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Synergistic Antibacterial Effects of Leaf Extracts of *Acacia nilotica* and *Psidium guajava* against Extended Spectrum Beta Lactamase (ESBL) Producing *E. coli* and *Klebsiella pneumoniae*

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ABSTRACT

The World Health Organization (WHO) recognizes traditional medicine, particularly plant medicine as an important alternative healthcare delivery system for most of the world's population. The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants. Hence, the combined antibacterial effects of leaf extracts of *A. nilotica* and *P. guajava* against ESBLs-producing *E. coli* and *K. pneumoniae* were investigated. Fresh leaves of the plants were collected, processed, and then subjected to three different organic solvents (Methanol, Dichloromethane, and N-Hexane) with varying polarities (high, moderate and low) and water extraction through the maceration method. The various concentrations of the leaf extracts of both plants were subjected to antimicrobial susceptibility testing individually and in combination using agar well diffusion technique. The MIC and MBC were determined according to standard microbiological protocols. The phytochemical analyses revealed the presence of bioactive compounds with exception of anthraquinone and reducing sugars in methanol leaves extracts (ALME+PLME). Methanol extracts of both plants showed highest antibacterial activity against the isolates. The MIC of the plants part was detected at 2.5% and 5% (mg/ml) while the MBC was detected at 5% and 10% (mg/ml). The findings prove that the plants part could be useful in new natural antimicrobial drug development which could serve as potential treatment for the infections caused by the test organisms.

Keywords: Synergy, Antibacterial Effects, Leaves Extracts, *Acacia nilotica*, *Psidium guajava*,

ESBL *E. coli*, ESBL *K. pneumoniae*

INTRODUCTION

The World Health Organization (WHO) recognizes traditional medicine, particularly plant medicine, as an important alternative healthcare delivery system for most of the world's population. In Ghana, traditional medicine, especially plant medicine, provides many citizens with affordable healthcare services. Nyarko et al (2005) reported that traditional medicine, especially plant medicine in Ghana provides many citizens with affordable healthcare services. Since

prehistoric times man has used plants for various purposes and he will continue to do so as long as life continues on this planet. (Abbiw, 1990). Man's symbiotic relationship over time with plants has given the world many invaluable benefits. Apart from the raw materials that go from our variety of foods, the most important plant products are medicines, cosmetics, and flavour products, as well as other pharmaceuticals. (Sofowora, 1996). Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi-synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products. The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted worldwide to prove antimicrobial activities from medicinal plants (Paz *et al.,* 1995;). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (Santos *et al.,* 1995) medicinal plants would be the best source to obtain a variety of drugs. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils as well as tannin (Saxena *et al.,* 1994). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. Among the medicinal plants *Azadirecta Indica* and *Psidum gujava are* rich in antibacterial activity. The use of plant materials to prevent and treat infectious diseases successfully over the years has attracted the attention of scientists worldwide (Falodun *et al*., 2006). *Acacia nilotica* (bagaruwa in Hausa) has been designated and used as medicinal plant in parts of Northern Nigeria, West Africa, North Africa, and other parts of the world. The plant is used to treat infections such as diarrhea, dysentery, leprosy, cancers, ulcer, and diabetes (Aliyu, 2006). Antimicrobial drug resistance is not only on the increase; it is also a serious problem to the medical profession. The Guava tree, *Psidium guajava* Linnaeus belongs to the family Myrtacae, is a tropical hardwood plant that can reach a height of 10m. It is considered native of Mexico and extends to throughout South America, Europe, Africa and Asia. Guava is used medicinally in many parts of the world as anti-inflammatory, and antiseptic as well as in the treatment of diabetes, hypertension, pain, fever, respiratory disorders, gastroenteritis, diarrhoea and dysentery (Nwinyi *et al*., 2008).

Beta-lactamases are enzymes produced mostly by Gram-negative bacteria. They are often responsible for resistance to β-lactam antibiotics by organisms possessing them (Bush *et al.,* 1995). ESBLs are chromosomal or plasmid-mediated and occur as a result of spontaneous mutations that take place in the serine active site of the old beta lactamase enzyme, adding 4-6 new amino acids that extend their hydrolytic substrate (Steward *et al*., 2001). These enzymes mediate resistance to oxymino-cephalosporins (ceftriaxone, ceftazidime, cefotaxime, cefepime) and monobactams (aztreonam) with the exception of carbapenems. The first β-lactamase with extended-spectrum was detected in *Klebsiella pneumoniae* in Garmany in 1983 (Knothe *et*

al., 1983) and later in the western parts of Europe, probably because extended-spectrum β-lactam antibiotics were first used there clinically. They occur predominantly in members of the Enterobacteriaceae with *Klebsiella pneumoniae* and *Escherichia coli* being the most commonly reported worldwide. They are responsible for 5-20% of outbreaks of nosocomial infections in intensive care units, burn, oncology, and neonatal units (Kotra *et al.,* 2002). Therefore, combined antibacterial effects of leaves extracts of *A. nilotica* and *P. guajava* against ESBLs producing *E. coli* and *K. pneumoniae* was investigated.

MATERIALS AND METHODS

Plants Collection and Preparation

The fresh leaves of the plants were collected aseptically using a cleaned cutlass in Gombe State, Nigeria, and were transported in cleaned polythene bags to the laboratory. A soft brush was used to remove any dirt/debris from the plant materials (Sarker *et al.*, 2006). The plant parts were identified at the Botany Herbarium of the Biological Sciences Department, Gombe State University. Samples were air-dried under a shade for two weeks and pulverized to powder form using mortar and pestle (Aliyu *et al.,* 2008; Esimone *et al.,* 2012).

Extraction of Plant Materials

Water extraction

Using the method of Esimone *et al.,* (2012), 50 g of each of the powdered samples of the plants were extracted by boiling in 200mls of distilled water for 30 minutes, the extracts were then filtered using Whatman filter paper no. 2, and then the filtrate was evaporated to dryness at 40°C using water bath, and then stored in air-tight containers in a refrigerator at 5°C before use.

Organic solvent extraction

Three different organic solvents with varying polarities (high, moderate, and low polarity) were used in order to exploit the varying solubility of the plant constituents (Sarker *et al.*, 2006). The three organic solvents chosen are; *n*-hexane (low polarity solvent), Dichloromethane (moderate polarity solvent), and Methanol (high polarity solvent). Exhaustive extraction using cold maceration procedure was used so as to extract as many compounds as possible from the plant materials adopting the method of Sarker *et al.*, (2006) as described below;

50 g of the pulverized plant materials were soaked separately in different closed conical flasks containing 200mls each, of the three solvents separately at room temperature. The flasks were then kept for 2 weeks, so as to ensure exhaustive extraction. After the 2 weeks, the residual plant material was separated from the solvent by decanting and filtration, and then centrifugation. The filtrate was evaporated to dryness at 40^{0}C using water bath, and then stored in air-tight containers in a refrigerator at 5°C before use. Before use, the plant extracts were sterilized by filtering through a membrane filter of 0.22µm pore size (Bhojwani, 2013).

Isolation of Test Organisms

Using sterile plane containers, urine and stool samples were aseptically collected from in and outpatients attending Federal Teaching Hospital Gombe, the urine and stool samples collected from each patient were inoculated onto MacConkey and Blood agar by spreading and the plates were incubated for 24hours at 36°C. The colonies were further sub-cultured to obtain pure culture as described by Cheesbrough 2006.

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Antimicrobial Sensitivity Testing

Susceptibility was determined by the Kirby Bauer disk diffusion method as described by Clinical Laboratory Standards Institute (CLSI, 2006). Bacteria were grown on nutrient broth at 37°C overnight. The suspension was visually adjusted with normal saline to 0.5 Macfarland turbidity standard. Each inoculum was separately swabbed across the entire surface of the Muller Hinton agar plate (Biotech) using a sterile swab stick and the plate was rotated approximately 60°C between streaking to ensure even distribution. Inoculated plates were left to stand for at least 3 minutes before the disks were placed on the inoculated plates. Commercial antibiotics disks (Abtex Biological Ltd) used include: Ceftazidime (30µg), Cefuroxime (30µg), Cefotaxime (30µg), Gentamicin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Augumentin (30µg), Nitrofuratoin (300 µg), Ampicilin (10µg) and Imipenem (10µg). The plates were incubated within 15 minutes of the application of the disks at 37°C for 24 hours. The inhibition zone diameters around the disks were measured and interpreted according to the CLSI guideline.

Screening for Extended Spectrum Beta-Lactamases

The double disc synergy test (DDST) method described by CLSI (2021) was employed. Standardized inocula of the test organisms were inoculated on Muellar Hinton Agar (MHA) using sterile swab sticks as previously described. Amoxicillin/clavulanic acid disc (20/10µg,) was placed at the center of each inoculated MHA. Ceftazidime (30µg) and Cefotaxime (30µg) were placed 15 mm center to center from the Amoxycillin/clavulanic acid disc. The plates were incubated at 37oC for 24 hours. After incubation, enhancement of the zone of inhibition of either or both the Ceftazidime and Cefotaxime discs towards the Amoxycillin/Clavulanic acid discs is indicative of ESBL production.

Preparation of turbidity standard

Adopting the method described by CLSI (2021), one percent (1% v/v) solution of sulphuric acid was prepared by adding 1 ml of concentrated H_2SO_4 into 99 ml of distilled water. One percent (1%w/v) solution of barium chloride was also prepared by dissolving 0.5g of dehydrated barium chloride in 50ml distilled water, then 0.6ml of the barium chloride solution was combined with 99.4ml of sulphuric acid solution to yield 1% w/v barium sulfate suspension. The turbid solution formed was then transferred into the test tube as the standard for comparison.

Standardization of inoculum

Using inoculation platinum wire loops, enough material from an overnight culture of the test organisms were transferred into test tubes containing normal saline until the turbidity of the suspension matched the turbidity of the 0.5 McFarland Standard (CLSI, 2021).

Antibacterial Activity Tests

Activity testing of all crude extracts

This assay was conducted using the agar-well diffusion method of Esimone *et al.,* (2012) as described below;

An 80 mg/ml concentration of each of the different extracts were constituted by dissolving 0.08 g in 2 ml each of 20% v/v dimethyl sulfoxide (DMSO) and 2-fold serial dilutions were made. A single colony of the test isolate was suspended in 2mls of sterile Muller Hinton Broth. The suspension of the isolate was standardized as stated previously, and used to inoculate the surface of the Muller Hinton agar, and the excess fluid was drained into a disinfectant jar. The inoculated agar surfaces were allowed to dry and the plates were appropriately labelled.

Using a cork borer, four wells of 8 mm in diameter were bored in the inoculated Muller Hinton agar. Using a micropipette, 50µl of each concentration of the test extracts were delivered into each well. The plates were left on the bench for 30 minutes to allow the extracts to diffuse into the agar. Thereafter, the plates were incubated at 37°C for 24 h. After incubation, the plates were observed for inhibition zones around the wells. The diameters of the zones were measured with meter ruler to the nearest whole millimeter. Each test was carried out thrice and the mean inhibition zones diameter were recorded to the nearest whole millimetre. The test was done separately for all the different extracts of all the plant materials.

Test for synergistic activity

This test was carried out for only extract combinations that have independently shown significant activity in the sensitivity test above. This assay was conducted using agar-well diffusion method of Esimone *et al.,* (2012) as has been described above.

Minimum inhibitory concentration (MIC)

This test was carried out for the independent extracts, and the extracts combinations. This was carried out by adopting agar dilution method following the procedure outlined by CLSI in 2021 as described by Esimone *et al.,* (2012), For each extract (single or combined), 80 mg was weighed and dissolved in 2 ml of 20% v/v DMSO to get a stock solution with concentration of 40 mg/ml. Sterile test tubes were arranged on a test tube rack and 1 ml of sterile distilled water was dispensed into them. From the stock solution, 1 ml was transferred into the first test tube and serial dilution of the extracts were carried out until the resultant concentrations in the test tubes become 40, 20, 10, 5, 2.5, 1.25, 0.625, 0.3125 mg/ml. 1 ml of the extracts dilution were added to 19 ml of sterile molten Muller Hinton agar, mixed thoroughly and poured into sterile Petri dishes. The plates were allowed to solidify and then labelled appropriately. The plates were kept overnight in the incubator to check for their sterility. A single colony of each test isolates were picked from the subcultured plates with wire loop and inoculated into 2 ml sterile Muller Hinton broth to make a suspension of the test isolates. Each suspension was standardized as stated previously. Using a micropipette, 10µl of the standardized broth cultures were placed on the surface of the plates containing various concentrations of the extracts. Plain Muller Hinton agar (that is, without the extract) were also streaked and served as negative control. Inoculated plates were then incubated at 37°C for 24 h and observed for any visible bacterial growth. MICs were taken as the lowest concentration of extract that resulted in no visible growth on the surface of the agar. The tests were done separately for the different extracts, and then in combination.

Minimum bactericidal concentration (MBC)

The MBCs of the extracts were determined by adopting the method described by Esimone *et al.,* (2012), after completion of the MIC procedure above, the agar plates showing no growth in the MIC tests were used for the determination of the MBC. Blocks were cut out from the plates that showed no growth in the MIC test and transferred to a corresponding test tube of fresh Muller Hinton broth, to act as the recovery medium. The absorbance of the newly inoculated broth medium was taken, and then the tubes were incubated for 24 h at 32°C. At the end of incubation, microbial growth was ascertained by checking the turbidity of the medium.

The absence of change in turbidity in the recovery medium was used as evidence of total cell death, and vice-versa. This test was done for all the extracts both individually and

in combination. Care was taken to note the inhibition zone of MICs was larger in the combination than the sum of zones of individual assays for synergistic interactions and also the inhibition zone of MICs was smaller in combination, then antagonistic interactions were noted (Van Vuuren and Viljoen, 2011). The above experiments were carried out on all 4 types of extracts of all the plant materials. The results of all the independent and combined sensitivity assays, MICs, and MBCs were presented in separate tables with respect to the plant part, the type of extraction used, and the plant combination.

Qualitative Phytochemical Screening of Plant Materials

The qualitative phytochemical composition of all the extracts of all the plant materials that showed antibacterial activity was analyzed for the presence of alkaloid, saponin, anthraquinone, steroids, tannin, flavonoid, reducing sugars, and cardiac glycosides according to standard methods of Odebiyi and Sofowora, (1978) and Sofowora, (1982), and modified by Aiyelaagbe and Osamudiamen, (2009).

RESULTS

Table 1: Percentage yields of *Acacia nilotica* and *Psidium guajava* Extracts

Plant name	Methanol extract			Dichloromethane Extract			N-Hexane Extract			Water Extract		
	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%	Initial (g)	Final (g)	%
Acacia nilotica	50.00	14.03	28.06	50.00	4.07	8.14	50.00	4.20	8.4	50.00	14.75	29.5
Psidium guajava	50.00	15.63	31.26	50.00	10.80	21.6	50.00	3.38	6.76	50.00	25.02	50.04

Table 2: Physical Properties of Leaf extracts of *A. nilotica* and *P. guajava*

Extracts	Colour	Texture
ALME	Dark Green	Gummy
ALDE	Dark Green	Gummy
ALHE	Dark Green	Granular
ALWE	Dark Brown	Gummy
PLME	Dark Green	Gummy
PLDE	Dark Green	Gummy
PLHE	Dark Green	Granular
PLWE	Brown	Granular

Key: A= *Acacia nilotica*, P= *Psidium guajava*, L= Leaf, ME= Methanol Extract, HE= Hexane Extract, DE= Dichloromethane Extract, WE= Water Extract

Table 3: Occurrence of ESBLs in Test Isolates

Samples	No. of samples	Isolates	No. of positive isolates	No. screened	No. (%) ESBL Producers
Urine	20	*E. coli*	16	2	1(50)
Stool	10	*K. pneumonioae*	7	6	4(66.7)
Total	30		23	8	5(62.5)

Table 4: Antibacterial Activity of *Acacia nilotica* and *Psidium guajava* against ESBL *E. coli* and *Klebsiella pneumoniae*

Plant	Diameter of Zones of Inhibition (mm)/Extract Concentration (mg/ml)																Positive Ctl	Neg. Ctl
Name of plant	**Methanol**				**Dichloromethane**				**Hexane**				**Water**				**Gen 40** µg	DMSO
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80		
ESBL E. coli																		
Acacia nilotica	0	0	18	20	0	0	0	0	0	0	0	0	0	0	12	17	28	0
Psidium Guajava	0	0	15	18	0	0	0	0	0	0	0	0	0	0	15	20	28	0
ESBL K. pneumoniae																		
Acacia Nilotica	0	8	16	18	0	0	0	0	0	0	0	8	0	0	15	16	26	0
Psidium guajava	0	0	12	14	0	0	0	0	0	0	0	0	0	0	0	8	26	0

Key: Ctl= Control, Gen= Gentamycin, DMSO= Dimethylsulfuroxide

Table 5: Minimum Inhibitory and Minimum Bactericidal Concentrations (mg/ml) of *Acacia nilotica* and *Psidium gujava* against ESBL *E. coli* and *K. pneumoniae*

Plant name	**Methanol**		**Dichloromethane**		**Hexane**		**Water**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
ESBL E. coli								
Acacia nilotica	10	20	-	-	-	-	20	40
Psidium Guajava	10	20	-	-	-	-	10	20
ESBL K. pneumoniae								
Acacia nilotica	10	20	-	-	20	40	10	20
Psidium Guajava	10	20	-	-	-	-	20	40

Key: - = No Activity

Table 6: Antibacterial Activity of Combinations of Extracts against ESBL *E. coli* and *K. pneumoniae*

Extract Combination	Diameter of zones of Inhibition (mm)/Extract Concentration (mg/ml)				Positive Ctrl
	10	20	40	80	Gen 40 µg
E. coli					
ALME+PLME	10	13	27	40	28
ALWE+PLWE	8	11	18	30	28
K. pneumoniae					
ALME+PLME	10	14	30	37	26
ALWE+PLWE	8	10	15	26	26

Key: ALME= Acacia Leaf Methanol Extract, PLME= Psidium Leaf Methanol Extract, ALWE=Acacia Leaf Water Extract, PLME=Psidium Leaf Water Extract

Table 7: Minimum Inhibitory and Minimum Bactericidal Concentrations (MIC/MBC) in mg/ml of Extracts Combination of *Acacia nilotica* and *Psidium guajava* against *E. coli* and *K. pneumoniae*

Extracts Combination	MIC	MBC
E. coli		
ALME +PLME	2.5	5
ALWE + PLWE	5	10
K. pneumoniae		
ALME +PLME	2.5	5
ALWE + PLWE	5	10

Table 8: Determination of Phytochemicals Present in *Acacia nilotica* and *Psidium guajava* Extracts

Extracts	Phytochemicals							
	Alk	Sap	Anth	Str	Tann	Flv	Cgs	Rds
				Acacia nilotica				
ALME	+	+	-	+	+	+	+	-
ALWE	+	-	+	+	+	+	+	+
				Psidium guajava				
PLME	+	+	-	+	+	+	+	-
PLWE	-	+	+	+	+	+	+	+

Key: ALME= Acacia Leaf Methanol Extract, PLME= Psidium Leaf Methanol Extract, ALWE=Acacia Leaf Water Extract, PLME=Psidium Leaf Water Extract, Alk= Alkaloid, Sap= Saponin, Anth= Anthraquinone, Str= Steroids, Tan= Tanins, Flv= Flavonoids, Cgs= Cardiac glycosides, Rds= Reducing sugar, += Presence, - = not detected

DISCUSSION

The results of the percentage yield of *A. nilotica* and *P. guajava* were presented in Table 1. The Table showed that water extracts had the highest percentage yields for both plant parts (Leaves) of 29.5% and 50.04% for *A. nilotica* and *P. guajava* respectively, which was followed by methanol extracts with 28.06% and 31.26% percentage yields for both plants respectively. N-hexene extract recorded

8.4% for *A.nilotica* as the third percentage yield while dichloromethane extract had the least percentage yield for *A. nilotica* of 8.14%. However, dichloromethane extract recorded 21.6% for *P. guajava* as the third percentage yield while N-hexene had least percentage yield of 6.76% for *P. guajava*. Table 2 above showed the physical properties of the tested plants. All the leaf extracts were gummy and granular in texture and brown, dark brown, and dark green in colour.

Table 3 showed the occurrence of Extended Spectrum Beta Lactamase producing test isolates. Multi-drug-resistance (MDR) Gram-negative bacteria-induced infections have been reported with an increasing frequency in tertiary healthcare facilities in Nigeria and they have been found to be associated with significant morbidity and mortality (Yusuf *et al*., 2012). Members of Enterobacteriaceae are the most common causative agents of nosocomial and community bacteria-acquired infections (Coque *et al*., 2008). The overall prevalence of ESBL producers in this study was 62.5%, this was however in disagreement with the findings of Eze *et al*., (2015) who reported the overall prevalence of ESBL producers to be 29.2%, the results obtained from this study was also higher than the observation shown by Aibinu *et al*., (2003) who repotted ESBL production of 20.8% in *E. coli* and *Klebsiella* spp in Lagos, Nigeria. The results from this study was not in line with the report of Yusuf *et al*., (2011) who reported a prevalence of 37.1% in Kano. The prevalence of ESBL in this study was also higher than those reported by Bouchillion *et al*., (2012) from Egypt which showed ESBL production among *E. coli* and *Klebsiella* spp to be 40.9%. These variations could be due to difference in sample size, local antibiotics, and prescribing habits, and antibiotic selection pressure which differ from state to state, institution to institution, and from country to country however, the results obtained (Table 3) from this study tends to agree with the findings of Iroha *et al*., (2010) who reported the prevalence of 58.6%.

The distribution of ESBL among clinical specimens was also determined. Urine had the highest prevalence of 75% while stool had the least prevalence of 25%. The results of the findings were in agreement with Doughari and Akafa (2009), who reported a higher prevalence rate of 91% in urine, Iroha *et al*., (2010) who reported a high prevalence of 60.3% in urine, and Osazuwa and Osazuwa (2011) who also found that ESBLs prevalence was high in urine (61.4%). The high prevalence of ESBL in urine may be attributed to factors like age gap, gender, contraception, urinary tract obstruction, sexual activity, pregnancy, neurological dysfunction, poor hand washing techniques among health care practitioners, and antimicrobial use, which are some of the factors that can predispose one to urinary tract infection (UTI) development. From the results obtained in this study, the highest prevalence of ESBLs were detected in *Klebsiella pneumoniae* at 66.7% while *E. coli* had a 50% prevalence of ESBL.

Antimicrobial drugs provide the main basis for the treatment of various infections, however, the high genetic variability of some microorganisms enables them to rapidly develop antimicrobial resistance. Thus, there has been a continuing search for new potent antimicrobials (Hsueh *et al*., 2002). According to the World Health Organization (WHO) report on infectious diseases, overcoming resistance is the major issue of the WHO for the next millennium. Hence the last decades witnessed an increase in the evaluation of plants as a source of human diseases management (Prashanth *et al*., 2001).

Table 4 showed the individual activity of both plants against the tested organisms (ESBL *E.*

coli and *Klebsiella pneumoniae*). The results from both Table revealed that all the leaves the part of the plants used showed antibacterial activities against the tested organisms with the methanol extracts of both plants showed the highest activities in Table 4 with the exception of the water extract of *P. guajava* which showed the highest activity against *E. coli*, followed by the water extracts of both plants used and at the concentrations mostly of 40mg/ml and 80mg/ml while hexane extracts showed the least antibacterial activities in Table to an extent that it did not show activities at all in some parts of both plants used at different concentrations. This may be due to its low polarity in nature which makes it impossible to extract all the phytocompounds present in the part of the plants. These results agreed with the findings of Abeer and Sanaa (2007) who reported ethanol extract of *A. nilotica* exhibited higher antibacterial activities than chloroform extract on some bacterial isolates. The results obtained from this study are also in contrast with the findings of Abd-Ulgadir *et al*., 2015 who reported that methanolic extract of *Accacia nilotica* ssp. Tomentosa stem bark had higher antibacterial activity against *E. coli*. The results obtained from this study is line with the findings of Iroha *et al*., (2015) who reported that methanolic and ethanolic extracts of leaf and stem bark extracts of *P. guajava* exhibited high antibacterial activity against bacterial isolates. However, the results obtained from this study was in contrary to the findings of Abubakar, 2009 who reported aqueous extracts were more potent in inhibiting the growth of pathogenic *Proteus mirabilis, Streptococcus pyogenes, Escherichia coli, Staphylococcus aureus and pseudomonas aeruginosa* than the organic extracts.

The results of the antibacterial activity of extracts combinations against ESBL *E. Coli* and *Klebsiella pneumoniae* were presented in Table 6. The results obtained from Table 6 showed that methanolic extracts of leaves combinations of both plants had the highest antibacterial activity against the tested organisms of 40mm and 37mm at 80mg/ml respectively, followed by the water leaf extracts combinations of 30mm and 26mm at the same concentration respectively although there are activities at various concentrations used which may be attributed to the synergistic effects of the plants part. Although all extract combinations were significant when compared with the CLSI (2017) guidelines for antimicrobial susceptibility testing. These significant activities may have resulted from reactions between components of the plant extracts that were not active themselves, to improve the stability, solubility, bio-availability, or half-life of some other components, thereby making them very bioactive (Bone and Mills, 2013). Therefore, the results obtained from this study are in line with the findings of Oseni and Owusu (2012) who reported that a multi-concoction containing *F. albida*, *P. guajava*, and *F. exasperata* plant parts displayed synergistic activity on some pathogenic bacteria. Philips (2010) reported that tannins and alkaloids are natural products that have medicinal properties. The activity exhibited may be a result of the phytochemicals contained. Components like tannins, saponins, flavonoids, and terpenes have been severally reported to exhibit antibacterial and antioxidant activities (Kunle and Egharevba 2009, Ayoola *et al*., 2008).

The results of the MIC and the MBC for individual sensitivity testing conducted were presented in Table 5. The results obtained showed that the growth of ESBL *E. coli* and *Klebsiella pneumoniae* were inhibited at concentrations ranging from 10mg/ml to 20mg/ml respectively and were killed at concentrations ranging from 20mg/ml to

40mg/ml respectively. The results obtained from the study are lower than the findings of Okoro *et al*., (2014) and Gislene *et al*., (2000) who reported MIC and MBC of *A. nilotica* extracts and other plants on bacteria isolates to be 12.5mg/ml to 50mg/ml and 50mg/ml to 400mg/ml, this variation may be because of differences in the methods used while the results obtained for the combined (synergy) extracts (Table7) showed that the growth of both ESBL *E. coli* and *Klebsiella pneumoniae* were inhibited at concentrations ranging from 2.5mg/ml to 5mg/ml respectively and were killed at concentrations ranging from 5mg/ml to 10mg/ml respectively. The MIC test for the extract combination showed that the ALME + PLME combination had MIC values of 2.5mg/ml for both ESBL *E. coli* and *Klebsiella pneumoniae* respectively, which is lower than the MIC reported by Aliyu *et al,* (2008) for *F. albida* stem bark (3 mg/ml) on MRSA, and also lower than the MIC for *P. guajava* stem bark (5 mg/ml) on MRSA reported by Ibe *et al.,* (2013).

The phytochemical screening of the leaves of both plants revealed the presence of alkaloids, saponins, steroids, tannins, flavonoids, and cardiac glycosides, except anthraquinone and reducing sugar which were not detected as shown in Table 8, these results obtained in this study is similar to the results of presented by Owoyale *et al*., 2005 and Makinde *et al*., 2007. The inhibitory activities exhibited by the extracts tend to be in line with the reports of Levin *et al*., 1979 and Elmahmood *et al*., 2008 all of whom linked antimicrobial properties of plants to the presence of bioactive metabolites like alkaloids, tannins, saponins, and flavonoids, phenol, glycosides, and diterpenes. Drugs present in plants are known as active principles and these serve to protect the plants themselves against microbial attacks as well as predation by pests and animals (Elmahmood and Amey, 2007). Several plants which contain alkaloids, saponins, and glycosides have been shown to possess antimicrobial activity against a number of microorganisms as investigated by Adebajo, 1983.

CONCLUSION

Based on the findings obtained from this study, it was revealed that ESBLs producing *E. coli* and *K. pneumoniae* exhibited susceptibility to various concentrations of combined methanol leaves extracts of both plants. This high susceptibility was attributed to the presence of bioactive compounds (alkaloids, saponins, steroids, tanins, flavonoids and cardiac glycosides) in the plants part as widely referred in the literature. However, the findings from this study indicated that leaves extracts of *A. nilotica* and *P. gujava* could serve as a valuable panacea to antimicrobial resistance or pharmacological prospect for new natural antimicrobial drug development which could be useful in the treatment of infections caused by ESBLs producing *E. coli* and *K. pneumoniae.*

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