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Antimicrobial Activity of *Andrographis paniculata* against Profiled Multidrug-Resistant Isolates from Asymptomatic Bacteriuria

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Abstract

Asymptomatic bacteriuria (ASB) is the presence of actively multiplying bacteria in urine of any individual without any visible signs or symptoms of urinary tract infection (UTI) such as painful urination, frequent urination, fever, abdominal pain, cloudy or strong smelling urine. In this research on antimicrobial activities of *Andrographis paniculata* against profiled multi drug resistant clinical isolates from asymptomatic bacteriuria, seventy (70) clinical isolates collected from apparently healthy female students of Renaissance University Enugu were used. The isolates were identified and biochemical tests were carried out according to standard methods. The antibiotic susceptibility tests of the isolates was done using the single disk diffusion method according Kirby-Bauer. The leaves of *Andrographis paniculata* were extracted by cold maceration using methanol as the solvent and their phytochemical analysis done. The antimicrobial susceptibility pattern of the extract against the clinical isolates were determined using the agar disk diffusion technique. The minimum inhibitory concentration (MIC) and the Minimum Bactericidal Concentration (MBC) were done using standard method. The plasmid profiling of the 16 multidrug isolates that were resistant to the extract and ciprofloxacin (positive control) was done using standard method. Furthermore, plasmid curing was done using sodium dodecyl sulphate (SDS). Their antibacterial susceptibility testing of the resistant isolates were repeated with the leaf extract to confirm their curing. The result of the study showed that 70 identified isolates occurred in the frequency of 15 *K. pneumoniae*, 10 *S. aureus*, 16 *E. coli*, 5 *E. fecalis*, 2 *P. mirabilis*, 4 *P. aeruginosa*, 7 *E. aerogenes*, 1 *S. saprophyticus*, 2 *S. epidermis*, 8 *E. cloacae*. The antibiotic susceptibility result showed that the isolates showed varying degrees of resistance to Nitrofurantoin, Gentamicin, Cefotaxime, Ofloxacin, Ampiclox, Cefixime, Ceftriaxone Sulbactam, Amoxicillin clavulanate, Nalidixic acid, Cefuroxime, Levofloxacin, Imipenem, The phytochemical analysis of the leaves showed that they contain bioactive compounds such as tannins, alkaloids, saponins, steroids, flavonoids, terpenoids, proteins, carbohydrates, reducing sugars, anthocyanin, and phenols. The extract of *A. paniculata* had the highest invitro antimicrobial activity against *K. pneumoniae* (31.60±0.85mm at 100mg/ml) while the least antimicrobial activity was on *S. aureus* (4.83±0.00 mm at 50mg/ml). The minimum inhibitory concentration of the extract of *A. paniculata* showed inhibition of the bacterial isolates at 3.125mg/ml and 12.5mg/ml for strains of *E. cloacae* and *P. aeruginosa*. The minimum bactericidal concentration of *A. paniculata* was 6.25mg/ml against strains *E. cloacae*. Plasmid profile gel images of 16 selected bacterial isolates that were resistant to extract and ciprofloxacin showed 10 samples out of 16 selected bacterial isolates have mild resistant genes in them. The result of the repeated antimicrobial susceptibility after curing with SDS showed that 8 of the bacterial resistant isolates became susceptible to the extract and or ciprofloxacin to which they previously showed resistance prior to curing while the rest of the selected bacterial isolates were not affected by the curing agent as they still maintained their resistance and this showed that their resistance could be plasmid mediated. The result in our findings showed the relationship of plasmids with antibiotic resistance and that methanol extract of *A. paniculata* has broad-spectrum antimicrobial activity and can serve as natural therapeutic agent against asymptomatic bacteriuria pathogens.

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Keywords: Antimicrobial resistance; Asymptomatic bacteriuria; *Andrographis paniculata*; Plasmid profiling and Plasmid curing

1. Introduction

Asymptomatic bacteriuria (ABU) is the presence of bacteria in urine ($\geq 10^5$ CFU/ml) without any signs or symptoms of urinary tract infections (Nicole *et al.*, 2005). This condition is particularly common among certain populations such as the elderly, pregnant women and individuals with diabetes and is characterized by having a significant number of bacteria in the urine ($\geq 100,000$ CFU/mL) without the classic signs of infection like dysuria (Gupta *et al.*, 2020). The dangers of untreated asymptomatic bacteriuria can lead to misdiagnosis or over diagnosis which exposes bacteria to antibiotics without a justified need and this can enhance the chances of developing resistance as bacteria adapt to the pressure of the antibiotics, share resistant genes with other microorganisms and potentially lead to the spread of resistant strains within the community or healthcare settings. This is because they act as silent reservoirs for the spread of resistant gene. The knowledge of the genetic basis of the antimicrobial resistant especially plasmid mediated mechanisms is essential in controlling this rising global resistant threat.

Antimicrobial Resistance (AMR) occurs when bacteria, viruses, fungi and parasites no longer respond to antimicrobial medicines making infections harder to treat thereby, increasing the risk of disease spread, severe illness and death (WHO, 2014; Ventola, 2015; Prestinaci, 2015). The emergence of these resistant pathogens has been one of the most critical public health problems in the last decades (Doyle, 2015). Humans develop this resistance through genetic mutations, misuse and abuse of antibiotics in the hospitals, other individuals carrying the microbes, livestock, poor infection control, environmental contamination caused by companion animals and use of antimicrobials in agricultural settings as growth enhancers. Antimicrobial resistance by these microorganisms can develop as a result of enzymatic degradation of antibacterial drugs by the microorganisms (Bush *et al.*, 2010), modification of bacterial proteins that are antimicrobial targets (Wilson, 2014), biofilm formation which are made up of complex communities that adhere to surfaces and are resistant to antimicrobials (Stoodley *et al.*, 2009), horizontal gene transfer and changes in membrane permeability to antibiotics (Nordmann *et al.*, 2014). The global rise in antibiotic resistance poses a significant threat, frustrating the efficacy of common antibiotics against widespread bacterial infections. This resistance can be plasmid mediated or maintained on the bacterial chromosome.

Plasmids are independent, circular, self-replicating extra chromosomal DNA elements with characteristic ability to copy numbers within the host and they replicate independently of the bacterial chromosome (Wang *et al.*, 2011). They play a crucial role in the emergence of antibiotic resistance, endangering human health by allowing pathogenic bacteria to pick up numerous resistance genes in a single transfer event (Peterson *et al.*, 2018; Carattoli, 2005). Plasmids are also very important tool in the field of molecular biology and genetics specifically in the area of genetic engineering (Bruce *et al.*, 2002; Bulut *et al.*, 2005). The study of plasmids is important to medical microbiology because apart from encoding genes for antibiotic resistance or virulence factors, plasmids serve as markers of various bacterial strains when a typing system referred to as plasmid profiling or plasmid finger print is used. According to Carattoli (2013), plasmid profile typing has been used to investigate outbreaks of many bacterial diseases and trace inter and intra species spread of antibiotic resistance. Plasmids on bacteria can be removed by a process known as curing.

Plasmid curing is the removal of plasmids from bacteria cells during cell division (Freifeld, 1983). Plasmid curing can be achieved through physical, chemical or biological methods. Chemical agents like acridine orange, ethidium bromide and sodium dodecyl sulphate (SDS). During plasmid curing, the plasmids that code for antibiotics resistance are lost thereby, making the bacteria susceptible to antibiotic attack but if the gene that codes for the antibiotic resistance is chromosomal, then the bacteria will still be resistant after curing (Freifeld, 1983).

In response to the limitations of conventional antibiotics and the global rise of multi-drug resistant bacterial infections, there have been a renewed interest in medicinal plants as an alternative non-toxic therapeutic options. Medicinal plants have been used for centuries as natural remedies for various diseases in humans, animals and they continue to serve as a source of pharmaceutical development (Ghorbani, 2005). They contain bioactive compounds such as alkaloids, flavonoid, terpenoid, tannins and saponins which have therapeutic effects such as antimicrobial, anti-inflammatory, anti-fungal, anti-viral, antioxidant and immune boosting properties (WHO, 2002; Sammy *et al.*, 2000). This study was carried out to determine antimicrobial activities of *Andrographis paniculata* against plasmid profiled multi drug resistant clinical isolates from asymptomatic bacteriuria with the aim of identifying potential therapeutic agents to fight antimicrobial resistance.

2. Materials and methods

2.1. Resuscitation and Standardization of Multidrug-Resistant Bacterial Isolates for Antimicrobial Studies

A total of seventy previously isolated multidrug-resistant bacterial strains, originally obtained from asymptomatic female students of Renaissance University, Enugu, were utilized for this study. These clinical isolates were resuscitated by sub-culturing them in batches onto freshly prepared nutrient agar plates and incubated at 37°C for 24 hours. Following incubation, the bacterial suspensions were standardized to match the 0.5 McFarland turbidity standard, in accordance with Cheesbrough (2006). The McFarland standard was prepared by mixing 99.5 mL of 1% sulfuric acid with 0.5 mL of 1% barium chloride to achieve the desired turbidity, corresponding to an approximate bacterial density of 1.5×10^8 CFU/ml

2.2. Identification and Confirmation of Test Isolates

Bacterial colonies that appeared on the culture plates were counted and expressed as colony-forming units per milliliter (CFU/mL), in accordance with the method described by Cheesbrough (2012). The identification of bacterial isolates was initially based on the observation of colony morphology on appropriate culture media. Distinct characteristics such as colony size, edge, shape, elevation, surface appearance, pigmentation, and consistency were carefully noted to guide the preliminary classification.

Following morphological assessment, bacteriological techniques were applied, beginning with Gram staining to differentiate the isolates based on cell wall composition, distinguishing between Gram-positive and Gram-negative organisms. A motility test was also conducted to determine whether the isolates were capable of movement, often associated with the presence of flagella and useful in differentiating motile from non-motile species.

Further biochemical identification involved the catalase test, which detects the ability of bacteria to break down hydrogen peroxide into water and oxygen, indicated by the formation of bubbles. The urease test was performed to evaluate the ability of isolates to hydrolyze urea into ammonia and carbon dioxide, which raises the pH and causes a color change in the medium. The sugar fermentation test was used to assess the capacity of the bacteria to ferment specific sugars, often accompanied by acid and/or gas production.

In addition, a coagulase test was carried out to differentiate *Staphylococcus aureus* (which produces coagulase, an enzyme that clots plasma) from coagulase-negative *staphylococci*. This test is especially useful in distinguishing pathogenic staphylococci from less virulent strains.

Finally, the findings from all morphological, staining, and biochemical assessments were compared with established criteria in Bergey's Manual of Determinative Bacteriology to confirm the identities of the bacterial isolates.

2.3. Collection of *Andrographis paniculata* plant material

Fresh and healthy leaves of *Andrographis paniculata* were collected from a garden at Nibo in Anambra state. The leaves were identified in the Botany department of Nnamdi Azikiwe University, Awka Anambra state by Dr. C. F. Iroka with Herbarium number NAUH-198^A. The freshly collected leaves were washed with water to remove soil and dirt particles, shredded with a knife and room dried. The dried leaf materials were blended to form a fine powder using an electric blender and stored in airtight bottles.

2.4. Preparation of the Leaf Extract

Bioactive compounds were extracted from *Andrographis paniculata* leaves using the cold maceration method. A conical flask filled with 200 milliliters of methanol was filled with precisely 20 grams of the powdered leaf material. To guarantee even mixing and effective phytochemical extraction, the flask was incubated on a rotating vacuum evaporator for 48 hours after being sealed with a stopper to prevent solvent evaporation. After incubation, the mixture was filtered using Whatman No. 1 filter paper to remove residual plant debris. The resulting filtrate was subjected to gentle evaporation using a water bath maintained at 45°C until complete dryness was achieved. This process helped preserve the integrity of heat-sensitive bioactive compounds. The dried extract was then weighed and stored in sterile, airtight containers for subsequent analyses (Karabi *et al.*, 2015).

2.5. Phytochemical Screening of the Extracted Leaves

Phytochemical screening involves the qualitative detection of bioactive constituents present in plant extracts. In this study, the crude methanolic leaf extract of *Andrographis paniculata* was subjected to preliminary phytochemical

analysis using standard protocols as described by AOAC (2011). The bioactive components tested included tannins, alkaloids, saponins, steroids, flavonoids, terpenoids, phenols, carbohydrates, and proteins. The procedures and indicative observations for each compound are described below.

To test for tannins, 0.2 g of the leaf extract was dissolved in 10 mL of distilled water and filtered. One milliliter of 5% ferric chloride (FeCl_3) was added to the filtrate. A dark blue precipitate indicated the presence of tannins, consistent with the findings of Mann et al. (2008).

For alkaloids, 2 mL of the extract was mixed with Dragendorff's reagent and allowed to stand for 5 minutes. The development of an orange-brown precipitate confirmed the presence of alkaloids (Mann et al., 2008).

The frothing test was employed for detecting saponins. A mixture of 2 mL of the extract and three drops of olive oil was vigorously shaken in a test tube for 2 minutes. The formation of a stable emulsion indicated the presence of saponins (Mann et al., 2008).

Steroids were identified using the Salkowski test. A few drops of concentrated sulfuric acid were added to 1 mL of the extract. The appearance of a reddish-brown ring at the interface was indicative of the presence of steroid compounds.

To detect flavonoids, 2 mL of the extract was treated with two drops of dilute sodium hydroxide. A yellow solution that turned colorless upon the addition of 1% hydrochloric acid indicated the presence of flavonoids (Mann et al., 2008).

For terpenoids, 2 mL of the extract was mixed with thionyl chloride. The development of a pink coloration was suggestive of terpenoid presence (Mann et al., 2008).

The presence of phenols was assessed by adding a few drops of 5% FeCl_3 to 2 mL of the extract in a test tube. A green coloration signified the presence of phenolic compounds.

Carbohydrates were tested using Benedict's reagent. A mixture of 3 mL of the extract and Benedict's reagent was heated in a water bath. The appearance of an orange-red precipitate indicated the presence of reducing sugars (Mann et al., 2008).

Finally, the presence of proteins was determined by mixing 2 mL of the extract with 1 mL of 40% sodium hydroxide and 2 mL of copper sulfate solution. A purple precipitate formation was taken as a positive result for proteins (Mann et al., 2008).

These results provide initial evidence of the potential bioactivity of *Andrographis paniculata* extract and justify further investigation into its pharmacological and antimicrobial properties.

2.6. Preparation of leaf extracts stock concentration

Stock concentration of the *Andrographis paniculata* leaf extract was prepared by weighing and dissolving 0.4 g of each extracts in 4mls of Dimethyl sulfoxide (DMSO) to get 100ml dilution.

2.7. Standardization of the Bacterial Isolates

The seventy (70) multi-drug resistant isolates were sub cultured in a nutrient slant for 24hours. The bacterial density was adjusted to 0.5 McFarland turbidity standards according to Cheesbrough, (2006).

2.8. Antibacterial Assay of the Leaf Extracts Using Disc Diffusion Method

The antimicrobial activity of the leaf extract (*Andrographis paniculata*) was done using disc diffusion method as described by Ekwealor et al., (2016); Geetha et al. (2017) with slight modification. A 20mls of Muller Hinton agar was prepared as described by Tagoe et al. (2011); Onifade et al. (2018) and was allowed to set. A 1ml of each standardized 70 pure multi-drug bacterial isolates were put in each of the agar plates and the inoculation done by spread plate method using a swab stick. Sterile filter paper discs loaded with different concentration of the extracts (100mg/ml, 50mg/ml and 25mg/ml) were introduced into each of the bacterial inoculated plates using disc diffusion method. Sterile filter paper discs seeded with Dimethyl sulfoxide (DMSO) was used as the negative control while sterile filter paper discs seeded with 1 μ g of ciprofloxacin was used as the positive control. The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the zones of inhibition around each disk was measured and recorded in millimeter representing the antimicrobial activities of the extracts (Ahmad et al., 2001). This was compared with Clinical and

Laboratory Standards Institute's standard for sensitivity or resistance (CLSI, 2017). The mean zone of the inhibition was calculated, recorded and interpreted as susceptible, intermediate or resistant using CLSI (2009) guideline

2.9. Determination of Minimum Inhibitory Concentration (MIC) Of the Leaf Extracts

The minimum inhibitory concentration (MIC) is the lowest concentration able to inhibit any visible bacterial growth (Balouiri *et al.*, 2016, CLSI, 2020). This was done using tube dilution method with Mueller Hinton broth (Isunu *et al.*, 2022). A series of Muller Hinton broths (2mls) prepared according to the manufacturer's instructions were put in test tubes and sterilized using an autoclave at 121°C for 15 minutes. A 2mls of double fold serial dilution of the two leaf extracts were done (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml, 3.125mg/ml, 1.56mg/ml) using DMSO as the solvent as described by Iqbal *et al.* (2001). Each of the test tubes containing mixtures of Muller Hinton broth and different diluted leaf extract concentrations were inoculated with 0.1ml standardized bacterial isolates that were susceptible to the *A paniculata* leaf extract. They were incubated at 37°C for 24 hours and then examined for growth by observing turbidity (Kaya *et al.*, 2012). Tubes containing both Muller Hinton agar broth and DMSO served as control. The lowest dilution of the extracts that yielded no single bacterial colony was taken as the minimum inhibitory concentration (Akinyemi *et al.*, 2005).

2.10. Determination of Minimum Bactericidal Concentration (MBC) Using Broth Diffusion

The minimum bactericidal concentration (MBC) is the lowest concentration of an antibacterial agents required to kill a bacteria over a period of time such as 18-24hrs. It was determined by sub culturing the broth dilution of the MIC testing to the highest concentration. The MBC were carried out according to CDC. (2019), from each negative tube to the MIC assay. 1 ml was transferred into the surface of a freshly prepared nutrient agar plates (without the test agents) using spread plate method and incubated at 25°C for 48hrs. The lowest concentration of MIC tubes with no visible bacterial growth on the nutrient agar plates were regarded as the minimum bactericidal concentration (Pallet, & Hand, 2011).

The MBC is complimentary to the MIC, whereas MIC test determines the lowest level of antibacterial agents that greatly inhibits microbial growth, the MBC demonstrates the lowest level of antimicrobial agents resulting in microbial death.

2.11. Plasmid Profiling

This is a molecular technique used to characterize and identify plasmid DNA present in bacteria isolates. 16 samples of the bacterial isolates which were resistance to leaf extract (*Andrographis paniculata*) and the positive control (ciprofloxacin) were taken for profiling. The plasmid Profiling was done at CHISCO Research Institute at Nnamdi Azikiwe University, Awka, and Anambra State.

Plasmid DNA extraction was carried out employing the standard alkaline lysis protocol delineated by Sambrook *et al.* (1989). Three complex solutions such as solution 500 µl of each sample was added to a micro centrifuge tube and centrifuged at full speed for 20 seconds and the resulting supernatant was discarded. The bacteria pellets were re-suspended with 250µl of ZymoPURE™ P1 (red) and completely mixed by vortexing. 250µl of ZymoPURE™ P2 (green) was added and immediately mixed by gently inverting the tube 6-8 times and allowed to sit at room temperature for 2-3 minutes. 250µl of ice cold ZymoPURE™ P3 (yellow) was added and mixed thoroughly by inversion. The sample was then left to incubate on ice for 5 minutes. The sample was then centrifuged at 16000xg for 5 minutes. 600µl of the supernatant was carefully transferred into a new 1.5ml micro centrifuge tube. 275µl of ZymoPURE™ binding buffer was added to the transferred supernatant and mixed thoroughly by inverting the tube 8 times. The entire mixture was then transferred to a Zymo-spin™ II-P column in a collection tube and incubated at room temperature for 2 minutes before centrifuging at 5000xg for 1 minute after which the flow through was discarded. 800µl of ZymoPURE™ wash 1 was added to the Zymo-spin™ II-P column and centrifuged for 5000xg for 1 minute after which the flow through was discarded. 800µl of ZymoPURE™ wash 2 was added to the Zymo-spin™ II-P column and centrifuged for 5000xg for 1 minute after which the flow through was discarded. 200µl of ZymoPURE™ wash 2 was added to the Zymo-spin™ II-P column and centrifuged for 5000xg for 1 minute after which the flow through was discarded. The Zymo-spin™ II-P column was centrifuged at ≥10000xg for 1 minute in order to remove any residual wash buffer. The Zymo-spin™ II-P column was transferred to a clean 1.5ml tube and 25µl of ZymoPURE™ Elution Buffer was added directly to the column matrix and allowed to incubate at room temperature for 2 minutes and then centrifuged at ≥10000xg for 1 minute. The eluted plasmid DNA was then stored at ≤ -20°C.

Finally, the plasmid DNA was visualized performing 1.5% agarose gel electrophoresis using ethidium bromide as a staining agent under UV light (Naraian *et al.*, 2012).

2.12. Plasmid Curing

The Isolates that were positive for the plasmid genes were subjected to standard curing method using Sodium dodecyl sulphate (SDS). An overnight culture (0.1ml) was inoculated into 9 ml of freshly prepared nutrient broth and incubated for 4hrs for the minimal growth of microorganisms (Uba *et al.*, 2018). 1ml of SDS which is the curing agent used was added sufficiently to the mixture to bring the concentration to 10% and then incubated for 24hrs at 37°C. The cured organisms (0.1ml) were sub cultured on a sterile nutrient agar plates and incubated at 37°C for 24hrs. Susceptibility testing using the *A. paniculata* leaf extract and ciprofloxacin as control were repeated for the bacterial isolates that showed resistance previously to them prior to curing. The changes in resistant patterns were noted.

2.13. Statistical Analysis

Data was analyzed using statistical package for social sciences (SPSS) software version 21.0 Inc. Chicago, USA was used for the analysis of variance (ANOVA). A value of $P < 0.05$ was considered significant. Triplicate determinations were carried out and standard errors were calculated for all results.

3. Results

Table 1 shows the morphology and biochemical characterization of the clinical isolates that enhanced their identification. The table shows shapes, biochemical results and identities of the clinical isolates while table 2 shows the frequency of occurrence of the clinical isolates and their percentage calculation.

Table 1 Biochemical of the Bacterial Isolates from Asymptomatic Bacteriuria

Isolate Codes	GS	CAT	IND	UR	COAG	MOT	CIT	OXI	ISOLATES IDENTITIES
28F	-	+	-	+	-	+	+	-	<i>Proteus mirabilis</i>
29F	+	+	-	+	-	-	-	-	<i>Staphylococcus saprophyticus</i>
41F	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
42F	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
44F	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
54F	-	+	-	-	-	+	+	-	<i>Enterobacter cloacae</i>
60FA	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
60FB	+	+	-	-	+	-	-	-	<i>Pseudomonas aeruginosa</i>
61M	+	-	-	-	-	-	-	-	<i>Enterococcus faecalis</i>
62M	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
63F	+	-	-	-	-	-	-	-	<i>Enterococcus faecalis</i>
67F	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
68FA	-	+	-	+	-	+	+	-	<i>Proteus mirabilis</i>
68FB	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
70FA	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
73M	-	+	-	+	-	-	+	-	<i>Enterobacter cloacae</i>
74M	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
80F	+	+	-	-	-	-	-	-	<i>Staphylococcus epidermidis</i>
81F	-	+	-	+	-	-	+	-	<i>Enterobacter cloacae</i>
82F	-	+	-	-	-	+	+	-	<i>Enterobacter aerogenes</i>
84F	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
85F	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>

87F	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
92F	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
95F	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
98F	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
101F	-	+	-	+	-	-	+	-	<i>Enterobacter cloacae</i>
102F	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
105F	-	+	-	-	-	+	+	-	<i>Enterobacter aerogenes</i>
121F	+	-	-	-	-	-	-	-	<i>Enterococcus faecalis</i>
128F	+	+	-	-	+	-	-	-	<i>Pseudomonas aeruginosa</i>
131M	+	-	-	-	-	-	-	-	<i>Enterococcus faecalis</i>
155M	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
156M	-	+	-	-	-	+	+	-	<i>Enterobacter aerogenes</i>
157F	-	+	-	+	-	-	+	-	<i>Enterobacter cloacae</i>
162M	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
175M	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
178F	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
179F	-	+	-	+	-	-	+	-	<i>Enterobacter cloacae</i>
181M	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
187F	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
192F	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
193F	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
198F	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
211M	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
240M	-	+	-	+	-	-	+		<i>Klebsiella pneumoniae</i>
245M	+	-	-	-	-	-	-	-	<i>Enterococcus faecalis</i>
258F(018F)	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
263F	+	+	-	-	+	-	-	-	<i>Pseudomonas aeruginosa</i>
295F(55F)	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
302M	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
312F(72F)	+	+	-	-	+	-	-	-	<i>Pseudomonas aeruginosa</i>
318F(78F)	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
319F(79F)	-	+	-	-	-	+	+	-	<i>Enterobacter aerogenes</i>
320FB	+	+	-	-	+	-	-	-	<i>Pseudomonas aeruginosa</i>
323F	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
325M(085M)	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
326M	+	+	-	-	+	-	-	-	<i>Staphylococcus aureus</i>
330M (090F)	-	+	-	+	-	-	+	-	<i>Enterobacter cloacae</i>
335M(095M)	-	+	-	-	-	+	+	-	<i>Enterobacter aerogenes</i>

345F(0136F)	-	+	-	+	-	-	+	-	<i>Klebsiella pneumonia</i>
364F									<i>Escherichia coli</i>
387M(0147M)	-	+	-	-	-	+	+	-	<i>Enterobacter aerogenes</i>
418F (0178M)	-	+	-	+	-	-	+	-	<i>Klebsiella pneumoniae</i>
419F (0179M)	-	+	-	+	-	-	+	-	<i>Enterobacter cloacae</i>
432F	+	+	-	-	-	-	-	-	<i>Staphylococcus epidermidis</i>
459F	-	+	-	-	-	+	+	-	<i>Enterobacter aerogenes</i>
0413F	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>
444M(0175)	-	+	-	-	-	+	+	-	<i>Enterobacter aerogenes</i>
(0136M) (453M)	-	+	+	-	-	+	-	-	<i>Escherichia coli</i>

Key: Positive (+) = Presence; Negative (-) = Absence; GR= Gram reaction; OX= Oxidase; CAT; Catalase; Coag= Coagulase; Ind= Indole; Mot= Motility; UR= Urease

Table 2 Frequency of Occurrence of the Bacterial Isolates from Asymptomatic Bacteriuria

Names of organism	Frequency of occurrence	% Frequency (n=100)
<i>Klebsiella pneumonia</i>	15	21.43%
<i>Staphylococcus aureus</i>	10	14.28%
<i>Escherichia coli</i>	16	22.86%
<i>Enterococcus faecalis</i>	5	7.14%
<i>Proteus mirabilis</i>	2	2.86%
<i>Pseudomonas aeruginosa</i>	4	5.71%
<i>Enterobacter aerogenes</i>	7	10.00%
<i>Staphylococcus saprophyticus</i>	1	1.43%
<i>Staphylococcus epidermis</i>	2	2.85%
<i>Enterobacter cloacae</i>	8	11.43%
Total	70	100

Table 3 shows the phytochemical analysis of *Andrographis paniculata* leaf extracts. The table shows that *A. paniculata* contains tannins, saponins, phenols, steroids, alkaloids, terpenoids, and flavonoids while table 4 depicts the antimicrobial susceptibility patterns of the different concentration *Andrographis paniculata* against MDR bacteria isolates at 100mg/ml, 50mg/ml and 25mg/ml. The table shows that more 20 Of the multidrug resistant (MDR) isolates became susceptible to *A. paniculata*. The *P*-value was 1.53E-11 (0.000000000153) which is significant (≤ 0.05).

Table 3 Phytochemical Analysis of *Andrographis Paniculata* Leaf Extracts

Parameters	Experimental method	<i>Andrographis paniculata</i>
Alkaloids	Dragendorff' test	++
Phenols	5 % FeCl ₃	++
Flavonoids	Alkaline test	++
Tannis	Ferric Chloride	++
Saponnins	Foam test/ Froting test	++
Steroids	Salkowski's test	+
Terpenoids		+++
Protein		+
Reducing sugar	Benedict's test	++
Carbohydrate		++
Anthocyanin		-

KEYS: + =presence in trace quantities; ++ = present in moderate high concentration; +++ = present in high concentration; - =not detected

Table 4 Antibacterial Susceptibility Patterns of the Isolates (>10⁵cfu/ML) From Asymptomatic Bacteriuria Using *Andrographis Paniculata* Leaf Extract

Isolates	Zones of Inhibition (mm)				
	<i>Andrographis paniculata</i> leaf extract (mg/ml)			Ciprofloxacin. (positive control)	DMSO (negative control)
	100	50	25		
29F	-	-	-	45.00±2.00	-
41F	-	-	-	30.00±0.00	-
42F	-	-	-	15.00±1.00	-
44F	13.00±0.50	-	-	30.00±0.00	-
60FA		-	-	9.00±1.00	-
70FA	18.00±0.50	4.83±0.00	-	12.33±1.04	-
80F	22.87±0.40	17.00±1.04	-	15.00±0.50	-
82F	-	-	-	6.67±0.76	-
84F	18.00±1.04	-	-	23.00±1.00	-
85F	24.57±0.35	15.00±0.50	-	37.83±0.29	-
98F	-	-	-	25.00±2.52	-
101F	-	-	-	48.00±0.00	-
128F	-	-	-	25.00±2.00	-
131M	26.50±1.30	18.00±0.40	-	21.00±1.30	-
157F	-	-	-		-
181F	18.12±0.35	12.00±0.50	-	22.00±2.00	-

211M	-	-	-	12.00±0.35	-
240M	31.60±0.85	15.00±0.50	-	38.00±2.00	-
263M (23F)	-	-	-	20.00±1.00	-
295M (55F)	-	-	-	34.00±1.00	-
302M (62M)	-	-	-	14.00±0.06	-
312F	-	-	-	9.93±0.12	-
319F	-	-	-	40.12±0.35	-
323F	19.83±1.63	14.33±0.58	13.00±0.00	22.67±1.23	-
54F	20.17±0.29	17.33±1.15	-	12.12±0.31	-
60FB	-	-	-	15.01±0.60	-
62M	22.67±0.76	14.83±0.29	13.00±0.30	35.83±0.76	-
68FB	26.10±0.36	10.00±0.30	-	14.00±0.00	-
73F	20.20±0.70	10.00±0.00	-	29.83±0.76	-
81F	21.57±0.49	10.00±0.20	-	29.83±0.29	-
92F	11.07±0.60	5.03±0.65	-	24.43±0.74	-
121F	-	-	-	19.90±0.17	-
155M	-	-	-	29.87±0.23	-
156F	11.93±0.80	10.00±0.40	-	47.00±0.97	-
162M	-	-	-	13.83±0.06	-
318M (078F)	-	-	-	24.97±0.06	-
320FB	14.7±0.35	10.97±0.45	5.00±0.30	20.00±1.04	-
0326M	-	-	-	20.00±0.30	-
0136F (345M)	-	-	-	19.98±0.27	-
325M (085F)	-	-	-	11.07±0.60	-
335M (095M)	-	-	-	11.47±0.84	-
0413F	-	-	-	30.43±0.93	-
459M (0178M)	-	-	-	18.34±67	-
178F	-	-	-	12.97±0.06	-
192F	27.73±0.92	20.03±0.75	10.07±0.40	38.00±.00	-
330M(090F)	-	-	-	20.77±0.80	-
282FB	10.10±0.66	-	-	40.23±0.46	-
0175M (415F)	-	-	-	15.07±0.80	-
74M	-	-	-	22.06±0.40	-
61M	-	-	-	12.47±0.59	-

73F	-	-	-	21.33±0.97	-
157F	-	-	-		-
193F	20.00±0.00	-	-	14.50±0.87	-
0147M(387)	25.00±1.73		-	10.90±0.40	-
95F	-	-	-	19.83±0.65	-
67M	-	-	-	24.07±0.61	-
0179M (419F)	17.00±1.83	-	-	25.12±0.78	-
198F	30.10±0.87	14.97±0.06	10.33±0.76	21.03±0.61	-
018F (258F)	20.77±0.70	15.00±0.00	12.00±0.20	20.77±0.15	-
187F	20.33±1.80	-	-	24.70±0.80	-
121F	-	-	-	20.10±0.46	-
102F	-	-	-	14.37±0.42	-
28F	13.33±1.53	13.67±1.15	-	10.07±0.40	-
325M (085M)	27.00±0.60	21.00±0.90	13.30±0.96	30.27±0.59	-
245M	-	-	-	20.73±21	-
432F	-	-	-	22.73±4.5	-
162M	20.00±1.04	-	-	15.20±0.46	-
60FA	-	-		10.13±35	-

KEY: Values and means (n=3) of triplicate analysis ± standard deviation; - = **Not detected**, *P*-value is 1.53E-11 (0.0000000000153); *P*- Value < 0.05

Table 5 shows the minimum inhibitory concentration (MIC) of the *Andrographis paniculata* leaf extract against the different bacteria isolates at various concentration of 200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.75mg/ml, 3.125mg/ml, 1.56mg/ml. The table shows that the MIC of *A. paniculata* was 3.125mg/ml and 12.5mg/ml against *E. cloacae* and *P. aeruginosa* respectively while Table 6 shows the minimum bactericidal concentration (MBC) of the *Andrographis paniculata* leaf extract on the selected MDR isolates from the result of the MIC of *A. paniculata*. The MBC of *A. paniculata* was 6.25mg/ml

Table 5 Minimum Inhibitory Concentration (MIC) Of *Andrographis Paniculata* Leaf Extract against the Bacterial Isolates

Isolates	Concentration (mg/ml)							
	200	100	50	25	12.5	6.25	3.125	1.56
0147M	-	+	+	+	+	+	+	+
018F	-	+	+	+	+	+	+	+
085M	-	+	+	+	+	+	+	+
44F	-	+	+	+	+	+	+	+
70FA	-	+	+	+	+	+	+	+
73M	-	-	-	+	+	+	+	+
80F	-	-	-	-	+	+	+	+
84F	-	-	+	+	+	+	+	+

85F	-	-	-	+	+	+	+	+
090M	-	-	-	-	-	-	-	+
92F	-	-	-	+	+	+	+	+
95F	-	+	+	+	+	+	+	+
121F	-	-	-	+	+	+	+	+
131M	-	-	-	+	+	+	+	+
156M	-	-	-	+	+	+	+	+
181M	-	-	-	+	+	+	+	+
187M	-	-	+	+	+	+	+	+
198F	-	-	+	+	+	+	+	+
240M	-	-	+	+	+	+	+	+
245F	-	-	+	+	+	+	+	+
192F	-	-	+	+	+	+	+	+
295F	-	+	+	+	+	+	+	+
302M	-	-	+	+	+	+	+	+
320FB	-	-	-	-	-	+	+	+
459F	-	+	+	+	+	+	+	+

KEY: + = Presence of growth (turbidity in test tubes); - = No visible growth (no turbidity in test tube)

Table 6 Minimum Bactericidal Concentration of *Andrographis Paniculata* Leaf Extracts against the Bacterial Isolates

Isolates	Concentration (mg/ml)							
	200	100	50	25	12.5	6.25	3.125	1.56
0147M	-	+	+	+	+	+	+	+
018F	-	+	+	+	+	+	+	+
085M	-	+	+	+	+	+	+	+
44F	-	+	+	+	+	+	+	+
70FA	-	+	+	+	+	+	+	+
73M	-	-	+	+	+	+	+	+
80F	-	-	-	+	+	+	+	+
84F	-	-	+	+	+	+	+	+
85F	-	-	+	+	+	+	+	+
090M	-	-	-	-	-	-	+	+
92F	-	-	+	+	+	+	+	+
95F	-	+	+	+	+	+	+	+
121M	-	-	-	+	+	+	+	+
131M	-	-	+	+	+	+	+	+
156M	-	-	+	+	+	+	+	+

181M	-	-	+	+	+	+	+	+
187M	-	+	+	+	+	+	+	+
198F	-	+	+	+	+	+	+	+
240M	-	+	+	+	+	+	+	+
245F	-	+	+	+	+	+	+	+
192F	-	+	+	+	+	+	+	+
295F	-	+	+	+	+	+	+	+
302F	-	+	+	+	+	+	+	+
320FB	-	-	-	-	+	+	+	+
459F	-	+	+	+	+	+	+	+

KEY: + = Presence of growth, - = No visible growth

Table 7 shows the plasmid profile of the multi-drug resistant isolates using the commercial kit method while Table 8 Antibacterial Activity of *A. paniculata* extract and Ciprofloxacin against the 10 Selected Profiled Multi Drug Resistant Isolates Prior to and after curing with SDS. The table shows that seven (8) of the profiled MDR isolate were cured by SDS as they became susceptible to ciprofloxacin and the *A. paniculata* leaf extract in this frequency: (1) bacterial isolate became susceptible to *A. paniculata* only, (6) bacterial isolates were susceptible to ciprofloxacin only and (1) bacterial isolate was susceptible to *A. paniculata* and ciprofloxacin. Bacterial isolates that displayed clear changes in resistant patterns to *Andrograhis paniculata*, and or Ciprofloxacin after curing were regarded as bearing their resistant factors in their plasmids.

Table 7 The Plasmid Profile of the 17 Selected Multi Drug Resistant Isolates

S/N	Isolate codes	Plasmids	Isolates identity
1	42F	Positive	<i>Staphylococcus aureus</i>
2	54F	Positive	<i>Enterobacter cloacae</i>
3	60FA	Positive	<i>Klebsiella pneumoniae</i>
4	62M	Positive	<i>Staphylococcus aureus</i>
5	74M	Positive	<i>Escherichia coli</i>
6	82F	Positive	<i>Enterobacter aerogenes</i>
7	95F	Positive	<i>Klebsiella pneumoniae</i>
8	102F	Negative	<i>Klebsiella pneumoniae</i>
9	157F	Negative	<i>Enterobacter cloacae</i>
10	162F	Positive	<i>Escherichia coli</i>
11	211M	Negative	<i>Escherichia coli</i>
10	245F	Negative	<i>Enterococcus faecalis</i>
12	302M	Positive	<i>Klebsiella pneumoniae</i>
13	312F	Negative	<i>Pseudomonas aeruginosa</i>
14	459M	Positive	<i>Enterobacter aerogenes</i>
15	0136F	Negative	<i>Enterobacter aerogenes</i>
16	0175M	Negative	<i>Escherichia coli</i>

Table 8 Antibacterial Activity of *A. paniculata*, and Ciprofloxacin against the 10 Selected Profiled Multi Drug Resistant Isolates Prior to and after curing with SDS

S/N	Isolate codes	<i>Andrographis paniculata</i>			Ciprofloxacin			Isolate identities
		BC	AC	ZI (mm)	BC	AC	ZI (mm)	
1	42F	R	S	20	R	R	R	<i>S. aureus</i>
2	54F	R	R	-	R	S	15	<i>E. cloacae</i>
3	60FA	R	R	-	S	S	35	<i>K. pneumonia</i>
4	62M	R	R	-	S	S	30	<i>S. aureus</i>
5	74M	R	R	-	S	S	40	<i>E. coli</i>
6	82F	R	R	-	R	R	-	<i>E. aerogenes</i>
7	95M	R	R	-	S	S	29	<i>K. pneumonia</i>
8	162M	R	R	-	S	S	39	<i>E. coli</i>
9	302M(102F)	R	S	9.7	S	S	22	<i>K. pneumonia</i>
10	450M	R	R	-	R	R	-	<i>E. aerogenes</i>

KEY: BC= Before Curing, Ac= After Curing, ZI= Zones of Inhibition; R= Resistance, S= Susceptible, - = no inhibition detected

4. Discussion

Multi drug resistance by asymptomatic bacteriuria poses a serious threat and has become a global health concern. Therefore, understanding the resistant pattern and finding a suitable, non-harmful alternative therapy has become imperative in winning this war. The following organisms were isolated; *Klebsiella pneumoniae* (15), *Staphylococcus aureus* (10), *Escherichia coli* (16), *Enterococcus fecalis* (5), *Proteus mirabilis* (2), *Pseudomonas aeruginosa* (4), *Enterobacter aerogenes* (7), *Staphylococcus saprophyticus* (1), *Staphylococcus epidermis* (2), *Enterobacter cloacae* (8). The frequency of occurrence of the bacteriuria as indicated in the result showed that *E.coli* was predominant and this correlates with the findings of Ayodele *et al.* (2013); Adikwu *et al.* (2020), who opined that the high presence of *E.coli* could be attributed to the fact already established that the organism is a commensal found in the gut but disagreed with work of Ekwealor *et al.* (2016) where *S. aureus* was found to be the predominant and most frequently isolated urinary pathogen in antimicrobial evaluation of bacterial Isolates from Urine Specimen of Patients with Complaints of Urinary Tract Infections in Awka, Nigeria.

With rising cases of antibiotic resistance, adverse effects of synthetic drugs, non-affordability and non-accessibility of prescribed antibiotics especially in developing world, the need to use indigenous plants extract has become imminent to combat resistant pathogens (Williams, 2016 Anibijuwon *et al.*, 2017). Leaf extracts of some medicinal plants have been proven experimentally to have a wide spectrum of pharmacological activities.

Andrographis paniculata has a long history of being utilized as an oriental and Ayurvedic medicine. Phytochemical analysis of *Andrographis paniculata* showed that they contain alkaloids, tannins, saponins, terpenoids, steroids, etc. which conferred on them their antimicrobial ability and this agreed with works done by Nnaebue *et al.* (2024); Nwanjo *et al.* (2006). In addition, Mastan *et al.* (2023) opined that phenols and phenolic acids are among the simplest bioactive phytochemicals known to be toxic to microorganisms and the mechanisms thought to be responsible for phenolic toxicity to microorganisms include enzyme inhibition by the oxidized compounds. This is possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins. Also, according to Aiyelaagbe *et al.* (2009), flavonoids have been referred to as nature's biological response modifiers, because of their inherent ability to modify the body's reaction to allergies and virus. Flavonoids has anti-allergic, anti-inflammatory, anti-microbial and anti-cancer activities. Alkaloids have been used to treat diseases like malaria, pain killers and managing heart diseases. (Oomah, 2013).

Antimicrobial bioassay using methanol leaf extracts showed that 68.57% of the Isolates which were resistance to the antibiotic discs used previously were susceptible to the leaf extracts. The antibacterial action of the extracts could be attributed to the presence of phenolic compound detected in the plant extract which included saponins, tannins, alkaloid etc. The result of the anti-bacterial assay of *A. paniculata* showed activity against strains of *Staphylococcus aureus*,

Klebsiella pneumoniae, *Enterococcus cloacae*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and this agrees with the work of Radha *et al.* (2011); Dubey *et al.* (2019), who 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 pyrogenes*. The methanol extract of *A. paniculata* has the highest invitro antimicrobial activity against *Klebsiella pneumoniae* (31.60±0.85 mm at 100mg/ml) while the least antimicrobial activity was on *Staphylococcus aureus* (4.83±0.00 mm at 50mg/ml). There was observed significant differences ($P \leq 0.05$) in different concentration of the extracts. This shows that the antibacterial activity of *A. paniculata* is concentration dependent and it supports its use as an antibacterial agent. The MIC of the methanol extract of *A. paniculata* inhibited the growth of the bacterial isolates at 3.125mg/ml and 12.5mg/ml for strains of *Enterococcus cloacae* and *Pseudomonas aeruginosa* respectively and this agrees with the work of Isunu *et al.* (2002) on antibacterial activity of *Andrographis paniculata* methanol leaf extract on bacteria consortia isolated from blood of diabetic patients. The minimum bactericidal concentration (MBC) of *A. paniculata* was 6.25mg/ml against strains of *Enterococcus cloacae* and this agrees with work of Isunu *et al.* (2002); 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; *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Hemophilium influenza*, and *Klebsiella pneumoniae* with two gram positive bacteria organisms also common with blood specimens of diabetes patients like *Staphylococcus aureus* and *Streptococcus pneumoniae*. The result shows that *Andrographis paniculata* can be used against clinical isolates of asymptomatic bacteriuria and this may be directly proportional to the source of the leaves, drying process used, diluent and solvent used.

The plasmid profiling of the selected sixteen (16) MDR bacterial isolates that were resistance to *Andrographis paniculata*, and ciprofloxacin (positive control) was done. The results from the gel images showed 10 samples out of 16 selected bacterial isolates (162F 302M, 459M, 42F, 54F, 60FA, 62M, 74M, 82F and 95F) had mild resistant genes in them. Though there was no relation between the antibacterial resistant pattern and the plasmid profile analysis. Rozwandowicz *et al.* (2018) opined that these self-governing DNA molecules (plasmids) can transmit between cells and turn on a portion of the chromosome via high-recurrence recombination. Plasmids often carry genes for antibiotic resistance or virulence, so their removal can help study bacterial genetics or reduce undesirable traits. The profiled MDR bacteria isolates that showed mild resistance were subjected to curing by the use of sodium dodecyl sulfate (SDS) and their antibacterial susceptibility testing repeated with leaf extracts and ciprofloxacin (positive control). The result showed that the seven(8) MDR profiled isolates became susceptible to the extracts after curing with SDS in this frequency: one (1) bacterial isolate became susceptible to *A. paniculata* only, (6) bacterial isolates were susceptible to ciprofloxacin only and (1) bacterial isolate was susceptible to *A. paniculata* and ciprofloxacin which they previously showed resistance prior to curing while the rest of the selected MDR bacterial isolates were not affected by the curing agent as they still maintained their resistance to the leaf extracts. Analysis of plasmids and their functions provide essential insight into antibiotic resistance while genotyping and other molecular characterization of plasmids could help determine the reasons for the presence of antibiotic-resistant gene. Ahmed *et al.* (1993) opined that the greater the susceptibility with post plasmid curing when compared to pre-plasmid curing proved that plasmids harbor inhibitory actions against antimicrobials and this is in line with our study on antimicrobial activities of *Andrographis paniculata* against profiled plasmid multi-drug resistant isolates from asymptomatic bacteriuria.

5. Conclusion

The incidence of antibiotic resistance is increasing and has become a public health concern. Our study confirmed the presence of phytochemicals such as alkaloids, saponins, flavonoids, tannin and anthocyanin in *Andrographis paniculata* which confers on them antimicrobial activities. The bioactive compounds from this extract has potential application in the development of antimicrobials, antioxidants, anti-inflammatory and other disorders.

The partial or complete elimination of plasmids from the treated bacterial isolates from our result obtained in this study indicates that these leaf extracts contain bioactive compounds that can compromise plasmid stability and replication. This may lead to the development of a potential antibiotics against *asymptomatic bacteriuria* from the extract of *A. paniculata* leaves in combating the growing antibiotic resistant infections.

Recommendation

I recommend deep translational research in herb use and funds provided for more researches to be carried out in order to determine the various solvents for extraction for these leaves. Further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activeness of these plant parts and standardization of the required dosage for safe consumption. In addition, more than one extraction method is recommended during studies

on plant extract to ensure all bioactive components of the plant are recovered and additional work is encouraged to elucidate the possible mechanism of action of these extracts.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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