Ten milliliters (10 ml) of the various isolated component concentrations

prepared in 10 % dimethyl sulfoxide (2, 1, 0.5, 0.25, 0.125, 0.06 and 0.031 mg/ml) were mixed with 10 ml of double strength Molten Mueller-Hinton agar (prepared by doubling the manufacturer's recommended weight of the agar to be dissolved in the same volume water) at 45 °C and poured into Petri-plates aseptically and allowed to solidify. The mixture gave a final various component concentration of 1, 0.5, 0.125, 0.0625, 0.031, 0.0156 and 0.0078 mg/ml for the study. After setting, sterile paper discs (6 mm) were applied at equidistance to the surface of the set agar containing the various isolated component concentrations. Ten microliters (10 µl) of each standardized organism/inoculum (10<sup>6</sup>cfu/ml) were then spot-inoculated on each disc and allowed to diffuse for 30 minutes before incubating at 37 °C for 18 hours. Growth control was prepared by inoculating 10 µl of each culture suspension on 15ml MHA medium without any compound or solvent (drug-free medium). Solvent control was prepared by pouring 1 ml of 10% DMSO into 14 ml of MHA medium followed by seeding of cultures. The first lowest concentration that showed no visible growth of the inoculated test organism was recorded as the MIC of the isolated compound for the test organism after overnight incubation ((Ali et al., 2011).

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

All inoculated paper discs showing no visible growth from MIC determination were

aseptically transferred to 5 ml of sterile nutrient broth containing 5 % yeast extract and 3 % Tween 80 to neutralize the effect of the components and incubated for another 18 hours at 37 °C. The discs from the lowest concentration of each isolated compound that showed no visible growth (cloudiness) was taken as the MBC of each plant extract against the test organism.

#### **Bioautographic studies**

For the bioautographic assay, the agar overlay method developed by Fisher and Lautner (1961) as adapted by Choma and Grzelak (2015) and Udobi et al. (2010) was adopted. Ten (10) ml of molten nutrient agar (NA) was seeded with 0.1 ml of standardized culture of the susceptible test organisms (*Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) and shaken very well. The seeded molten agar was poured/spread thinly over the developed TLC plates earlier air-dried for 24 hours in Petri-dishes. It was allowed to solidify and pre-diffuse for 2 hours before wrapping the plates with foil paper and incubating at 37 °C for 24 hours. The plates were then sprayed with an aqueous solution (2 mg/ml) of methylthiozolyltetrazolium chloride (M.T.T) and further incubated at 37 °C for 2 hours for detection of dehydrogenase activity. Microbial growth inhibition was observed as clear spots/bands against a purple background. The Rf values of spots/bands showing inhibition were determined.

### Isolation of bioactive compounds from the most active fraction

Bioactive spots/bands showing inhibition zones on the bioautogram were further purified using multiple development preparative thin layer chromatography (PTLC). In this step, the preparative glass chromatographic technique was employed for the separation and isolation of the bioactive components. The most active column fraction (A5) was spotted along a horizontal line of the activated glass preparative (20 x 20 cm, thickness 0.5 mm) at room temperature. After a proper run of the solvent system (hexane: ethyl acetate, 80:20), the plate was taken out of the chromatographic chamber and air-dried for 24 hours. The plates were visualized under ultraviolet light (254 and 360 nm). The straight-line bands that developed on the plate were scrapped off using a clean razor blade along the sorbent into a conical flask. Chloroform (10 ml) was added and the

suspension obtained was left to stand for 30 minutes to facilitate leaching of the compound into the solvent and then filtered. This isolation process was subjected to repeated preparative TLC (X 5) to ensure maximum recovery. The filtrates were left in an open crucible for the chloroform to evaporate. The compounds recovered from the PTLC were weighed and evaluated for antibacterial activity using the agar-well and dilution methods.

### Results

The TLC analysis, characteristics and percentage yields of isolated bioactive components as presented in Table 1 showed that the percentage yield was poor (component A was 7.66, B 7.76 and C 1.21 %). Component A was brown with a melting point of 133-143, B was light brown with a melting point of 130-140 and C was dark brown with a melting point of 130-142. The R<sub>f</sub> values of each component (A, B and C) were 0.594, 0.89 and 0.94 respectively.

**Table 1: TLC Analysis, Characteristics and Percentage Yield of Isolated Bioactive Components**

Bioactive component	Percentage yield (%)	RF value	Description	Melting point (°C)
A	7.66	0.594	Brown powder	133-143
B	7.76	0.891	Light brown powder	130-140
C	1.21	0.938	Dark green powder	130-142

**Table 2: Zone of inhibition of the Bioactive Components in the Pooled Column Fractions from Chloroform Fraction of *A. paniculatus* Root on TLC bioautogram**

Isolated components	Test bacteria (zone of inhibition-mm)		
	<i>S. pyogenes</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
A	17.5±0.71	NA	16.0±0.00
B	36.5±0.71	16.0±0.00	11.0±0.00
C	16.5±0.71	15.0±0.00	10.5±0.71
Rf Value of component A	0.26	NA	0.55
Rf Value of component B	0.85	0.87	0.25
Rf Value of component C	0.9	0.44	0.21

Values are mean inhibition zone (mm) ± S.D of two replicates

NA = No activity

**Table 3: Antibacterial Activity (inhibition zone diameter) of the Bioactive Components in the Pooled Column Fraction from Root Chloroform Fraction of *A. paniculatus*.**

Isolated component (1 mg/ml)	Test bacteria/ zone of inhibition (mm)		
	<i>S. pyogenes</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
A	23.5±0.71	19.5±0.71	17.5±0.710
B	30.0±0.00	28.0±0.00	18.5±0.71
C	24.5±0.71	20.5±0.71	17.0±0.00
Ofloxacin (5 µg)	32	30	31
DMSO (10 %)	NA	NA	NA

Values are mean inhibition zone (mm) ± S.D of two replicates

NA = No activity



**Table 4: Minimum Inhibitory Concentration (MIC) of Bioactive Components from Chloroform Fraction of the Chloroform Root Extract of *A. paniculatus***

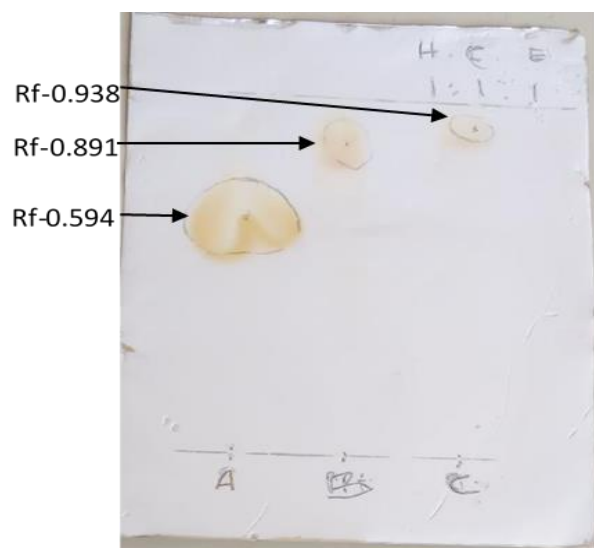
Isolated Component	MIC ( $\mu\text{g/ml}$ )		
	<i>Strep</i>	<i>Staph</i>	<i>Pseudo</i>
A	125	250	250
B	31	62	125
C	62	125	250
Ofloxacin	10	10	10
DMSO (10 %)	NA	NA	NA

NA = No activity

**Table 5: Minimum Bactericidal Concentration (MBC) of Bioactive Components from Chloroform Fraction of the Chloroform Root Extract of *A. paniculatus***

Isolated Component	MBC ( $\mu\text{g/ml}$ )		
	<i>Strep</i>	<i>Staph</i>	<i>Pseudo</i>
A	250	500	500
B	62	125	250
C	125	250	500
Ofloxacin ( $\mu\text{g/ml}$ )	10	10	10
DMSO (10 %)	NA	NA	NA

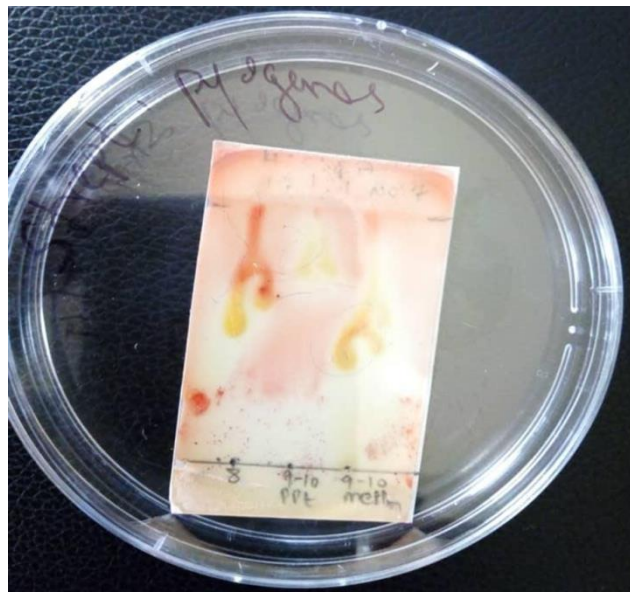
NA = No activity



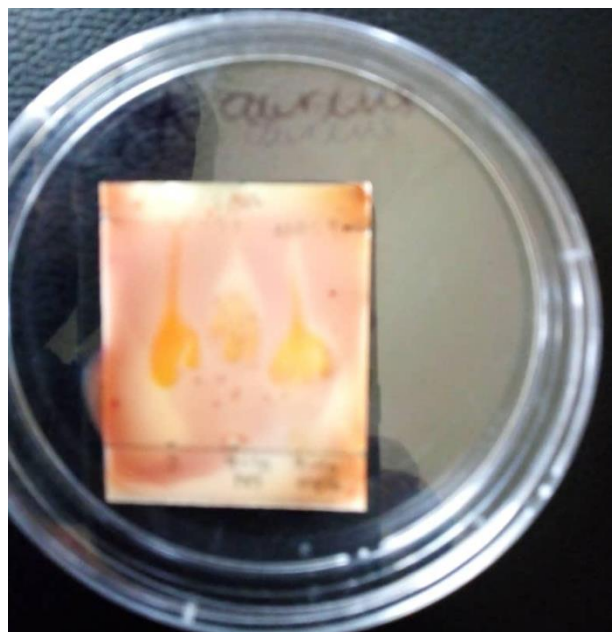
**Plate 1:** Thin layer chromatogram of the bioactive components showing three spots (A, B and C) in the chloroform fraction from chloroform root extract of *A. paniculatus*



**2. Bioautogram of bioactive compounds in the chloroform fraction against *Pseudomonas aeruginosa***



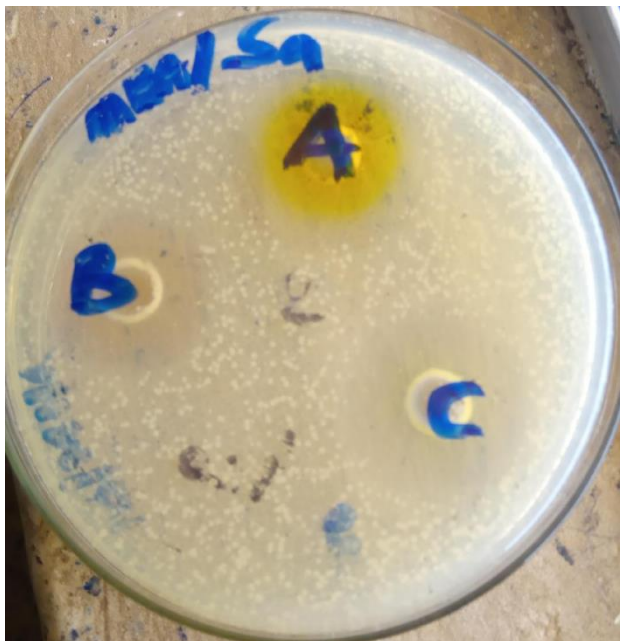
**3: Bioautogram of bioactive compounds in chloroform fraction against *Streptococcus pyogenes***



**4: Bioautogram of bioactive compounds in chloroform fraction against *Staphylococcus aureus***



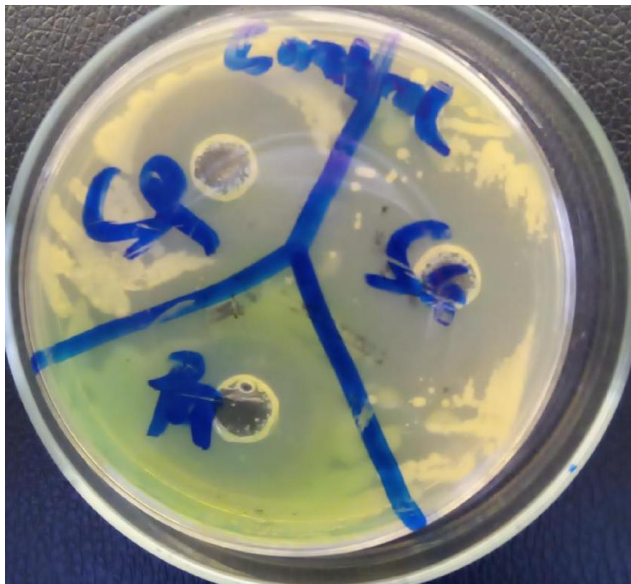
1: Sensitivity of *Streptococcus pyogenes* to isolated bioactive components A, B and C



2: Sensitivity of *Staphylococcus aureus* to isolated bioactive components A, B and C



3: Sensitivity of *Pseudomonas aeruginosa* to isolated bioactive components A, B and C



4: Sensitivity of *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* to the referenced antibiotic-Ofloxacin as positive control

## Discussion

The result of the TLC analysis showed that the percentage yield of the fraction was poor with the highest at only 7.76 %. The result of the study showed that the chloroform root extract exhibited *in vitro* antibacterial activity on three of the four selected test bacteria at a concentration of 100 mg/ml. The chloroform root extract was further subjected to vacuum liquid chromatography (VLC). The VLC separation of the chloroform extract yielded several fractions and a total of 14 fractions which pooled together based on their TLC characteristics into six combined fractions. The result of the antibacterial *in vitro* study of the six combined fractions indicated that only two combined fractions (A4 and A5 fractions) at 20 mg/ml were active against only three (3) of the four (4) pathogens tested; namely, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* and *E. coli* with zones of inhibition measuring 13.0, 10.5 and 10.00 mm (A4) and 28.5, 23.0 and 13.5 mm (A5), respectively. It was not active on *E. coli*.

The combination fraction (A5), which showed better antibacterial activity, was subjected to a bioautography study. The result showed the appearance of white areas against a purple-red background on the chromatograms, denoting inhibition of growth of the bacteria (Figure 1-3), which was due to the presence of compound(s) that

inhibit their growth. The zone of inhibition spots/bands exhibited the isolated bioactive components presented in Table 2 were 17.5 mm and 16.0 mm for bioactive component A against *Streptococcus pyogenes* and *Pseudomonas aeruginosa*, respectively. The absence of antibacterial activities against *Staphylococcus aureus* could be due to evaporation of the active compound (photo-oxidation) or due to very little amount of the active compound (Masoko and Eloff, 2005). Bioactive component B has inhibition of 36.5 mm, 16.0 mm and 11.0 mm against *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, respectively, while component C showed 16.5 mm, 15.0 mm and 10.5 mm for the same organisms, respectively. The  $R_f$  values of the bioactive component A corresponding to the inhibition zones/bands on *Streptococcus pyogenes* and *Pseudomonas aeruginosa* were 0.26 and 0.55, respectively, and no inhibition of microbial growth was observed on *Staphylococcus aureus*. The  $R_f$  values of the bioactive component B corresponding to the inhibition zones/bands on *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 0.85, 0.87 and 0.25, respectively. The  $R_f$  values of the bioactive component C corresponding to the inhibition zones/bands on *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were 0.90, 0.44 and 0.21, respectively. It is possible that

synergism played a major role in extracts that were active when the minimum inhibitory concentration of the mixture was determined, but the separated compounds had no antimicrobial activity based on bioautography (Eloff et al., 2011).

Actively growing microorganisms can reduce methylthiozolytetrazolium chloride (M.T.T) to a purple-red color (Begue and Klein, 1972). In the presence of active plant compounds on the chromatograms, the growth of the organism was inhibited. Because the  $R_f$  value is constant for the same compound under defined conditions, the presence of clear bands with the same  $R_f$  value may mean that the same compounds are probably responsible for the antimicrobial activity in the same bioactive component tested against the test bacteria. This would suggest a non-selective antimicrobial activity.

Each isolated component exhibited *in vitro* antibacterial activity on the three sensitive test bacteria at a concentration of 1 mg/ml as presented in Table 3. Bioactive component A showed antibacterial activity against the three test bacteria (*S. aureus*, *S. pyogenes* and *P. aeruginosa*) with inhibition zone diameters ranging from 17.5 to 23.5mm. Bioactive component B showed antibacterial activity against the three test bacteria (*S. aureus*, *S. pyogenes* and *P. aeruginosa*) with inhibition zone diameters ranging from 18.5

to 30.0mm. Bioactive component C showed antibacterial activity against the three test bacteria (*S. aureus*, *S. pyogenes* and *P. aeruginosa*) with inhibition zone diameters ranging from 17.0 to 24.5mm. The largest inhibition zone diameter (30 mm) was produced by bioactive component B against *S. pyogenes*, *S. aureus* (28 mm) and *P. aeruginosa* (18.5 mm). The minimum inhibitory concentration of the component A against *S. pyogenes*, *S. aureus* and *P. aeruginosa* were 125, 250 and 250  $\mu\text{g/ml}$ , respectively, while the minimum bactericidal concentrations for the three test bacteria were 250, 500 and 500  $\mu\text{g/ml}$ . The minimum inhibitory concentration of component B against *S. pyogenes*, *S. aureus* and *P. aeruginosa* were 31, 62 and 125  $\mu\text{g/ml}$ , respectively, while the minimum bactericidal concentrations for the three test bacteria were 62, 125 and 250  $\mu\text{g/ml}$ . The minimum inhibitory concentration of component C against *S. pyogenes*, *S. aureus* and *P. aeruginosa* were 62, 125 and 250  $\mu\text{g/ml}$ , respectively, while the minimum bactericidal concentrations for the three test bacteria were 125, 250 and 500  $\mu\text{g/ml}$  as presented in tables 4 and 5 respectively. The inhibitory values of the isolated components were much lower (the lowest was 31  $\mu\text{g/ml}$ ) than those of both the extract and fractions (310 and 1,560  $\mu\text{g/ml}$ ). The MIC and MBC values of each the components against the test organisms were higher than the

control/standard drug. This is expected because the compounds may not be 100 % pure, unlike the control drug which is synthetic and 100 % pure.

Bioactive component B produced the highest zones of inhibition against the test bacteria, followed by bioactive component C, and then bioactive component A which did not show any activities on *S. aureus*. The superiority of activities of bioactive component B could mean that the component contained more potent antimicrobial compounds acting synergistically or that the component contained higher concentrations of the bioactive components.

### Conclusion

The three bioactive isolated compounds from *A. paniculatus* root showed antibacterial activities against *S. pyogenes*, *S. aureus* and *P. aeruginosa*. The diameters of the zones of inhibition of the most active component B were 30.00, 28.00 and 18.5 mm, respectively. The inhibitory effect of bioactive component B against the test bacteria was not statistically effective compared to the standard antibiotic (Ofloxacin) disc, which had the inhibitory zone diameters of 32.00, 30.00 and 31.00 mm, respectively. The inhibitory activities of the isolated bioactive compounds against the test organisms provide scientific support for the traditional uses of the plant for the treatment of mouth

and throat infections caused by any of the test bacteria. It also confirmed that the root of *A. paniculatus* contained bioactive compounds that had an inhibitory effect on the test bacteria. Further investigation will be required to characterize bioactive component B which showed the most potent antibacterial activity. This may serve as a template for drug discovery. Also, an investigation can be performed to isolate a single bioactive compound to see if the chemical structure can be modified to improve its antibacterial activity.

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