The Journal of Phytopharmacology 2020; 9(5): 318-322
Online at: www.phytopharmajournal.com

The Journal of Phytopharmacology
(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X
JPHYTO 2020; 9(5): 318-322
September- October
Received: 22-07-2020
Accepted: 29-08-2020
©2020, All rights reserved
doi: 10.31254/phyto.2020.9505

Evaluation of the Antibacterial Activities, Acute Toxicity and Immuno-stimulatory Potential of *Adenodolichos paniculatus Chloroform* root Extract

Kyahar IF*, Onwuliri AE, Ehinmidu JO and Oladosu PO

Kyahar IF
Dept. of Pharmaceutical Microbiology and Biotechnology, University of Jos, Jos, Nigeria

Onwuliri AE
Dept. of Pharmaceutical Microbiology and Biotechnology, University of Jos, Jos, Nigeria

Ehinmidu JO
Dept. of Pharmaceutics and Pharmaceutical Microbiology, Ahmadu Bello University Zaria, Zaria, Nigeria

Oladosu PO
Dept. of Microbiology and Biotechnology, National Institute for Pharmaceutical Research and Development, Abuja, Nigeria

Correspondence:
Kyahar IF
Dept. of Pharmaceutical Microbiology and Biotechnology, University of Jos, Jos, Nigeria
Email: kyaharfriday@yahoo.com

ABSTRACT

Medicinal plants have been used in the treatment of illnesses from time immemorial. In Nigeria today a large population of people still depend on herbal medicines for their health needs. The medicinal plant, *Adenodolichos paniculatus* has been used traditionally as a remedy for mouth and throat infections in Pushit community of Plateau state, Nigeria. Five solvents (hexane, chloroform, ethyl acetate, methanol and water) were used sequentially for the isolation of bioactive compounds from the root of the plant *Adenodolichos paniculatus* and concentrated on rotary vacuum evaporator. Concentrated extracts were tested for their antibacterial activities against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* by agar-well diffusion method. Ofloxacin was the referenced drug. Chloroform root extract was more potent than the remaining four extracts. The extract at 100 mg/ml was active against only three (3) of the four (4) pathogens tested namely *Streptococcus pyogenes*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* with zones of inhibition measuring 25.00, 21.00 and 11.75 mm respectively. The minimum inhibitory concentration of the extract against the test organisms were 1,560, 6,250 and 25,000 µg/ml respectively. The minimum bactericidal concentration was 3,120, 12,500 and 50,000 µg/ml against the three organisms. The extract was found to be nontoxic with LD_{50} of above 2000 (mg//kg body weight) when administered orally in mice. The plant extract has immunostimulatory activity with a significant increase ($p<0.05$) in white blood cell proliferation when administered into mice at doses of 50, 25 and 100 mg/kg. Conclusion: The result of this investigation supports the use of this plant for the traditional remedy of mouth and throat infections possibly caused by these test organisms. The extract can serve as lead in phytomedicine development for the treatment of throat related infections.

Keywords: *Adenodolichos paniculatus*, Chloroform root extract, Antibacterial activity, Acute toxicity, Immune-stimulatory.

INTRODUCTION

For decades, plants have been used by man as a natural source of therapy for the management of several illnesses and diseases. The use of plant compounds for pharmaceutical purposes has increased in Nigeria. The full integration of herbal medicine into the mainstream primary health care programmes will require rigorous scientific study of both their therapeutic potentials as well as the assessment of safety issues. *Adenodolichos paniculatus* (a shrub of 4 - 5 m high found in the savanna, bush and jungle, from Guinea to Northern Nigeria, and across to Sudan (Burkill 1985a) [4] is also known by various ethnic names such as kpàrák (Berom), gargung (Mwaghavul), kilikainawa (Fulfulde) and waken wuta (Hausa) to mention but a few in Nigeria (Hutchison and Dalziel, 1958) [11]. It is one of the important medicinal plants with widespread traditional uses in the remedy and management of cough and sore throat infections in Pushit community. Other folkloric uses include dressing of burns, dysentery, liver trouble, venereal diseases, dysentery, diarrhea, blennorrhoea and also used as a pain-killer (Sani *et al*; 2010) [17].

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 ethnopharmacological information (Parekh, et al., (2005).

Group A *Streptococcus* (GAS), or *S. pyogenes*, is one of the major causes of acute respiratory tract infections. This pathogen can lead to severe invasive diseases, including pharyngitis and pyoderma. Recently, the increase in the incidence of antibiotic-resistant clinical isolates of *S. pyogenes* underscores the need for continuous surveillance of antimicrobial resistance patterns (Chen, et al., 2011) [6]. Macrolide resistance was first reported in 1955 and since then, the resistance has been progressively increasing worldwide (Sanders, 1968) [16]. The development of resistance to many antibiotics by *S. aureus* has

involved acquisition of determinants by horizontal gene transfer of mobile genetic elements (Mulyaningsih 2010). Methicillin-resistant Staphylococcus aureus have been found to be responsible for several difficult-to-treat infections including sore throat (Jensen and Lyon 2009). *Pseudomonas aeruginosa* displays resistance to a variety of antibiotics, including aminoglycosides, quinolones and β-lactams (Hancock and Speert, 2000) [10]. The high rate of antibiotic resistance among these pathogens poses serious problems for the treatment and management of bacterial infections.

There has been effort to document the phytochemicals present in methanolic leaf extract of *A. paniculatus* while investigating the analgesic properties of the plant (Sani *et al.,* 2010) [17] in Nigeria. Also, there has been effort to document the bioactive compounds present in ethyl acetate leaf extract of the plant while study on the isolation, characterization and antimicrobial activity of the plant (Isyaku, *et* al., 2017) [12]. To date there was no information in literature on the antibacterial activity of root extract of *Adenodolichos paniculatus*. It was not yet known which component of the root extract has antibacterial activity, if any. The present study, was, therefore, conducted to evaluate the antibacterial susceptibility of *Streptococcus pyogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *E. coli* to chloroform root extract of *A. paniculatus* and to assess its acute toxicity as well as its immunostimulatory potential.

MATERIALS AND METHODS

Collection, Identification and Extraction of Plant Materials*:* The plant specimens were harvested from the wild plant through consulting with the herbalist between the months of October, 2018 and March 2019 as his experience was used to find out the right place of the plant within Pushit district of Mangu LGA of Plateau state. The roots collected 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 labelled with the plant name and date of collection and kept/stored at room temperature (25 °C) until ready for extraction. The plant 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 system (n-hexane, ethyl acetate, chloroform, methanol and water). This was to ensure that a wide polarity range of compounds could be extracted. Serial maceration extraction technique was employed whereby successive extraction was carried out with solvents of increasing polarity from a non-polar to a more polar solvent (Thakare, 2004) [19].

One kilogram (1 kg) of powered sample was extracted in 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 2 times with muslin cloth, then with vacuum pump filtration and the filtrate collected. The filtrate was concentrated using water bath at 40 ˚C. The concentrated/dried extract was collected into a pre-weighed sterile universal bottle and further 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 2-3 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:

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

Test Microorganisms: The typed and clinical isolates used for the study included:

Staphylococcus aureus-ATCC6538, *Escherichia coli* –ATCC 43888 and *Pseudomonas aeruginosa*-ATCC9027 were obtained from Bacteriology Unit-National Veterinary Research Institute (NVRI), Vom, Plateau state and *Streptococcus pyogenes*- Clinical isolate obtained from 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 incubated at 37 ˚C for 18 hours. The 18-hour broth culture was streaked unto 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 18-hour broth culture was streaked on selective agar and incubated at 37 °C for 18 hours. The cultural characteristics of *Escherichia coli* was 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* was checked on mannitol salt agar and *Streptococcus pyogenes* was checked on blood agar. The cultural characteristics were checked and compared with standard references (Murray et al, 2007) [14]. Further biochemical tests were carried out for their 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 [2] was employed. 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 x 10^6 cfu/ml. The turbidity of the cell culture was matched with that of the 0.5 McFarland standard (holding mixture and the standard in front of a light against a white background with contrasting black lines) through visual comparison with its density by addition of normal saline

Standard McFarland preparation: The solution of 0.5 ml of 0.048 M $BaCl_2$ was added to 99.5 ml of a 0.18M H_2SO_4 solution and vortex for 2 min. 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 and 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 crude extracts for antibacterial activity. Overnight broth cultures were diluted appropriately using McFarland scale (0.5 McFarland which is approximately 1.0 x 10^6cfu/ml). The molten sterile Mueller-Hinton agar (20 ml) was poured into 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 cork borer (6 mm). One drop of the molten agar was used to seal the bottom of the bored hole so that the extract would not sip beneath the agar. Five grams (5 g) of each dried crude extract was dissolved in 10 ml of 10 % dimethyl sulfoxide (DMSO) solution in water to obtain a stock solution of 500 mg/ml concentration. Using micropipette, two hundred microliters (200 µl) of the crude extract 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. These were allowed to diffuse into the agar at room temperature for one hour before incubation at 37 ˚C for 24 hours. The experiment was carried out in duplicates and the inhibition zones diameter were measured to the nearest millimeter (mm) and mean and standard deviation of the duplicates were recorded (Ali et al., 2011) [3]

Determination of Minimum Inhibitory Concentration (MIC)*:* 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 active crude extracts. From a stock concentration of 4 g/20 ml, different concentration of both aqueous and solvent extracts (200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 mg/ml) were prepared in 10 % dimethyl sulfoxide (DMSO). 10 ml of each of the prepared concentrations of the extract was mixed with 10 ml of double strength (doubling the agar manufacturer recommended weight of the agar to be dissolved in the same volume water) Molten Mueller-Hinton agar at 45 ^{0}C and poured into Petri-plates aseptically and allowed to solidify. The mixture gave a final various extract concentration of 100, 50, 25, 12.5, 6.25, 3.125, 1.56 and 0.78 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 extract concentrations. Ten microliters (10 µl) of each standardized organism/inoculum (10^{6}cfu/ml) was then spot-inoculated on each disc and allowed to diffuse for 30 minutes before incubating at 37 ˚C for 18 - 24 hours. Growth control was prepared by inoculating 10 µl of each culture suspensions on 15 ml MHA medium without any extract or solvent (drug-free medium). Solvent control was prepared by pouring 1 ml of 10% DMSO to 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 extract for the test organism after overnight incubation (Ali *et al*., 2011) [3].

Determination of Minimum Bactericidal Concentration (MBC): 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 extracts and incubated for another 18 hours at 37 °C. The discs from the lowest concentration of each extract that showed no visible growth (cloudiness) was taken as the MBC of each plant extract against the test organism.

Test Animals*:* The test animals included: Swiss albino mice (BALB/ c). They were obtained from the Pharmacology and Toxicology department of National Institute for Pharmaceutical Research and Development, Abuja.

Acute toxicity studies: The Organization for Economic Cooperation and Development (OECD-up and down) guidelines No. 425 guidelines (2008) was employed to determine the acute toxicity of the chloroform root extract. Five healthy Swiss albino female mice ages between 8-9 weeks old weighing 22, 22, 21, 19 and 25 g were brought into the laboratory and coded 24 hour prior to the test. They were fasted for 18 hours but had accessed to water and the fasted body weights were used to calculate the individual doses of the extract for oral administration.

On the day of the test, a mouse was picked at a time, weighed and dosed with 2000 mg/kg body weight of the extract dissolved in coconut oil. Dosed one mouse at the test dose of 2000 mg/kg body weight until a total of five mice were tested. Each mouse was given single oral dose in a volume of 1 ml/100 gm body weight through oral gavages using oral suitable intubation cannula. They were each placed in an observation cage. The mice were checked separately 30 mins after initial dosing, periodically during the first 24 hours, with special attention given during the first 4 hours and critically after first 8 hours of administration and daily thereafter for a total 14 days for acute toxicity signs of delayed activity (mortality), behavioral and body weight changes were observed and recorded on the chart. Individual weights of the mice were determined on the 14th day post-dosing. All observations were systematically recorded with individual records being maintained for each mouse. The results were evaluated as followed (0=survival, X=death). The LD_{50} is less than the test dose (2000 mg/kg) when three or more animals die, and the LD_{50} is greater than the test dose (2000 mg/kg) when three or more animals survived.

Immuno-stimulatory effect of *A. paniculatus* root extract on Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC): A modification of Chidume *et al.,* 2002 method was adopted to determine the immune-stimulatory impact of the chloroform root extract. Twelve (12) mice of average 21g weight were grouped into four and three in each group. The first group served as control, the second, third and fourth groups were administered with the extract doses at doses of 25, 50, and 100 mg/kg body weight intraperitoneally respectively on the day 1, 5, and 9. On the 10th day, blood samples were collected by applying pressure on their tails and the cutting off the tip of each tail for analyzes of total leucocytes count (TLC) and differential leucocytes count (DLC). The TLC was done by making 1:20 dilution of the blood samples with white cell diluting fluid and counting aid of improved Neubauer counting chamber under the microscope at X 10 magnification. The DLC was determined by making a thin film of the blood samples on microscopic slides and staining with Leishman's stain. The slides were air-dried at room temperature and examined under microscope in oil immersion (X 100 magnification). The changes in total differential leucocytes counts (TLC and DLC) were observed and recorded (Ali *et al*., 2013).

RESULTS

The result of the study showed that the extract exhibited in *vitro* antibacterial activity on three of the four selected test bacteria at a concentration of 100 mg/ml (100,000 µg/ml). The chloroform root extract showed antibacterial activity against three (*S. aureus*, *S. pyogenes* and *P. aeruginosa*) of the four bacteria tested with inhibition zone diameters ranging from 11.75 to 25.00 mm. The largest IZD (25 mm) was produced by chloroform root extract against *S. pyogenes*, *S. aureus* (21 mm) and *P. aeruginosa* (11.75 mm). The chloroform root extract showed no activity against *E. coli.* The MIC of the extract against *S. pyogenes*, *S. aureus* and *P. aeruginosa* were 1,560 6,250 and 25,000 µg/ml and the MBCs for the three test bacteria were 3,120, 12,500 and 50,000 µg/ml.

Table 1: Antibacterial Activity of Chloroform Extract (Root)

	Organisms	Diameter of Inhibition (mm)	MIC (µg/ml)	MBC (µg/ml)
Extract (100,000 µg/ml)	*Streptococcus pyogenes*	25.00±0.00	1,560	3,120
	Staphylococcus aureus	21.00±0.00	6,250	12,500
	Pseudomonas aeruginosa	11.75±0.35	25,000	50,000
	E. coli			
Control (Ofloxacin (5µg/disc)	*Streptococcus pyogenes*	28.00±0.00	10	10
	Staphylococcus aureus	25.00±0.00	10	10
	Pseudomonas aeruginosa	15.75±0.35	10	10
	E. coli	29.75±0.35	10	10

Table 2: Animal Acute Toxicity Profile of *Adenodolichos paniculatus* Chloroform Extract (Root) on Mice

Animal code (ID)	Fasted body weight (gm)	Dose administered (mg/kg)	Volume (ml)	Route of Adminnistration	Short-term result (8hrs)	Fasted body weight after 14 days (gm)	Long-term result (14 days)
Control	22	2000	0.22	Oral	Survival	22	Survival
BK	22	2000	0.22	Oral	Survival	21	Survival
LS	21	2000	0.21	Oral	Survival	21	Survival
RS	19	2000	0.19	Oral	Survival	19	Survival
TL	25	2000	0.25	Oral	Survival	25	Survival

The result of the study showed that the LD_{50} for the extract was greater than 2000 mg/kg of body weight in mice as presented in Table 2. The result of the study showed that the extract had immuno-stimulatory effect on non-specific immune response with significant increase ($p<0.05$) in the Total Leucocyte Count (TLC and Differential Leucocytes Count (DLC) in the three doses of 25, 50 and 100 mg/kg in mice as presented in Table 3.

Table 3: Effect of various Doses of Chloroform Root Extract of *A. paniculatus* on Total Leucocytes Count (TLC) and Differential Leucocytes count (DLC) in Mice

Groups	TLC (10^9/l)	Differential leucocytes count (%)			
		Neutrophils	Lymphocytes	Monocytes	Eosinophils
Control	5.40±0.72	16.00±2.00	73.33±3.05	3.33±2.30	3.00±1.73
25	8.83±3.65	28.66±4.04	74.00±8.71	0.33±0.57	0.00±0.00
50	15.60±2.64	19.66±5.13	78.66±4.72	0.33±0.57	1.33±1.15
100	7.50±1.12	25.33±1.15	76.00±5.56	0.00±0.00	0.66±1.15
Total	10.63±4.44	24.46±6.18	75.50±4.25	0.33±1.92	0.63±0.1.52

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

DISCUSSION

The result of the study showed that the extract exhibited in *vitro* antibacterial activity on three of the four selected test bacteria at a concentration of 100 mg/ml. This means none of the compounds extracted by chloroform have activity against E. coli. The inhibitory concentrations of the extract against the test organisms were significantly higher ($P<0.05$). This is not surprising considering that the extract is still in the crude form and therefore not as potent as the control which is a pure/synthetic drug.

The data revealed that LD_{50} of the extract was greater than 2000 mg/kg body weight and said to be non-toxic and safe when orally taken as no animal death was noticed. Similarly, no significant differences in body weight were observed at the end of the treatment period (14 days). This implies that the extract is practically nontoxic and safe when administered orally as no animal death was recorded. This result was similar to an earlier report by Sani *et* al. (2010) [17] who evaluated the analgesic and anti-inflammatory activities of the methanolic leaf extract of *A. paniculatus* in mice and reported that the plant extract was non-toxic with LD_{50} greater than 1131.3 mg/kg in mice. There is no toxic report of *A. paniculatus* in humans at the time of this study and probably responsible for its wide use in treatment of bacterial mouth and throat infections by the herbalist in Mangu LGA of Plateau state-Nigeria. The result shows that the oral administration of *A. paniculatus* extract produced no significant toxic effect in BALB/c mice. The extract can be a lead for research and development in pharmaceutical formulations.

The result presented showed that the extract caused increase in total leucocytes counts (TLC) and differential leucocytes counts (DLC) at the three doses/concentration (25, 50 and 100 mg/kg body weight) adopted in the study. The increase was significant ($P= 0.004<0.05$). The non-statistical values showed that 50 mg/kg produced more WBC, followed by 25 mg/kg, while 100 mg/kg produced less WBC ($x10^9$/l). There was also significant difference in the neutrophils (%) at three doses since $p=0.012<0.05$. This indicated that some particular doses have produced more neutrophils in the study, hence the non-statistical values showed that 25 mg/kg produced more neutrophils, followed by 100 mg/kg with the 50 mg/kg dose produced less.

There was significant difference in the lymphocytes count at three different doses since p=0.020<0.05. This indicated that some particular doses have produced more lymphocytes (%), hence the non-statistical values showed that 50 mg/kg produced more lymphocytes, followed by 100 mg/kg with the 25 mg/kg dose produced less. There was no significant difference in the monocytes count at three different doses. This indicated that no particular dose has produced more monocytes (%). There was no significant difference in the eosinophils count at three different doses. This indicated that no particular dose has produced more eosinophils (%).

The increase in both TLC and DLC indicated that the extract has immunostimulatory effect in mice. Phytochemicals such as flavonoids, tannins, saponins, diterpenoids and glycosides present in several plants are reported to have immune-modulatory properties (Jantan et al., 2015). The result of this study was in agreement with this report because the extract of *A. paniculatus* contained array of these phytochemicals.

CONCLUSION

Chloroform root extract of *A. paniculatus* showed antibacterial activity against three of the four test bacteria namely *S. pyogenes*, *S. aureus* and *P. aeruginosa.* The diameters of zones of inhibition were 25.00, 21.00 and 11.75 mm respectively. The extract showed inhibitory effects on the test bacteria with MIC of 1,560, 6,250 and 25,000 µg/ml and MBC of 3,120, 12,500 and 50,000 µg/ml respectively. The inhibitory effect of extract against the test bacteria was not statistically effective as the control antibiotic (Ofloxacin) disc which had inhibitory zone diameters of 28.00, 25.00 and 15.00 respectively. The inhibitory activity confirmed that there are potential antibacterial agents in the plant. Extract of *A. paniculatus* has shown good immune-stimulatory effects of the plant on test animals at LD_{50}.

Conflict of interest

The authors declare no conflict of interests

Funding

No funding was received for this study.

REFERENCES

1. Abdulsalam I.A, Ehinmidu, J.O and Igbadi E.O. *Evaluation of Antibacterial Properties, Acute Toxicity and Immuno-stimulatory Potential of Scoparia dulcis*. Nigerian Journal of Biotechnology, 2013; (26):21-25.
2. Adeshina, G., O., Onaolapo, J. A., Ehinmidu, J. O. and Odama, L. E. *Phytochemical and Antimicrobial Studies on the Ethyl acetate Extract of Alchornea cordifolia leaf found in Abuja-Nigeria*. Journal of Medicinal Plants Resources, 2010; 4(8):649-658
3. Ali A.I, Ehinmidu J.O and Ibrahim Y.K.E. *Preliminary Phytochemical Screening and Antimicrobial Activities of some Medicinal Plants used in Ebiraland*. Bayero Journal of Pure and Applied Sciences, 2011; 4(1):10-16
4. Burkill, H. M. *The Useful Plants of Tropical West Africa, Families A D Royal Botanical Garden*, Kew, 1985a, 1-254.
5. Burkill, H. M. (Ed.) *The Useful Plants of West Tropical Africa: Families A D.* Kew, Royal Botanic Gardens, 1985b, 1-319
6. Chen, I., Kaufsi, P and Erdem, G, Emergence of Erythromycin and Clindamycin Resistant-Streptococcus pyogenes emm 90 strains in Hawali. Journal of Clinical Microbiology, 2011; 49(1):439-441
7. Clinical Laboratory Standards Institute (CLSI) *Methods for dilution antimicrobial susceptibility test for bacteria that grow aerobically-approved guideline M7-A8. PA, USA*: Clinical Laboratory Standards Institute, 2009.
8. Clinical Laboratory Standards Institute (CLSI) *Performance standards for antimicrobial susceptibility testing: twenty second Information Supplement (M100-S22). PA, USA*: Clinical Laboratory Standards Institute, 2012.
9. Chidume, F., Gamaniel, K., Amos, S., Akah, P., Obodozie, O & Wambebe, C. *Pharmacological Activity of Methanolic Extract of Cassis nigricans leaves,* Indian Journal of Pharmacology, 2001; 33(5):350-356. Retrieved from http://medind.nic.in/ibi/t01/i5/ibit01i5p350.pdf
10. Hancock, R.E and Speert, D.P Antibiotic resistance in Pseudomonas aeruginosa: Mechanisms and Impact on Treatment. Drug Resistant Updat, 2000; 3:247-255
11. Hutchinson, J. and Dalziel, J. M. *Caesalpiniaceae, Mimosaceae and Papilionaceae.* Flora of West Tropical Africa*,* 1 1958; (2):439-587.
12. Isyaku, I, Bello, A.I, & Ndukwe, G.I. *Isolation, Characterization and Antibacterial Activity of Bioactive Constituents from the Leaf Extract of Adenodolichos paniculatus (Hua), & Dalz (Fabaceae)*. Unpublished Master's thesis. Ahmadu Bello University, Zaria, 2017.
13. Latha, M., Ramkumar, K.M., Pari, L., Damodaran, P.N., Rajeshkannam, V. and Suresh, T. Phytochemical and Antimicrobial Study of an Antidiabetic Plant: *Scoparia dulcis*. *Journal of Medicinal Food.* 2006; 9(3):391-39.
14. Murray, P. R., Baron, E. J., Jorgensen, J. H., Landry, M. L. and Pfaller, M. A. (ed.) Manual of Clinical Microbiology, 9th Edition. ASM Press, Washington, D. C., 2007.
15. *Organization for Economic Cooperation and Development (OECD) 425 guidelines*, 2008.
16. Sanders, E., Foster, M.T and Scott, D., Group A beta-hemolytic Streptococci Resistant to Erythromycin and Lincomycin. N. England. Journal of Medicine, 1968; 278:538-540
17. Sani, M., Anuka, J., Magaji, I., Yaro, A., Magaji, M. and Sani, Y. *Evaluation of Analgesic and Anti-inflammatory Activities of the Methanolic Leaf Extract of Adenodolichos paniculatus* (hua). Nigerian Journal of Pharmaceutical Sciences, 2010; 9(1):73-80.
18. Sedighinia F, Safipour A., A, Soleimanpour S, Zarif R, Asili J & Ghazvini K. *Antibacterial Activity of Glycyrrhiza glabra against Oral pathogens: an In- Vitro Study*. Avicenna Journal of phytomedicine, 2012; 2(3):118-24.
19. Thakare, M. *Pharmacological Screening of some Medicinal Plants as Antimicrobial and feed additive*. Masters' Thesis. Department of Animal and Poultry Science, Virginia Polytechnic Institute and State University, Blackburg, Virginia, USA, 2004.

HOW TO CITE THIS ARTICLE

Kyahar IF, Onwuliri AE, Ehinmidu JO and Oladosu PO. Evaluation of the Antibacterial Activity, Acute Toxicity and Immuno-stimulatory Potential of *Adenodolichos paniculatus* root Extract. J Phytopharmacol 2020; 9(5):318-322.