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ANTIBACTERIAL ACTIVITY OF *ANDROGRAPHIS PANICULATA* (BURM. F.) METHANOL LEAF EXTRACT ON BACTERIA CONSORTIA ISOLATED FROM BLOOD OF DIABETIC PATIENTS

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ABSTRACT

Introduction: *Andrographis paniculata* (Burm. f.) is a significant pharmacological plant and regularly used in different parts of the world. The antibacterial activity of the methanol leaf extract of *A. paniculata* against bacterial consortia from blood of diabetic patients was evaluated in this study.
Methods: The enumeration of bacteria from blood samples of diabetic patients and their antibiotic sensitivity pattern were done using standard techniques. The phytochemical analysis of *A. paniculata* methanol extract and antibacterial assay of the extract were also done using standard methods.
Results: *Staphylococcus aureus* had the highest occurring rate of 19.56 % while *Klebsiella pneumoniae* had the lowest occurring rate of 0.40 %. The isolates exhibited different sensitivity patterns to conventional antibiotics. There were variations in the zones of inhibition of *A. paniculata* methanol extract against the bacterial isolates as extract showed concentration was dependent on antibacterial activity with all the bacterial isolates susceptible to the extract. The minimum inhibitory concentration (mg/ml) of the *A. paniculata* methanol extract ranged from 5 mg/ml to 10 mg/ml while the minimum bactericidal concentration (mg/ml) of the bacteria isolated from diabetic patients ranged from 10 mg/ml to 20 mg/ml.
Conclusion: Findings revealed that the methanol leaf extract of *A. paniculata* very strong antibacterial activity for a wide range of bacteria from blood samples of diabetic patients and more reliable than commercially available antibiotics hence suggesting that leaves of *A. paniculata* can be used to develop novel antibacterial drugs.

Keywords: Diabetes, antibiotic, antibacterial, blood, antimicrobial

INTRODUCTION

The extensive administration of antimicrobial agents to treat diseases continues to lead to the development of antibiotic resistant bacteria, and this is of great concern to the public health **(Theuretzbacher, 2020)**. This study therefore suggested alternative treatment method for diabetic associated bacterial infection using *Andrographis paniculata*. *Andrographis paniculata* (Burm. f.) Wall. ex Nees (AP) belonging to the family Acanthaceae is an essential therapeutic plant commonly used around the world. Countries where *Andrographis paniculata* has been used for therapeutic purposes include Pakistan, Bangladesh, India, China, Hong Kong, Philippines, Malaysia, Indonesia, and Thailand **(Chauhan *et al.*, 2019; Kabir *et al.*, 2014)**, particularly for the treatment of snake bite, bug bite, diabetes, dysentery and fever. The presence of some phytochemical constituents has been reported to be responsible for its medicinal properties. **Radha *et al.* (2011)** reported the antibacterial activity of *A. paniculata* against both gram positive and gram negative bacteria including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*.
Diabetes mellitus (DM), usually referred to simply as diabetes is a group of metabolic disorders in which the blood sugar level remains high over an extended period of time **(Balaji *et al.*, 2019)**. Type 1 DM is as a result of the inability of the pancreas to produce enough insulin, formerly referred to as insulin-dependent diabetes mellitus (IDDM). Type 2 DM occurs as a result of the failure of the cells to respond properly to insulin, and this was formerly referred to as non-insulin-dependent diabetes mellitus (NIDDM) or adult-onset diabetes **(Al-Goblan *et al.*, 2014)**. The most prominent risk factors have been identified to be excessive body weight and inadequate exercise **(Al-Goblan *et al.*, 2014)**. There exists a third form of diabetes, known as gestational diabetes, and it occurs when a pregnant woman develops high blood sugar level, having to former history of diabetes.
In 2016, it was estimated that about 422 million people had diabetes globally **(Zhou *et al.*, 2016; Amiri, 2016)**, up from an estimated 382 million people in 2013 **(Shi and Hu, 2014)**. Accounting for the ever-changing age structure of the global population, the occurrence of diabetes is 8.5 % among adults, nearly double the rate of 4.7% in 1980 **(WHO, 2016)**. Type 2 DM makes up about 90 % of the cases **(Vos *et al.*, 2012)**.
Diabetic patients who also have ulcers usually become infected with organisms such as *Staphylococcus aureus, Enterococcus, Pseudomonas aeruginosa, Escherichia coli, Klebsiella species* and *Proteus species* **(Shankar et al., 2005)**. Consequently, many drugs have been discovered and used in the treatment of diabetic-associated bacterial infections, however the existing challenge of antibiotic resistance calls for the need to seek alternative trado-medical therapy. This study therefore assessed the antibacterial activity of *Andrographis paniculata* (Burm. f.) methanol leaf extract against bacteria isolated from blood of diabetic patients at the University of Medical Sciences Teaching Hospital (formerly State Specialist Hospital), Akure, Nigeria.

MATERIAL AND METHODS

Collection of plant materials

The plant material, leaves of *Andrographis paniculata* were collected at Obakere, The Federal University of Technology, Akure (FUTA), Nigeria where they were growing naturally. The authentication of the plant was done at the Department of Crop, Soil and Pest Management Department Federal University of Technology, Akure, Ondo State, Nigeria.

Collection of blood samples

The blood samples were collected into EDTA bottles which were labelled with information such as name, sex, age of patient, and date of collection, after which they were transported to the laboratory in an ice pack container for screening for the presence of bacteria.

Isolation of bacteria from the blood samples of diabetes patients

The isolation and identification of bacteria in the blood of patients were carried out according to the methods of **Fawole and Oso (2007)** and **Cheesbrough (2006)**. Colonial growth was subcultured into aseptically prepared McConkey agar, mannitol salt agar (MSA) and blood agar and incubated at 35 ± 2 °C for a duration of 24hrs **(Florio *et al.*, 2018)**.

Characterization of bacterial isolates

The pure culture of each isolate was examined. Microscopic examination, staining techniques and biochemical tests were carried out on the isolates according to the methods described by **Olutiola *et al.* (2000)**.

Standardization of test bacteria

A loopful of the bacterial culture was aseptically inoculated into freshly prepared sterile nutrient broth and incubated for 24 hours, after which 0.2 ml was pipetted from the 24 hours old broth culture of the test organism and was dispensed into 20

ml sterile nutrient broth and incubated for another 4 hours to standardize the culture to 0.5 McFarland's standard (10^6 cfu/ml) before use **(Oyeleke *et al.*, 2008)**.

Antibiotic sensitivity testing

The antibiotic sensitivity test was performed to investigate the antibiotic sensitivity profile of the microorganisms to different conventional antibiotics using the method described by **Bauer *et al*. (1966)**. Diameters of zone of inhibition was measured with a calibrated ruler and then compared with Clinical and Laboratory Standards Institute's standard for their sensitivity or resistance **(CLSI, 2017)**. Seeded plates without antibiotic disks served as the control. The antibiotic sensitivity profile was carried out in triplicates.

Preparation of plant extracts

The air dried sample of *Andrographis paniculata* were powdered in a table model grinder for extraction. The powdered materials were extracted using methanol. Three hundred gram (300 g) of the ground samples were weighed into three different containers, and was labelled. Then 2000 ml of the solvents were added, covered, shaken and mixtures were left for about 72 hours, after which the solvents along with the extracts were drained out with muslin cloth, filtered with no 1 Whatman filter paper and semi-solid extracts were obtained in using rotary evaporator (RE-52A Union Laboratories, England).

Phytochemical analysis of *A. paniculata* leaves methanol extract (qualitative)

The methanol leaf extract of *A. paniculata* was analysed for the presence or absence of different phytochemicals such as alkaloids, saponin, tannin, phlobatanin, anthraquinone, flavonoid, steroids, terpenoid and cardiac glycosides using standard methods described by **AOAC (2011)**.

Quantitative phytochemical analysis of *A. paniculata* methanol leaf extract

Determination of tannin

To determine the tannin content, the method described by **Iwuozor (2019)** was used. From the finely ground sample, 1g was weighed into 10ml distilled water and thoroughly shaken. The preparation was allowed to stand for 30 mins at room temperature while stirring was done intermittently at 5 mins interval. After the 30 mins duration, the solution was centrifuged, and the supernatant was obtained. Exactly 2.5ml of the obtained supernatant was pipetted into 50ml volumetric flask. Likewise, 2.5ml of standard tannin acid was dispersed into another 50ml flask, after which 1ml Folin-Denis reagent was dispersed into respective flask, which was immediately followed by the addition of 2.5ml of sodium carbonate (Na_2CO_3) solution. The resultant solution was diluted and made up to the 50ml mark of the flask and was thereafter incubated at room temperature for about 90 mins. The absorbance was read at 250nm using a UV spectrophotometer and readings were taken with the blank sample at zero.

Determination of total flavonoid

To determine the total flavonoid content of the extract, the method described by **Vuong *et al.*, (2013)** was used. This was done by mixing 0.5ml of the extract with 0.15ml of 5% sodium nitrate ($NaNO_3$). After 5 mins, 0.3ml of aluminum chloride (AlCl3) was added, after which 1ml of 1M sodium hydroxide (NaOH) and 2ml of distilled water were added to the mixture and thoroughly shaken. The absorbance was read using a spectrophotometer at 510nm.

Determination of saponin

To determine the saponin content, the spectrophotometric method described by **Dan *et al.*, (2009)** was used. From the finely grinded sample, 2g was weighed into 250ml beaker and 100ml of Isobutyl alcohol was added. The mixture was filtered with Whatman filter paper into 100ml beaker which contained 20ml of 40% saturated solution of magnesium carbonate ($MgCO_3$). The resultant solution was thereafter filtered with Whatman filter paper. A colourless solution was taken into 50ml volumetric flask and 2ml of 5% iron (III) chloride ($FeCl_3$) solution was added to make up with the mark of the distilled water. This setup was allowed to stand for 30mins for colour development. The absorbance is then read at 380nm.

Determination of alkaloid

To determine the alkaloid content, 5g of the sample was weighed into a 250ml beaker and 200ml of 10% acetic acid was added and allowed to stand for 4 mins. It was then filtered using Whatman filter paper and the extract was concentrated on a water bath to one quarter of the initial volume. Concentrated ammonium hydroxide was added drop by drop to the extract until precipitation was accomplished. The entire solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide, and then filtered. The residue was dried and weighed **(Harbone, 1973)**.

Determination of cardiac glycosides

To determine the cardiac glycosides content, the method described by **Sofowora (1996)** was employed. From the extract, 10ml was taken and pipetted into a 250ml conical flask, after which 50ml of chloroform was added and shaken on vortex mixer. The resultant solution was filtered into 100ml conical flask, after which 10ml of pyridine and 2ml of 29% of sodium nitroprusside were added and thoroughly shaken for 10 mins. 3ml of 20% NaOH was added to develop a brownish yellow colour. Glycosides standard (Digitoxin) with concentration ranging from 0 - 5mg/ml were prepared from stick solution and the absorbance was read at 510nm.

Determination of terpenoid

To determine the terpenoid content, the method described by **Sofowora (1996)** was used. From the finely grounded sample, 5g was weighed into a 50ml conical flask, and 20ml of chloroform/methanol solution in ratio 2:1 was added to the sample, shaken thoroughly and then allowed to stand for 15mins at room temperature. The suspension was centrifuged at 3000rpm. The supernatant was discarded and the precipitate was re-washed with 20ml of the chloroform/methanol solution in ratio 2:1 and then re-centrifuged. The precipitate was dissolved in 40ml of 10% sodium dodecyl sulfate (SDS) solution. 1ml of 0.01m ferric chloride was added and allowed to stand for 30mins before taking the absorbance at 510nm.

Determination of steroid

To determine the steroid, 5g of the finely grounded sample was weighed into 100ml conical flask, followed by the addition of 50ml of pyridine and the shaken thoroughly for 30mins at room temperature. 3ml of 250mg/ml copper (I) oxide (Cu_2O) and allowed to incubate for 1 hr in the dark and the absorbance was measure at 350nm against reagent blank **(Sofowora, 1996)**.

Antibacterial assay of *A. paniculata* methanol extract

The assay for the antibacterial activity of *A. paniculata* methanol leaf extract was carried out as described by **Geetha *et al*. (2017)** with slight modifications. 1g of the extract was dissolved in 10ml of Dimethyl sulfoxide (DMSO) for reconstitution of the extract. A sterile borer was used to make wells of 5mm diameter each on the already solidified Mueller Hinton agar prior to streaking of the plate with the bacterial culture. After the streaking had been completed, 0.1ml of the extract in peptone water was directly applied to each well on the agar. Positive control was maintained with ciprofloxacin, which is a standard antimicrobial drug, while wells containing the solvent alone were maintained as the negative control. The plates were then incubated for 18hours at 37°C and the diameter of zones of inhibition were measured in mm.

Minimum inhibitory concentration (MIC)

The MIC assay was performed using tube dilution method with Mueller Hinton broth. A series of broth tubes containing varying concentrations of the *A. paniculata* methanol leaf extract ranging from 5mg/ml to 100mg/ml were prepared and incubated with standardized density of the test organisms. The lowest concentration of *A. paniculata* extract resulting in no growth following visual inspection after 18-24 hrs of incubation for bacteria using a spectrophotometer was recorded as the MIC.

Minimum bactericidal concentration (MBC)

The MBC assay was performed using tube dilution method with Mueller Hinton broth. A series of broth tubes containing varying concentrations of the *A. paniculata* methanol leaf extract ranging from 5mg/ml to 100 mg/ml were prepared

and incubated with standardized density of the test organisms. The lowest concentration of *A. paniculata* extract resulting in no growth following visual inspection after 18-24 hrs of incubation for bacteria using a spectrophotometer was sub-cultured on fresh solid medium and further incubated at 37ºC for 18-24hrs. The lowest concentration of MIC tubes with no visible bacterial growth on solid medium was regarded as MBC.

Statistical analysis of data

Data obtained were subjected to one-way analysis of variance while the means were compared by Duncan's New Multiple Range Test at 95 % confidence interval using Statistical Package for Social Sciences version 23.0. Differences were considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Rate of occurrence of different bacteria isolated from diabetes patients blood samples

Staphylococcus aureus had the highest percentage positivity of 19.56% while *Klebsiella pneumoniae* had the lowest percentage positivity of 0.40% as shown in Table 1.

The most repeatedly isolated bacteria from blood of diabetic patients was *Staphylococcus aureus* with highest rate of occurrence of 19.56 %. This could be due to the fact that *S. aureus* is one the most common normal flora of human which could find its way into the blood stream of the diabetes patient through the slow healing wound. **Rajalakshmi and Amsaveni (2012)** reported that common pathogens isolated from the diabetic patients include *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas* sp., *Escherichia coli*, *Klebsiella* sp. and *Proteus* sp., an observation which is in accordance with the findings of this study.

Table 1 Rate of occurrence of different bacteria isolated from diabetes patients blood

Bacteria	Number of Patient tested positive	% Positivity
Staphylococcus aureus	98	19.56
Escherichia coli	80	15.97
Salmonella typhi	81	16.17
Streptococcus pyogenes	73	14.57
Streptococcus pneumoniae	50	9.98
Salmonella typhimurium	49	9.78
Pseudomonas aeruginosa	47	9.38
Haemophilus haemolyticus	11	2.20
Haemophilus influenza	10	2.00
Klebsiella pneumonia	2	0.40
Total	501	100.0

Susceptibility patterns of gram negative bacteria isolated from diabetes patients blood

Bacteria isolates were more susceptible to ciprofloaxacin with the zone of inhibition ranged from 8.33±0.58mm (*Haemophilus haemolyticus*) to 16.33±0.58mm (*Salmonella typhi*) and were least susceptible to sparfloxacin as shown in Table 2.

Susceptibility patterns of gram positive bacteria isolated from blood of diabetes patient

The isolates were more susceptible to ciprofloxacin with zone of inhibition ranged from 18.33±0.58mm (*Staphylococcus aureus*) to 24.33±0.58mm (*Streptococcus pneumoniae*) and least susceptible to zinnacef with zone of inhibition ranged from 8.67±0.58mm (*Staphylococcus aureus*) to 17.67±0.58mm (*Streptococcus pneumoniae*). *Staphylococcus aureus* was not susceptible to erythromycin and gentamycin as displayed in Table 3.

Table 2 Antibiotic susceptibility pattern of gram negative bacteria isolated from blood samples of diabetes patients

Antibiotics	*Salmonella typhi*	*Salmonella typhimurium*	*Escherichia coli*	*Pseudomonas aeruginosa*	*Haemophilus influenzae*	*Haemophilus haemolyticus*
Ciprofloxacin (30µg)	16.33±0.53^{e}	12.33±0.58^{d}	10.67±0.58^{e}	14.00±0.00^{e}	13.33±0.57^{f}	8.33±0.50^{b}
Chloramphenicol (30µg)	12.33±0.58^{c}	14.33±0.58^{e}	8.67±0.42^{d}	6.33±0.58^{b}	12.00±0.00^{e}	14.67±0.58^{d}
Septrin (30µg)	0.00±0.00^{a}	12.00±0.00^{d}	6.33±0.58^{c}	8.33±0.58^{c}	0.00±0.00^{a}	8.33±0.60^{b}
Amoxillin (30µg)	12.33±0.51^{c}	6.33±0.58^{b}	0.00±0.00^{a}	12.33±0.51^{d}	14.66±0.58^{g}	10.33±0.58^{c}
Augmentin (30µg)	12.33±0.58^{c}	0.00±0.00^{a}	10.00±0.00^{e}	8.67±0.58^{c}	6.33±0.58^{c}	4.33±0.58^{a}
Gentamycin (10µg)	0.00±0.00^{a}	8.33±0.57^{c}	6.67±0.58^{c}	4.33±0.51^{a}	12.33±0.58^{e}	8.33±0.58^{b}
Perfloxacin (30µg)	6.67±0.58^{b}	8.33±0.57^{c}	4.00±0.00^{b}	8.33±0.58^{c}	10.33±0.58^{d}	8.33±0.58^{b}
Streptomycin (30µg)	14.33±0.52^{d}	12.33±0.58^{d}	6.33±0.58^{c}	8.00±0.00^{c}	4.00±0.00^{b}	8.00±0.00^{b}
Tarivid (10µg)	12.33±0.50^{c}	8.00±0.00^{c}	4.33±0.58^{b}	12.33±0.53^{d}	16.33±0.58^{h}	8.33±0.58^{b}
Sparfloxacin (10µg)	6.00±0.00^{b}	8.33±0.58^{c}	4.00±0.00^{b}	12.33±0.58^{d}	14.33±0.58^{g}	8.00±0.00^{b}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different ($p<0.05$).

Table 3 Antibiotics susceptibility pattern of gram positive bacteria isolated from blood samples of diabetes patients

Antibiotics	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Streptococcus pyogenes*
Ciprofloaxacin (30µg)	24.33±0.50^{f}	18.33±0.58^{g}	22.33±-0.58^{g}
Streptomycin (30µg)	17.33±0.58^{b}	14.33±0.58^{f}	18.67±0.58^{d}
Septrin (30µg)	20.00±0.00^{c}	10.33±0.58^{d}	19.33±0.50^{de}
Erythromycin (30µg)	16.33±0.60^{a}	0.00±0.00^{a}	20.33±0.58^{f}
Perflaxin (30µg)	21.00±0.00^{d}	20.67±0.61^{h}	16.00±0.00^{c}
Gentamycin (10µg)	23.00±0.00^{e}	0.00±0.00^{a}	12.33±0.58^{b}
Ampiclox (30µg)	20.33±0.58^{cd}	12.33±0.54^{e}	18.67±0.58^{d}
Zinnacef (30µg)	17.67±0.58^{b}	8.67±0.58^{c}	16.33±0.52^{c}
Amoxacillin ((30µg)	20.33±0.58^{cd}	6.33±0.58^{b}	20.00±0.00^{ef}
Rocephin (25µg)	20.67±0.58^{cd}	12.00±0.00^{e}	10.33±0.58^{a}

Data are presented as Mean ± S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different ($p<0.05$).

The antibiotic sensitivity profile of bacteria isolated from diabetes patients in this study is analogous with result observed by **Vasudeva *et al.* (2016)** who observed appreciable level (85%) of *Staphylococcus* species susceptibility against ciprofloxacin. Similarly, the intermediate susceptibility and resistance of ciprofloxacin and chloramphenicol recorded against *E. coli* respectively in this study is also in agreement with the findings of **Vasudeva *et al.* (2016)** who revealed 33% sensitivity and 0% resistance for ciprofloxacin and chloramphenicol against *E. coli.*

Qualitative and quantitative phytochemical screening of *Andrographis paniculata* methanol leaf extract

The phytochemical screening carried out the extract revealed the presence of saponin, tannin, flavonoid, steroid, terpenoid, alkaloid, phytate, oxalate and cardiac glycosides, while phlobatannin and anthraquinone were not detected. This result is presented in table 4.

Table 4 Qualitative phytochemical screening of *Andrographis paniculata* leaf extract

Component	Presence/Absence
Saponin	+
Tannin	+
Phlobatannin	-
Flavonoid	+
Steroid	+
Terpenoid	+
Alkaloid	+
Anthraquinone	-
Phytate	+
Oxalate	+
Cardiac glycosides	+

Key: - = absent, + = present

The quantitative phytochemical screening carried out on the extract revealed that saponin is the most predominant phytochemical present in the extract with a mean value of 39.7 ± 2.6 mg/ml while oxalate is discovered to be the least predominant with a mean value of 1.5 ± 0.2 mg/ml. This result is shown in figure 1.

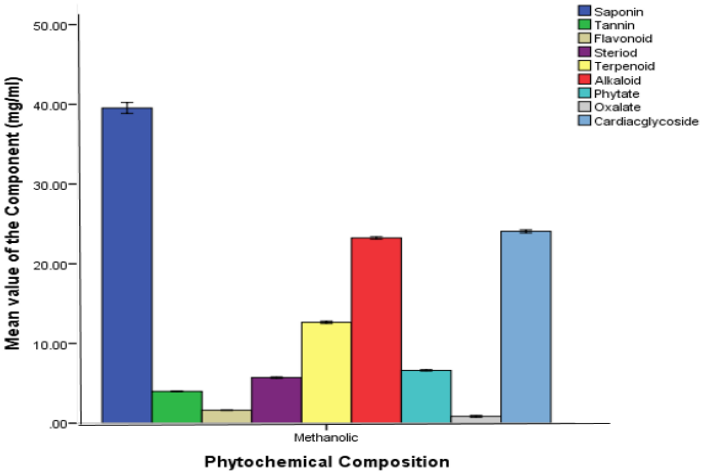

Figure 1 Quantitative phytochemical screening of *A. paniculata* leaf methanol extract

The result of the qualitative phytochemical analysis of the methanol leaf extract of *A. paniculata* shown in this study is in agreement with **Radha *et al.* (2011)** who also detected the presence of flavonoids, alkaloids, glycosides, steroids, phenols, tannins and saponins in the methanol extract of the plant leaves.

Antibacterial activities (mm) of *A. paniculata* extract against bacteria isolated from diabetes patients blood

There were variations in the zones of inhibition of extract against bacterial isolates, the inhibition of chloramphenicol (control) against the tested bacterial isolates was significantly ($p<0.05$) higher than what was observed in the extract. However, extract showed concentration dependent antibacterial activity. Also, all the isolates were susceptible to the extract as illustrated in Table 5.

Table 5 Antibacterial Activities (mm) of *A. paniculata* Leaves methanol extract against bacteria isolated from diabetes patients blood

Andrographis paniculata extract	A	B	C	D	E	F	G	H	I
10mg/ml	8.33±0.58^{a}	10.33±0.58^{a}	8.00±0.00^{a}	6.33±0.47^{a}	8.33±0.58^{b}	5.33±0.38^{a}	4.33±0.58^{a}	7.33±0.58^{a}	8.33±0.58^{a}
50mg/ml	18.33±0.61^{b}	19.67±0.58^{b}	20.33±0.58^{b}	12.33±0.68^{b}	6.33±0.58^{a}	12.67±0.58^{b}	10.33±0.58^{b}	14.33±0.58^{b}	8.67±0.52^{a}
100mg/ml	20.00±0.00^{c}	21.67±0.58^{c}	20.33±0.58^{b}	18.00±0.00^{c}	16.33±0.58^{c}	14.33±0.58^{c}	12.00±0.00^{c}	16.33±0.50^{c}	20.33±0.58^{d}
500mg/ml	22.33±0.58^{d}	23.33±0.58^{d}	19.67±0.58^{b}	20.33±0.58^{d}	21.33±0.58^{d}	22.00±0.00^{d}	18.33±0.58^{d}	17.00±0.00^{c}	19.33±0.58^{c}
1000mg/ml	25.33±0.58^{e}	24.33±0.58^{de}	23.33±0.58^{c}	25.67±0.58^{e}	23.67±0.58^{e}	24.33±0.58^{e}	19.33±0.58^{e}	22.67±0.58^{d}	16.33±0.33^{b}
Chloramphenicol	26.67±0.58^{f}	25.33±0.50^{e}	23.33±0.58^{c}	26.67±0.55^{f}	24.67±0.58^{e}	27.33±0.58^{f}	22.33±0.58^{f}	25.00±0.00^{e}	20.00±0.52^{cd}

Data are presented as Mean±S.D (n=3). Values with the same superscript letter(s) along the same column are not significantly different ($p<0.05$). Key: A = *Streptococcus pneumoniae*, B = *Staphylococcus aureus*, C = *Streptococcus pyogenes*, D = *Salmonella typhi*, E = *Salmonella typhimurium*, F = *Escherichia coli*, G = *Pseudomonas aeruginosa*, H = *Haemophilus influenzae*, *I = Haemophilus haemolyticus*

The antibacterial activity of *Andrographis paniculata* methanol leaf extract documented in this study might be due to the presence of the observed phytochemical constituents which include saponin, tannin, flavonoid, steroid, terpenoid, alkaloids, phytate, oxalates and cardiac glycosides as reported by **Nwanjo and Alumanah, (2006)**. The result of the bacterial assay of methanol extract of *A. paniculata* is also in line with **Shalini and Narayanan (2015)** who observed the antibacterial activity of the extract against selective human pathogens such as *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa*. Among the different solvents employed in their study, methanol extract showed greater antibacterial activity against *E. coli, S. typhi, P. aeruginosa* and *S. aureus*. **Dada-Adegbola *et al.* (2014)** observed antibacterial activity of methanol extract of *A. paniculata* against *S. aureus, E. coli, S. typhi, P. aeruginosa* as **Radha *et al.* (2011)** also revealed the antibacterial proficiency of the methanol extract of *A. paniculata* against *S. pyogenes.*

Minimum inhibitory and minimum bactericidal concentration (mg/ml) of *A. paniculata* leaves methanol extract against bacteria isolated from diabetes patients

The minimum inhibitory concentration (mg/ml) of the *A. paniculata* methanol extract range from 5 mg/ml to 10 mg/ml while the minimum bactericidal concentration (mg/ml) of *A. paniculata* methanol leaf extract against bacteria isolated from diabetes patient range from 10 mg/ml to 20 mg/ml as shown in a Table 6.

Table 6 Minimum inhibitory and minimum bactericidal concentration (mg/ml) of *Andrographis paniculata* leaves methanol extract against bacteria isolated from diabetes patients blood

Bacterial isolates	MIC (mg/ml)	MBC (mg/ml)
Salmonella typhi	10	10
Haemophilus influenza	10	10
Klebsiella pneumoniae	5	10
Staphylococcus aureus	5	10
Salmonella typhimurium	5	50
Escherichia coli	5	10
Pseudomonas aeruginosa	5	10

Key: MIC = Minimum Inhibitory Concentration, MBC= Minimum Bactericidal Concentration

The result of the minimum inhibitory concentration (MBC) of *A. paniculata* observed in this study is in agreement with **Banerjee *et al.*, (2017)** who revealed the antibacterial property of andrographolide; a bioactive ingredient of the methanol extract of A. paniculata against some gram negative organisms implicated in blood samples of diabetes patients including; *E. coli, P. aeruginosa, S. typhimurium, H. influenzae,* and *Klebsiella pneumoniae* with two gram positive bacteria organisms also common with blood specimens of diabetes patients like *S. aureus* and *S. pnenmoniae*.

CONCLUSION

From the result, it was revealed that the methanol leaf extract of *A. paniculata* showed strongest antimicrobial activity for a wide range of bacterial pathogens from diabetes patients blood samples and also more reliable than commercially available antibiotics. Therefore, the result suggests that the leaves of *A. paniculata* possess potential properties and bioactive ingredients in the search for novel anti-diabetes drugs. Further studies on the *Andrographis paniculata* leaves should be carried out to augment the pharmacological and phytochemical proficiency to find new bioactive compounds as well as conservation of this plant.

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Authors' Contributions: FOO designed the study. FOO and AOO jointly supervised the study. LEI developed the methodology and acquired the data. KOA performed the analysis and interpretation of data. OJB and MTB wrote the first draft of the manuscript and managed literature search. FOO and AOO reviewed and revised the manuscript. All authors read and approved the final manuscript.

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