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Effect of *Ficus exasperata* VAHL Extracts on Bacterial Isolates Associated with HIV Infection

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Abstract

Opportunistic infections are mostly associated with HIV infected patients owing to their immune-compromised situation. This study is aimed at investigating the antibacterial effects of *Ficus exasperata* leaf extracts on bacterial isolates from blood samples of HIV infected patients attending antiretroviral therapy (ART) clinics in Akure, Nigeria. A total of 233 blood samples were each collected and subjected to bacteriological analyses. Phytochemical constituents of the leaf extracts were done using standard methods. Extracts were further purified using column chromatography. Antibiotics sensitivity test and antibacterial activity of *F. exasperata* extracts on the isolated bacteria was done using disc and agar well diffusion test respectively. Plasmid profile of multiple resistant bacterial isolates was also investigated. Ciprofloxacin exhibited the highest efficacy across all the isolates compared to other antibiotics. Methanol extract at 400 mg/ml compared favorably well with ciprofloxacin and exhibited the highest antibacterial efficacy on *E. coli* (22.13 ± 0.25^{h}) while chloroform extract showed the least. Purified extracts recorded higher efficacy compared to the crude extracts. The highest activity was recorded for methanol extract at 200 mg/ml against *K. pneumoniae* (32.98 ± 0.82^{d}) while the least was aqueous extract against *E. coli* (17.96 ± 0.33^{a}). The isolates showed multiple bands containing plasmids; however, most of the resistance was not plasmid based. This antibacterial efficacy of *F. exasperata* leaf extracts justifies its ethnomedicinal use as an alternative or complementary drug for the treatment of secondary bacterial infections commonly associated with HIV infection.

Keywords: Antibacterial, HIV, Blood, Plasmid, Antibiotics, Resistance, Ethnomedicine, Infections

1. Introduction

Human immunodeficiency virus and acquired immunodeficiency syndrome is a global pandemic (Cohen *et al.*, 2008). As of 2017, approximately 37 million people have HIV infection worldwide with the number of new cases that year being about 2 million. Of these 37 million, more than half are women and 2.6 million are less than 15 years old. It resulted in about 1.2 million deaths in 2014, down from a peak of 2.2 million in 2005 (UNAIDS, 2018). The clinical manifestation of HIV secondary infections in developing countries, including Nigeria, shows a high prevalence of infections of the skin, gut, respiratory tract, tuberculosis, and malnutrition (Akinsete *et al.*, 1998). Bacterial bloodstream infections constitute a significant public-health problem and present an important cause of morbidity and mortality in HIV-infected patients (Adeleye *et al*., 2010). Plants have continued to be a major resource for therapeutic purposes. Ethnobotanical and ubiquitous plants serve as a rich resource of natural drugs for research and development (Kong *etal.,* 1999). The natural composition of medicinal plants may act as new alternative in treating various emerging infectious diseases (Wurocheke *et al*., 2008; Ncube *et al*., 2008; Oluduro and Aderiye, 2009). Herbal medicine as a form of complementary and alternative medicine in the treatment of diseases is becoming increasingly popular in both developing and developed countries (Egwaikhide and Gimba, 2007; Mustapha *et al*., 2009). Phytomedicine has demonstrated its contribution to the reduction of excessive mortality, morbidity and disability due to diseases such as HIV/AIDS, malaria, tuberculosis, sickle cell anaemia, diabetes, mental disorders (Elujoba *et al*, 2005) and microbial infections (Iwu *et al.*, 1999). *Ficus exasperata* belongs to the family Moraceae, with 800 species occurring in the warmer part of the world (Odunbaku *et al*., 2008). Nigeria are replete with over 45 different species of *Ficus* (Keay and Onochie, 1964), such as *F. glomosa, F. lecardi, F. goliath, F. capensis, F. ingens* and *F. elastica,* which can be found in the Savannah, rainforest, besides rivers and streams. *Ficus exasperata* is commonly known as sand paper tree ("Ewe ipin" in Yoruba) and is widely spread in West Africa in all kinds of vegetation and particularly in secondary forest re-growth. The leaf extract from *F. exasperata* has been reported to have diverse uses such as treating hypertensive patients (Buniyamin *et al*., 2007), heamostative opthalmia, coughs and haemorrhoid (Odunbaku *et al.*, 2008). In Nigeria, young leaves of *F. exasperata* are prescribed as a common anti-ulcer remedy. Various pharmacological actions such as anti-diabetic, lipid lowering andantifungal activities have been reported for *F. exasperata* (Sonibare*et al.,* 2006). Other industrial uses are for polishing woods (Cousins and Michael, 2002), stabilization of vegetable oils, suppression of foaming, supplement as food stock and antimicrobial (Odunbaku *et al.,* 2008). The activities of leaf extract of *F. exasperata* against some pathogenic

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organisms have been extensively investigated (Buniyamin *et al.*, 2007; Odunbaku *et al*., 2008). However, recent toxicity studies in rats involving crude aqueous and ethanol extract of the leaves have indicated potential hepatic and renal toxicity as reflected by significantly increased serum transaminases and bilirubin (Irenell and Chukwunonso, 2006). Among different parts of *F. exasperata*, leaves have received much attention from the researchers across the world and have been widely studied for various pharmacological activities such as antidiabetic, hypotensive, antioxidant, anti-inflammatory, antiarthritic, antinociceptive, anticonvulsant, anxiolytic, antiulcer, antipyretic, uterotonic and antimicrobial activities (Faiyaz *et al*., 2012). Antibiotics can literally save lives and are effective in treating illnesses; however, they have the potential to cause unwanted side effects. Medicinal plant products when compared to their synthetic counterparts minimize these adverse side effects (Gislence *et al.*, 2000). Bacterial blood stream infections caused by multi-drug resistant bacteria are the major cause of morbidity and in chronic stage mortality in patients with human immunodeficiency virus. Thus, this study aimed at evaluating the *in-vitro* antimicrobial effects of the methanol, aqueous and chloroform extract of the leave of *F.exasperata* on bacterial isolates from the blood samples of human immunodeficiency virus infected patients.

2. Materials and Methods

2.1. Ethical approval

Ethical approval was obtained from the Health Research Ethics Committee (HREC) of State Specialist Hospital, Akure, Nigeria. Patient consent was sought before the collection of samples.

2.2. Collection of blood samples and plant materials

In total, 233 blood samples from serologically confirmed HIV-1 infected patients attending the HIV Clinic State Specialist Hospital, Akure, Ondo State, Nigeria, were collected in EDTA bottles and immediately transported to the Laboratory for microbiological analyses. Leaves of *F. exasperata* were harvested and authenticated at the Department of Crop, Soil and Pest Management, Federal University of Technology, Akure, Nigeria.

2.3. Bacteriological analyses

Culture media including brain heart infusion broth, MacConkey agar, blood agar, chocolate agar, nutrient agar, Mueller Hinton agar, and nutrient broth were prepared according to manufacturer's specification. The blood samples were each cultured on the media and further identified using cultural and biochemical methods according to the methods of Cheesbrough (2010).

2.4. Preparation of leaf extracts of F. exasperata

Leaves of *F. exasperata* were washed to remove particles and foreign materials, left to air dry at room temperature on a clean surface until a constant weight of the sample was observed and thereafter ground into fine powder using electronic blender. The dried powdered leaves were stored at room temperature (Shahidi-Bonjar, 2004). Exactly 200 g of *F. exasperata* was measured into a container and each soaked with methanol, chloroform and water. The mixture was allowed to stand for 72 hours with intermittent stirring. This was followed by repeated filtration using sterile muslin cloth, non-absorbent cotton wool and Whatman filter paper. The filtrates were concentrated *in vacuo* at 40 ℃ using a rotary evaporator. The percentage yield of each extract was determined by comparing the weight of the yield and the initial weight of the powder extracted. The extracts obtained were preserved at 4 ℃ before use (Atata *et al.*, 2003).

2.5. Phytochemical analysis

Screening and identification of bioactive chemical constituents like alkaloids, glycosides, saponins, phenolic compounds, flavonoids, and tannins, in the medicinal plants under study were carried out using standard methods described by Trease and Evans (2002); Usman and Osuji (2007).

2.6. Antibiotics sensitivity test of bacterial isolates

Antibiotic sensitivity testusing commercially available antibiotics was performed on the bacterial isolates cultured on Mueller Hinton gar plates using standardized agar-discs diffusion technique as described by Clinical Laboratory Standard Institute (CLSI, 2016).

2.7. Antibacterial activity of F. exasperata leaf extract on bacterial isolates

Agar well diffusion technique was used to determine the *in-vitro* antibacterial activity of the crude extract. A 1 ml of 18 hours broth culture of each of the test bacterial suspension that have been adjusted to turbidity equivalent of 0.5 McFarland standard was pour plated on sterile Mueller-Hinton agar plates, 6 mm wells were bored and filled with 0.5 ml of each extracts. Dimethylsulfoxide (DMSO) and ciprofloxacin (500 µg) were used as the negative and positive control respectively.

2.8. Plasmid profile analyses of multi drug resistant bacterial isolates

Plasmid extraction and curing was carried out using methods described by Akinjogunla and Enabulele, (2010) on selected multiple drug resistant bacterial isolates.

2.9. Purification of extract using column chromatography

Purification of the extract was carried out using column chromatography as described by Atta *et al*. (2009) and Usha *et al.* (2010) using petroleum ether, chloroform and methanol in ratio 3:1:1 v/v as eluting solvent. The column was packed with silica gel (60-120 mesh). The fractions obtained were reconstituted with 30% DMSO and spotted on TLC plates. Fractions with the same retention factor (R_f) were pooled together.

2.10. Statistical Analysis

Data were presented as mean ± standard error (SE). The significance of difference between treatment groups was tested using two-way analysis of variance (ANOVA) and treatment means were compared with Duncan's New Multiple Range test using SPSS version 20 software, at $p < 0.05$ level of significance.

3. Results

3.1. Percentage occurrence of bacteria isolated from HIV infected blood

The occurrence of bacterial isolates from HIV infected blood samples is expressed as percentage as shown in Table 1. Nine bacteria including *Staphylococcus aureus, Streptococcus pneumoniae, S. pyogenes, Shigella* sp, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Salmonella typhi*were isolated. *Pseudomonas aeruginosa* had the highest occurrence (18%) while *P. mirabilis* had the least (6%).

Table 1. Percentage occurrence of bacteria isolated from HIV infected blood

Isolates	Frequency	Occurrence (%)
Pseudomonas aeruginosa	31	18
Klebsiella pneumoniae	28	16
Escherichia coli	24	14
Shigella sp	20	11
Streptococcus pneumoniae	19	11
Staphylococcus aureus	17	10
Salmonella typhi	15	09
Streptococcus pyogenes	12	07
Proteus mirabilis	10	06

3.2. Qualitative phytochemical constituents of F. exasperata leaf

Figure 1 shows the different phytochemicals inherent in the different extracts of *F. exasperata* leaf. It revealed the presence of flavonoids, tannins, terpenoids, steroidal and cardiac glycosides; alkaloids and saponins were found to be present. Alkaloid and flavonoids of methanolic leaf extract had the highest quantity of phytochemicals.

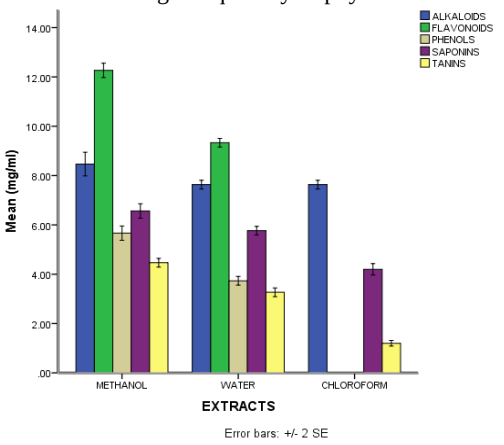

Figure 1. Quantitative Phytochemical Constituents of *F. exasperata* Leaf extract

3.3. Antibiotics sensitivity pattern of bacteria isolated from blood of HIV infected patients

Table 2 shows the antibiotics sensitivity patterns of Gram positive and negative bacteria isolated from the blood of HIV infected patients respectively. All the isolates showed multiple resistance to more than 3 antibiotics. Ciprofloxacin exhibited the highest efficacy across all the isolates compared to the other antibiotics.

Table 2. Antibiotics sensitivity pattern of bacteria isolated from HIV infected blood

Isolates	Zones of inhibition (diameter mm)									
Gram Positive	**ERY**	**OFL**	**STR**	**CHL**	**CEF**	**GEN**	**PEF**	**COT**	**CPX**	**AMX**
S. pyogenes	8.67±0.33^{f}	11.67±0.33^{a}	0.00±0.00^{e}	11.00±0.58^{b}	13.00±0.58^{b}	8.33±0.67^{a}	8.67±0.33^{d}	10.33±0.67^{a}	11.67±0.67^{a}	6.67±0.33^{b}
S. pneumoniae	13.33±0.33^{d}	19.00±0.58^{c}	17.67±0.89^{c}	24.33±0.33^{a}	17.00±0.58^{a}	17.00±0.58^{a}	19.67±0.33^{b}	15.00±0.80^{a}	15.00±0.58^{f}	20.67±0.67^{e}
S. aureus	9.00±0.58^{a}	0.00±0.00^{c}	10.33±0.58^{b}	12.33±0.58^{c}	11.00±0.67^{b}	1.67±0.33^{b}	12.00±0.58^{a}	9.67±0.33^{b}	10.00±0.58^{a}	11.67±0.67^{c}
Gram Negative	**AUG**	**TET**	**CEF**	**NIT**	**PEF**	**GEN**	**COT**	**OFL**	**AMX**	**CPX**
P. mirabilis	6.33±0.33^{d}	4.67±0.67^{c}	4.33±0.33^{a}	2.33±0.33^{a}	6.33±0.33^{c}	10.67±0.33^{a}	6.67±0.33^{b}	10.00±0.58^{d}	3.00±0.58^{a}	14.67±0.33^{a}
S. typhi	11.33±0.67^{b}	9.00±0.58^{c}	9.67±0.33^{a}	12.33±0.67^{a}	15.0±0.58^{d}	19.67±0.33^{a}	8.67±0.67^{f}	15.67±0.33^{a}	16.00±0.00^{b}	20.00±0.00^{e}
Shigella sp	9.33±0.33^{b}	10.67±0.33^{d}	22.67±0.33^{f}	16.33±0.3^{a}	15.67±0.30^{a}	17.67±0.33^{d}	20.00±0.58^{b}	19.00±0.58^{e}	15.00±0.58^{d}	20.67±0.33^{d}
E. coli	6.67±0.67^{e}	9.00±0.58^{g}	21.00±0.58^{b}	16.33±0.3^{a}	9.33±0.33^{f}	21.67±0.89^{e}	18.67±0.67^{f}	6.67±0.67^{b}	12.67±0.33^{a}	22.33±0.33^{f}
K. pneumoniae	8.33±0.33^{e}	0.00±0.00^{a}	6.00±0.00^{a}	6.67±0.67^{c}	4.67±0.33^{a}	7.00±0.58^{e}	0.33±0.33^{d}	0.00±0.00^{a}	0.00±0.00^{a}	15.33±0.33^{c}
P. aeruginosa	9.00±0.58^{a}	6.33±0.33^{a}	17.33±0.33^{h}	7.00±0.58^{b}	21.00±0.00^{f}	16.33±0.33^{d}	7.00±0.58^{f}	17.33±0.33^{a}	13.33±0.33^{b}	19.67±0.67^{d}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)
Keys: cotrimoxazole (25 µg) (COT), ciprofloxacin (CPX) (10 µg), tetracycline (30 µg) (TET), amoxicillin (30 µg) (AMX), ofloxacin (5 µg) (OFL), augmentin (30 µg) (AUG), nitrofurantoin (200 µg) (NIT), gentamycin (10 µg) (GEN), ceftriaxone (30 µg) (CEF), pefloxacin (5 µg) (PEF), streptomycin (10 µg) (STR), chloramphenicol (30 µg) (CHL) and erythromycin (15 µg) (ERY)

3.4. Antibacterial activity of F. exasperata leaf extracts on bacteria isolated from blood of HIV infected patients

Table 3 shows the antibacterial activity of varying concentrations of leaf extracts of *F. exasperata* on bacterial isolates from blood of HIV infected patients. Methanol extract at 400 mg/ml compared favorably well with ciprofloxacin (positive control) and exhibited the highest antibacterial efficacy on *E. coli* (22.13±0.25^{h}) while chloroform extract showed the least. Table 4 shows the antibacterial activity of the purified fraction of methanol and water extract of *F. exasperata*. A 200 mg/ml of the 3rd and 4th fraction of both purified extracts recorded higher antibacterial activity against the test isolates as compared to the crude extracts. The highest activity was recorded for methanol extract against *K. pneumoniae* (32.98±0.82^{d}) while the least was aqueous extract against *E. coli* (17.96±0.33^{a}). However, purified methanol extract of *F. exasperata* showed better antibacterial activity.

Table 3. Antibacterial activity of *F. exasperata* leaf extracts on bacteria isolated from HIV infected blood

Isolates	Zones of inhibition (diameter mm)									
	Methanol (mg/ml)			Chloroform (mg/ml)			Aqueous (mg/ml)			+ve ctrl
	50	200	400	50	200	400	50	200	400	CPX
S. pyogenes	3.07±0.21^{b}	9.17±0.15^{d}	11.63±0.64^{e}	1.20±0.10^{b}	6.80±0.10^{c}	8.27±0.15^{d}	2.23±0.31^{b}	6.10±0.20^{c}	10.00±0.17^{e}	11.67±0.67^{e}
S. pneumoniae	0.00±0.00^{a}	7.90±0.10^{c}	15.77±0.25^{f}	1.10±0.17^{b}	8.13±0.15^{d}	11.67±0.21^{d}	1.90±0.17^{b}	6.93±0.12^{c}	14.40±0.27^{e}	15.00±0.58^{f}
S. aureus	2.90±0.10^{b}	15.07±0.21^{f}	19.93±0.15^{g}	1.13±0.58^{b}	9.07±0.15^{d}	10.10±0.20^{d}	3.10±0.20^{c}	10.03±0.21^{d}	18.43±0.47^{f}	10.00±0.58^{d}
P. mirabilis	3.00±0.27^{c}	10.90±0.10^{e}	18.10±0.20^{g}	1.33±0.58^{b}	7.87±0.21^{c}	12.27±0.15^{e}	2.93±0.25^{b}	9.97±0.21^{e}	16.87±0.21^{f}	14.67±0.33^{e}
S. typhi	1.77±0.15^{b}	11.13±0.21^{f}	16.07±0.25^{f}	1.23±0.58^{b}	6.10±0.10^{c}	8.90±0.17^{d}	1.60±0.10^{b}	9.00±0.10^{d}	14.07±0.15^{e}	20.00±0.00^{g}
Shigella sp	1.13±0.58^{b}	8.10±0.17^{d}	11.30±0.61^{e}	0.00±0.00^{a}	3.93±0.15^{b}	7.37±0.15^{d}	0.00±0.00^{a}	5.97±0.12^{c}	9.83±0.25^{d}	20.67±0.33^{g}
E. coli	1.10±0.17^{b}	6.23±0.35^{c}	22.13±0.25^{h}	0.00±0.00^{a}	5.70±0.25^{c}	10.73±0.15^{e}	2.07±0.15^{b}	10.80±0.20^{e}	16.13±0.21^{f}	22.33±0.33^{h}
K. pneumoniae	3.10±0.20^{c}	11.07±0.21^{e}	15.90±0.10^{f}	1.30±0.17^{b}	6.93±0.15^{d}	11.37±0.21^{e}	1.90±0.17^{b}	9.83±0.15^{e}	16.70±0.53^{f}	15.33±0.33^{e}
P. aeruginosa	1.97±0.21^{b}	10.93±0.15^{e}	21.97±0.21^{h}	1.83±0.58^{b}	6.77±0.15^{d}	10.93±0.15^{d}	1.93±0.15^{b}	10.70±0.17^{e}	16.37±0.12^{f}	19.67±0.67^{f}

Data are presented as Mean ± S.E (n=3). Values with the same superscript letter(s) along the same column are not significantly different (P<0.05)
Keys: ciprofloxacin (CPX) (10 µg), +ve ctrl = Positive control

Table 4. Antibacterial activity of purified leaf extract of F. exasperata at 200 mg/ml on bacterial isolates from blood of HIV infected patients

Isolates	Methanol extract (mg/ml)		Aqueous extract (mg/ml)	
	Fraction 3	Fraction 4	Fraction 3	Fraction 4
Shigella sp	21.23±0.33^{c}	22.12±0.19^{d}	21.67±0.87^{c}	23.07±0.69^{b}
S. pyogenes	19.96±0.33^{a}	21.07±0.66^{a}	22.92±0.66^{c}	25.02±0.69^{a}
P. aeruginosa	23.46±0.31^{c}	25.76±0.33^{c}	23.96±0.33^{a}	26.07±0.30^{a}
S. pneumoniae	20.96±0.66^{c}	21.00±0.67^{a}	21.92±0.66^{c}	22.02±0.69^{d}
P. mirabilis	24.92±0.21^{e}	25.00±0.19^{e}	26.77±0.54^{c}	27.97±0.55^{c}
S. typhi	22.96±0.10^{e}	23.22±0.87^{b}	23.92±0.66^{a}	24.02±0.69^{a}
S. aureus	26.12±0.50^{e}	26.92±0.68^{e}	28.60±0.87^{c}	29.98±0.99^{c}
K. pneumoniae	29.04±0.01^{f}	32.98±0.82^{d}	30.18±0.01^{d}	30.15±0.31^{f}
E. coli	27.92±0.66^{b}	30.02±0.69^{b}	17.96±0.33^{a}	19.00±0.67^{a}

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

3.5. Plasmid profiling of multi drug resistant bacterial isolates.

The agarose gel electrophoresis of the plasmid DNA of the multi-drug resistant bacterial isolates is shown in Figure 2. Multiple bands were observed in each bacterial lane, showing that they possessed heavy plasmids, with the highest molecular weight of 23,130 bp. However, following plasmid curing, most of the isolates were not plasmid based, as resistance was still observed in almost all the tested isolates against the antibiotics as shown in Table 5.

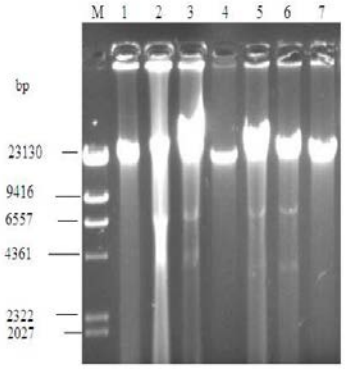

Figure 2. Plasmid profile of some selected multiple antibiotics resistant bacterial isolates from HIV infected blood samples. M: Marker, lane 1:*S. aureus*, lane 2: *P. aeruginosa*, lane 3: *K. pneumoniae*, lane 4: *E. coli*, lane 5: *P. mirabilis*, lane 6: *S. pneumoniae*, lane 7: *S. pyogene.s*

Table 5. Antibiogram profile of post cured bacteria isolates from blood of HIV infected patients

Isolates	Post cured resistant antibiotics
S. pyogenes	ERY, STR, GEN, AMX, PEF, COT
S. pneumoniae	ERY, COT, CPX
S. aureus	ERY, OFL, GEN, COT, STR
P. mirabilis	AUG, TET, CEF, NIT, PEF, COT, AMX
E. coli	AUG, TET, PEF, OFL
K. pneumoniae	AUG, TET, CEF, NIT, PEF, GEN, COT, OFL, AMX,
P. aeruginosa	AUG, TET, NIT, COT

Keys: cotrimoxazole (COT), ciprofloxacin (CPX), tetracycline (TET), amoxicillin (AMX), ofloxacin (OFL), augmentin (AUG), nitrofurantoin (NIT), gentamycin (GEN), ceftriaxone (CEF), pefloxacin (PEF), streptomycin (STR), and erythromycin (ERY)

4. Discussion

Bacteraemia is a common problem associated with HIV infected individuals, and this is usually associated with poor prognosis. Adeleye *et al*. (2008) reported that bloodstream infections constitute a significant public health problem and represent an important cause of morbidity and mortality in HIV/AIDS patients. Piroon (2007) reported that in HIV infected patients, community acquired and nosocomial bacteraemia were found in 78.5% and 25.1% respectively and are responsible for the immediate cause of death in up to 32% of HIV infected patients. Different factors may be responsible for the variation in the frequency and type of bacteria isolated from different HIV patients, such variations may be due to life styles, geographical differences, and use of intravenous drugs. Piroon (2007) also reported that bacterial infections are common in HIV infected patients because of abnormalities in humoral, cellular and mucosal immunity, and thus HIV infected patients have an increased risk of bacteraemia during bacterial infection. Bloodstream infections have been observed to appear more frequently in HIV/AIDS patients.

Gram negative bacteria were the most frequent pathogens isolated during this study. This corresponds with the findings of Piroon (2007), that bacteraemia in

HIV infected patients were usually caused by Gram negative bacteria, mostly belonging to the enterobacteriaceae family findings of Oladosu *et al*. (2016), revealed that the enterobacteriaceae family is the largest and most heterogeneous group of medically significant Gram negative bacteria and are most frequently isolated in clinical samples. They have been implicated in infections such as diarrhoea, dysentery, salmonellosis, haemolytic-uremic syndrome (HUS), necrotizing enterocolitis and various nosocomial infections (Oladosu *et al*., 2016). *Pseudomonas aeruginosa* was the highest occurring bacteria isolated in this research work (18%) and it corresponds with the findings of Ana *et al*. (2015). This bacterium has emerged as one of the most common causes of Gram negative bacteraemia and pneumonia in HIV-infected hospitalized patients, and its incidence in HIV/AIDS patients appears to be on the rise, with many studies demonstrating an annual increase in cases (Hart *et al*., 2000). Infections arising from them are hard to treat due to their natural resistance to antimicrobial agents (Obritsch *et al*., 2005). Blanc *et al*. (1998) also reported that *P. aeruginosa* is responsible for 10–15% of the nosocomial infections worldwide and has become an important cause of Gram negative infection, especially in patients with compromised immune system. *Klebsiella pneumoniae* was the most resistant isolates following post curing. This might be linked to its significant proportion in hospital-acquired urinary tract infections, pneumonia, septicemias, and other soft tissue infections. They are able to spread rapidly in the hospital environment, hence leading to nosocomial outbreaks (Podchun and Ullmann, 1998). The presence of *Shigella* spp in the blood stream of subjects analysed in this study is of great clinical concern. This high frequency of Shigella could be due to poor personal hygiene of the patients, poor food handling, preparation, bad feeding habit (malnutrition), and their choices food procurement. Kotloff *et al*. (1999) also submitted that HIV infected individuals are at high risk of recurrent, severe and fatal occurrences of bacteremia due to Shigella.

The highest occurring Gram positive bacteria from this research is *S. pneumoniae* (11%) which resides asymptomatically in healthy individuals or carriers. It has also been known to cause diseases such as community acquired pneumonia, meningitis, septicemia, bronchitis, otitis media in susceptible individuals with weaker immune system such as children, the elderly and HIV infected patients (Janoff *et al*., 1992). Other encountered Gram positive bacteria in this study, including *Streptococcus pyogenes* and *Staphylococcus aureus*, have been associated with respiratory infections from contaminated surfaces. Bianca *et al*., (2010) reported that individuals with HIV/AIDS infection are also at an increased risk of *S. aureus* bacteremia whose sources might be hospital acquired, community and health care.

The multi-drug resistance displayed by most of the organisms to the various test antibiotics is a major cause for concern because many clinicians fall back on the use of quinolones for the treatment of Gram-negative pathogens in the face of multi-drug resistance (Khaneja *et al.*, 1999). The high rate of resistance towards these antibiotics may be due to drug abuse because they are readily available over the counter, not completing a dose before beginning a new one. Genetic and non-genetic acquired resistance can lead to increased bacterial antibiotics resistance (Mutation or Plasmid acquired resistance). Ciprofloxacin, which belongs to the family of quinolone antibiotics, displayed the highest antimicrobial effect of all the antibiotics used in the antimicrobial susceptibility testing; this outcome agrees with a report from Oladosu *et al*. (2016). Prajna *et al*. (2001) reported that the ophthalmic solutions of ciprofloxacin antibiotics are effective and safe in the treatment of patients with culture positive bacterial keratitis. According to studies, the use of antibiotics, especially irrationally, is the reason for the emergence and spread of resistant strains of pathogens that are of clinical importance in both the community and hospital environments (Bradford, 2001; Schmitt *et al*., 2007).

Plants have a diverse range of bioactive molecules, making them rich sources of different types of medicines. These bioactive compounds, according to Aboaba *et al.* (2006) usually interfere with the growth and metabolism of microorganisms in a negative manner.The phytochemical test carried out on the powdered leaf extract revealed the presence of phytochemicals of pharmacological importance (Adebayo *et al*., 2009). The presence of flavonoid is an indication of natural occurring phenolic compound with beneficial effects in the human diet as anti-oxidant and free radicals (Adebayo and Ishola, 2009). The presence of flavonoid, tannins, saponins, phenolic constituents could provide synergistic effect which improves the efficacy of the active ingredients of the leaf (Takon *et al.*, 2013). The leaf extract of *F. exasperata* at 200mg/ml compared favorably with the commercial antibiotics. Methanolic extracts of *F. exasperata* leaves had greater antibacterial potency against the tested isolates compared to the aqueous and chloroform extracts. The presence of high amount of flavonoid which is a common secondary metabolite in medicinal plant may have accounted for this high antibacterial efficacy. The results of these inhibition growth zones of these is a confirmation of the ethno-medicinal significance of the plant, especially against secondary bacterial infections associated with HIV infection.

All the selected multi-drug resistant isolates harbor plasmids. Plasmids are extra-chromosomal pieces of DNA which are capable of replicating independently of the genome and have been directly implicated in the acquisition of resistance to many antibiotics (Weigel *et al.*, 2003).They have been documented to have encoded gene that provides resistance to occurring antibiotics in competitive environmental niche (Kroll *et al*., 2010). The post curing sensitivity test revealed that the resistance was not plasmid mediated, indicating chromosomal-borne resistant genes. Moreover, resistance to antibiotics by bacteria that is not due to plasmid or chromosome might be due to efflux pump mechanism (Poole, 2004) or other factors like mutation of gene encoding ribosomal protein which decrease permeability of the cell envelope in enteric bacteria (Isenberger *et al*., 2002).

5. Conclusion

It is important to monitor the susceptibility patterns of microorganisms as it contributes significantly to the burden of secondary bacterial infection in HIV infected patients.The antibacterial potential of the leaf extract of *F. exasperata* may be a source of new bioactive compounds

for drug development and also suggests it as a cost effective alternative therapy against opportunistic bacteria associated with HIV. Further purification of the extract and identification of the bioactive component is necessary to enhance greater antimicrobial potency.

References

Aboaba OO Smith SI and Olide FO. 2006. Antimicrobial effect of edible plant extract on *Escherichia coli* O157:H7. *Pakistan Journal of Nutrition*, **5:** 325-327

Adebayo EA and Ishola OR. 2009. Phytochemical and antimicrobial screening of crude extracts from the root, stem bark and leaves of *Bridelia ferruginea*. *African Journal of Biotechnology*, **8:** 650-653.

Adebayo EA Ishola, OR Taiwo OS Majolagbe ON and Adekeye BT. 2009. Evaluations of the methanolic extract of *Ficus exasperata* stem, bark, leaf and root for phytochemical analysis and antimicrobial activities. *African Journal of Plant Science*, **3:** 283-287.

Adeleye IA Akanmu AS Bamiro BS Obosi AC and Inem A. 2010. Bacterial Bloodstream Infections in HIV-infected Adults Attending a Lagos Teaching Hospital. *Journal of Health Population and Nutrition*. **28:** 318–326.

Adeleye A Smith S Akanmu S Bamiro S Sobande, E Igbinosum E and Inem V. 2008. Chromosomally mediated antibiotic resistance in non-typhoidal Salmonellae isolated from HIV patients in Lagos. *West Indian Medical Journal*. **57:**519-520

Akinjogunla OJ and Enabulele IO. 2010. Virulent factors, plasmid and curing analysis of multi-drug resistant *Staphylococcus aureus* and Coagulase negative *Staphylococcus* species isolated from patients with acute otitis media. *Journal of American Science*, **6:** 1022-1033

Akinsete I Akanmu AS and Okany CC 1998. Spectrum of clinical disease in HIV-infected adults at the Lagos University Teaching Hospital: a five year experience (1992-1996). *African Journal of Medical Science* **27:**147-51.

Ana ER Silvia RM Lilian AF Letícia PL and Elucir G. 2015. Prevalence of gram-positive bacteria in patients with HIV in specialized services. *Acta Paulista de Enfermagem* **28:**281-6.

Atata R Sani A and Ajewole SM 2003. Effect of stem back extracts of *Enantia chloranta* on some clinical isolates. *Biokemistri*, **15:** 84-92.

Atta HM Abul-hamd AT and Radwan HG. 2009. Production of Destomycin-A antibiotic by *Streptomyces* sp. using rice straw as fermented substrate. *Communications in agricultural and applied biological sciences*, Ghent University, **74:** 879-897.

Bianca SJ Jenny D Andreas P Robert S and Thomas B. 2010. Outcome and reinfection after *Staphylococcus aureus* bacteraemia in individuals with and without HIV-1 infection: a case–control study. *BMJ Open* **4:** e004075.

Blanc DS Petignat C Janin B Bille J and Francioli P. 1998. Frequency and molecular diversity of *Pseudomonas aeruginosa* upon admission and during hospitalization: a prospective epidemiologic study. *Clinical Microbiology and Infection* **4:** 242–247.

Bradford PA 2001. Extended-spectrum β-lactamases in the 21st century: Characterization, epidemiology and detection of this important resistance threat. *Clinical Microbiology Rev*iews, **14:** 933-951.

Buniyamin AA Eric KI and Fabian CA. 2007. Pharmacognosy and Hypotensive evaluation of *Ficus exasperata* Vahl (moraceae) leave. *Acta Poloniae Pharmaceutica-Drug Research*, **64:** 543-546.

Cheesbrough M. 2010. **District Laboratory Practice in Tropical Countries.** 2nd Edition. Cambridge University Press US. pp. 64-67.

Clinical and Laboratory Standards Institute (CLSI) 2016. **Performance Standards for Antimicrobial Susceptibility Testing**. 26th Informational Supplement. M100-S26. PA, USA.

Cohen MS Hellmann N Levy JA DeCock K and Lange J. 2008. The spread, treatment, and prevention of HIV-1: evolution of a global pandemic. *The Journal of Clinical Investigation*, **118:** 1244–54.

Cousins ON and Michael AH. 2002. Medicinal properties in the diet of Gorillas. An ethno-pharmacological evaluation. *African study monographs*. **23:** 65-89.

Egwaikhide PA and Gimba CE. 2007. Analysis of the phytochemical contents and antimicrobial activity of *Pletrabthus glandulosis* whole plant. *Middle East Journal of Scientific Research*, **2:** 135 – 138.

Elujoba AA Odeleye OM and Ogunyemi CM. 2005. Traditional medical development for medical and dental primary health care delivery system in Africa. *African Journal of Traditional, Complementary and Alternative medicines*, **2:** 46-61

Faiyaz A Mueen Ak Zainul A and Alias AK. 2012. Traditional Uses and Pharmacological Potential of *Ficus exasperata* Vahl. *Systematic Reviews in Pharmacy*, **3:**15-23

Gislence GF Juliana L Paulo FC and Giuliana SL. 2000. Antibacterial activity of plant extracts and phytochemicals on antibiotic resistant bacteria. *Brazilian Journal of Microbiology*, **31:**247-256.

Hart CA Beeching NJ and Duerden BI. 2000. Infection in AIDS. *Journal of Medical Microbiology*, **49:**947–67.

Irene II and Chukwunonso CA. 2006. Body and organ weight changes following administration of aqueous extracts of *Ficus exasperata* Vahl on white albino rats. *Journal of Animal and Veterinary Advances*, **5:**277-9.

Isenberger K Hill DW Jenkins IE and Mager JT. 2002. Clinical signs of the emergence of bacterial resistance in the hospital environment. *Journal of Applied Microbiology*, **92:**908-978.

Iwu MM Duncan AR and Okunji CO. 1999. New antimicrobials of plant origin In: Janick J (EDS.), **Perspectives in new crops and new uses.** ASHS Press, Alexandria, VA.

Janoff EN Breiman RF Daley CL and Hopewell PC. 1992. Pneumococcal disease during HIV infection: epidemiologic, clinical, and immunologic perspectives. *Annals of Internal Medicine*, **117:**314–324.

Keay RW and Onochie, CF. 1964. Nigeria Trees. *Department of Forest Reserves*, **2:** 16-187.

Khaneja M Naprawa J Kumar A and Piecuch S. 1999. Successful treatment of late onset infection due to resistant *Klebsiella pneumoniae* in an extremely low birth weight infant using ciprofloxacin. *Journal of Perinatology*. **19:** 311-4.

Kong JM Goh NK Chia IS and Chia TF. 1999. Recent advance in traditional plant drugs and Orchids. *Acta Pharmacologica Sinica*, **1:** 7-21.

Kotloff KL Winickoff JP Ivanoff B Clemens JD and Swerdlow DL. 1999. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bulletin of the world Health Organization*, **77:** 651-666.

Kroll J Klinter S Schneider C and Steinbucheal A. 2010. Plasmid addition system. Prespective and application of biotechnology. *Microbiology Journal of Biotechnology*. **3:**634-657.

Mustapha KA Abdulllahi BA and Rogo LD. 2009. Phytochemical properties and antibacterial activities of leaf and latex extracts of

Calotropis procera (AIT.F). *Bayero Journal of Pure and Applied Science*, **2:** 34 – 40.

Ncube NS Afolayan AJ and Okoh A. 2008. Assessment techniques of antimicrobial properties of natural compounds of origin: Current methods and future trend. *African Journal of Biotechnology*, **7:** 1797 - 1806.

Obritsch MD Fish DN MacLaren R and Jung R. 2005. Nosocomial infections due to multidrug-resistant *Pseudomonas aeruginosa*: epidemiology and treatment options. *Pharmacotherapy*. **25:**1353-64.

Odunbaku OA Ilusanya OA and Akasoro KS. 2008. Antibacterial activity of ethanolic leaf extract of *Ficus exasperata* on *Escherichia coli* and *Staphylococcus albus*. *Scientific Research and Essays*, **3:** 562-564.

Oladosu TO Adebolu TT and Oladunmoye MK. 2016. Evaluation of the Types of Bacteria in the Blood of HIV-1 Patients Attending ART Clinic at the FMC Owo, Nigeria and their Antibiogram Profile. *Current HIV Research*, **1:**103

Oluduro AO and Aderiye BI. 2009. Effect of *Moringa oleifera* seed extract on vital organs and tissue enzymes activities of male albino rats. *African Journal of MicrobiologyResearch*, **3:** 537 – 540.

Piroon M. 2007. Bacteremia in adult patients with acquired immunodeficiency syndrome in the northeast of Thailand. *International Journal of Infectious Diseases*, **11:** 226—231.

Podschun R and Ullmann U. 1998. *Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. *Clinical Microbiology Reviews*, **11:** 589–603.

Poole K. 2004. Acquired resistance, Principles and practice of disinfection, preservation and sterilization. Oxford: *Blackwell Scientific Publication*, p.170-83.

Prajna NV George C Srinivasan M Lu KL and McDonnell PJ. 2001. Bacteriologic and Clinical Efficacy of Ofloxacin 0.3% Versus Ciprofloxacin 0.3% Opthalmic Solutions in the Treatment of Patients with Culture-positive Bacterial Keratitis. *British Journal of Ophthalmology*, **20:** 175-178.

Schmitt JE Jacobs K and Schmidtt H. 2007. Molecular Characterization of extended-spectrum betalactamases in Enterobacteriaceae from patients of two hospitals in Saxony, Germany. *Journal of Medical Microbiology*, **56:** 241-249.

Shahidi-Bonjar GH. 2004. Evaluation of Antibacterial properties of Iranian Medicinal plants against *Micrococcus aureus, Serratia marcescens, Klebsiella pneunomiae and Bordella bronchoseptica*. *Asian Journal of Sciences* **3:**82-86.

Sonibare MO Isiaka AO Taruka MW Williams NS Soladoye M and Emmanuel O. 2006. Constituents of *Ficus exasperata* leaves. *Natural Product Communications*, pp 23-26.

Takon I Antai SP and Okon P. 2013. *In-vitro* evaluation of antibacterial activity of ethanolic and aqueous extracts of *Ficus exasperata* VAHL (Moraceae) leaves. *Journal of Microbiology and biotechnology research*, **3:**7-12.

Trease GE and Evans WC. 2002. Phytochemicals. In: **Pharmacognosy**, Saunders Publishers, London, 2002.

UNAIDS.UNAIDS data 2018. Geneva: UNAIDS; 2018. Available from: http://www.unaids.org/sites/default/files/media_asset/unaids-data-2018_en.pdf .

Usha R Ananthaselvi P Venil CK and Palaniswamy M. 2010. Anti-microbial and Anti-angiogenesis activity of *Streptomyces parvulus* KUAP106 from mangrove soil. *European Journal of Biological Research*, **2:**77-83.

Usman H and Osuji JC. 2007. Phytochemical and *in vitro* Antimicrobial Assay of the Leaf Extract of *Newbouldia laevis*. *The African Journal of Traditional, Complementary and Alternative Medicines*, **4:** 476-480

Weigel LM Clewell DB Gill SR Clark NC and McDougal LK. 2003. Genetic analysis of a high-level vancomycin-resistant isolate of *Staphylococcus aureus*. *Science*, **302:** 1569-1571.

Wurocheke AU Anthony EA and Obadiah W. 2008. Biochemical effects on the liver and the kidney of rats administered aqueous stem bark extracts of *Xemenia americana*. *African Journal of Biotechnology*, **1:** 2777 –2780.