Phytochemical and Antimicrobial Activities of *Bryophyllum pinnatum* and *Vernonia amygdalina* Leaves Extracts on Selected Microbial Isolates from Wound Infection

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ABSTRACT

Introduction: The therapeutic actions of plants may be due to the presence of some phytochemical components. Due to the increasing emergence of multi antibiotics resistance, wound pathogens are causing huge public health concerns. There is need for exploring some necessary alternatives for treatment of wound infections.
Aim: This study investigated the phytochemical and antimicrobial activities of *Vernonia amygdalina* and *Bryophyllum pinnatum* leaves extracts on *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* isolates from wound infection.
Methodology: The fresh leaves of both plants were extracted using Sofowora method and the phytochemicals were screened. Different concentrations of the extract, antibiotic and ethanol were tested against the isolates using disc diffusion technique.
Results: Alkaloids, flavonoids, tannins, anthraquinone and fixed oils were present in the both plant extracts but saponins were only found in *V. amygdalina* and cardenolide were only in *B. pinnatum.* Quantitatively, all the phytochemicals investigated in the study were present with *V. amygdalina* having the highest level of saponins than *Bryophyllum pinnatum* ($P<0.05$) and low steroids with $P=0.2879$ ($P>0.05$).The crude extract of *V. amygdalina* had the highest zone of inhibition compared to aqueous and ethanol extracts at 75 mg/ml concentration ($P<0.05$) but generally, the ethanol extracts of both plants had more inhibitions at varying concentrations. Thus comparing the antimicrobial activity of various extracts of both plants on the wound isolates and the controls (antibiotics and ethanol), there was significant variation in their zones of inhibition produce ($P<0.05$).
Conclusion: The results show that the zone of inhibition increases with the concentrations of the extracts. Therefore, the antimicrobial effect of these plants may depend on the concentration of the extract and the solvent used for extraction. This study showed that *B. pinnatum* and *V. amygdalina* could be used as an alternative therapy to antibiotics to treat wound infection caused by *P. aeruginosa, E. coli* and *S. aureus*.

KEY WORDS: *Bryophyllum pinnatum; Vernonia amygdalina;* Ethanol; *P. aeruginosa, E. coli* and *S. aureus;* Plant Extracts.

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1 INTRODUCTION

Wound is an opening or abrasion on the skin as a result of exposure of the subcutaneous layer of the skin that provides moist, warm and nutritious environment that favors the colonization and multiplication of microorganisms. Wound and other lesions are prone to infection due to multiplication of microbes from the environment or body surface (Oshim et al., 2016) [1]. Wound infection may occur due to contaminant that debase the cleaning effect of the host's immunity, colonizes and proliferate in the host. Wound infection may be exogenous or endogenous [2]. The endogenous infection or auto-infection occurs as a result of micro-organisms that are naturally in patient's body. Exogenous may occur through accident, trauma of the skin through surgical means or post-operative sepsis. Surgical site infection causes global Health Challenges [1]. Most bacteria enter the wound through external contamination from the environment, example; the bed, patient's body fluid, dressings, hands and or healthcare provider [3]. It was found that micro-organisms commonly found in infected wound includes *Staphylococcus aureus,* Methicillin resistant *Staphylococcus aureus, Streptococcus* species *Enterococci, Pseudomonas aeruginosa, Escherichia coli*, *Proteus* species and *Klebsiella* [3] [4]. Due to the increasing emergence of multi antibiotics resistance, wound isolates are causing huge public health concerns hence, the need for exploring some necessary alternatives for treatment of wound infections. Leaves and roots are useful therapeutic agents against numerous pathological infections [5]. Traditional treatment of circumcision wounds and chronic wound with locally prepared herbs and other natural occurring substances has been known for generation [6].

According to World Health Organization (WHO, [7], Medicinal plants are plants which one or more of its parts contain some substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [8]. Approximately, 80% of the world's population depend on herbal medicines for primary healthcare and plants have form the basis of strong traditional medicine systems that has provide needs for new drug development [7][9]. More so, Preethi et al. [10] stated that herbal medicine is the ancient form of healthcare known to man and over 50% of all modern drugs are of natural origin and natural products plays important role in drug development in the pharmaceutical industry. However, the increasing problems of Multi-Drug Resistance (MDR) bacteria is of great concern to both clinicians and pharmaceutical industries for this reasons, it is important to search for new drugs that are highly effective, affordable, acceptable and available [11]. Many of such plant used locally to reduce symptoms of illness includes; *Vernonia amygdalina* (Bitter leaf), *Allum sativa* (Garlic), *Ocimum gratissimum* (Scent leaf), *Zingiber officinale* (Ginger), *Bryophyllum pinnatum* (life plant), *Garcinia kola* and many others [12] [6]. *Vernonia amygdalina and Bryophyllum pinnatum* are plants that grow widely and used in folkloric medicine in tropical Africa, America, India,China Australia [13]. They possess a wide range of bioactive substances, including alkaloids, flavonoids, saponins, tannins, phenols, triterpenes, glycosides, steroids, lipids, organic acids and many others [13] [14]. These plants have been used in different ailments in traditional medicine. For example, novel of new born, convulsion, stomach ache, couph and more others. Different extracts from these plant have also been studied and reviewed that it posses pharmacological activities such as CNS depressant, antimicrobial, anti-inflammatory, immunomodulatory, analgesic, antitumor, antiulcer, antifungal, gastroprotective, insecticidal, antihistamine and many more [15].

However, in the past, the use of synthetic drugs from petroleum product yields decreased results in the pre-eminence of drugs from live plant sources. But with the recent trend of high percentage resistance of micro-organisms to present day antibiotic, efforts have been made by researchers to search for more source of antimicrobial agents from natural product (plants) to tackle the problems of drug resistance strains of micro organisms [16]. Nevertheless, for correct antimicrobial or phytotherapy for the treatment of wound infection, proper identification of microbes is important so that the healing activity of the wound can occur in less period of time [17]. This study investigates the phytochemical and antimicrobial activities of crude, aqueous and alcoholic extracts of *B. pinnatum* and *V. amygdalina* on *pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* isolated from wound infection.

2.0 MATERIALS AND METHODS

2.1 Study Area

This study was carried out between November, 2017 and July, 2018 at the University of Port Harcourt in the Department of Pharmacognosy and Phytotherapy and University of Port Harcourt Teaching Hospital Choba both in Obio/Akpor Local Government Area of Rivers State. The University of Port Harcourt Teaching Hospital is a tertiary health institution that accommodates both referrals and out patients from all parts of Rivers States and South – South geopolitical zone of Nigeria (Niger Delta). Nearly 200,000 patients are seen yearly in both inpatient and outpatient units as well as over 3000 surgical operations per annum in the University of Port Harcourt Teaching Hospital. It is located at 4^0 45N 6 ^{0}50'E/ 4.750^{0}N 6.833^{0}E of the Niger Delta with tropical rainforests and mangrove swamps. Port Harcourt is the biggest city in the South-South region of Nigeria with high economic importance as the centre of Nigeria's oil producer and also the political capital of the State with numerous medicinal plants such as Dongonyaro (*Azadiracha indica*), bitter leaf (*Vernonia amygdalina*), Scent leaf (*Ocimum gratissimum*) Africa never die (*Bryophyllum pinnatum*), Ginger (*Zingiber officinale*) and many others.

2.2 Source of Plant Samples

The plants used in this study were fresh leaves of *Vernonia amygdalina* and *Bryophyllum pinnatum* and were gotten from the University of Port Harcourt pharmacognosy and Phytotherapy Department garden and were identified by the Botanist.

2.3 Source of Microbial Isolates

The microbial isolates (*Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus)* used in this study had been isolated from wound infections in the University of Port Harcourt Teaching Hospital, Port Harcourt from the Department of Medical Microbiology Laboratory.

2.4 Sample Collection

The fresh leaves of *Bryophyllum pinnatum* and *Vernonia amygdalina* were removed from the stem, washed with clean tap water and rinsed in deionized water. After rinsing, they were transferred into a basket to drain the excess water and were sliced to pieces and three set of 200 g each were weighed. Using the laboratory mortar and electric blender, the leaves were grinded and each set transferred into 400 ml of 99% ethanol and 400 ml of de-ionize water in a 500 ml capacity flask and one set for crude extraction.

2.5 Sample Analysis

Basal medium such as nutrient agar, blood agar, peptone water, nutrient broth and other appropriate selective and differential media which include Mannitol Salt agar and MacConkey agar respectively were used in culturing and isolating the selected microbes in this study.

2.6 Medicinal Plants Extractions

2.6.1 Crude Extraction

The 200 g of the two plants were squeezed, pounded and some were blended with an electric blender (Moulinex, model F1 0027 412) and extracted using a double layer muslin cloth and then filtered through a Whatsman no.1 filter paper into different conical flask and stored in the refrigerator. After extraction and filtration, it was divided into two parts. One part for phytochemical assay and the other part was first stored in the freezer and then transferred to the freeze drying machine (Searchtech, Model: LGJ-10 freezing drier) to obtain the dried extracts before subjecting them to the microorganisms.

2.6.2 Aqueous (Water) Extraction

The 200 g in 400 ml of de-ionize water of both plants in different conical flask each was vigorously stirred respectively and extracted using a double layer muslin cloth and then filtered through a whatsman no.1 filter paper into different conical flask and stored in the refrigerator. The *Vernonia amygdalina* and *Bryophyllum pinnatum* aqueous extracts were transferred from the freezer to the freeze drying machine (Searchtech, Model: LGJ-10 freezing drier) after 24 hrs to obtain the dried extract for 13 hrs.

2.6.3 Ethanol Extraction

The mixture was vigorously stirred intermittently and then allowed to stand for 48 hrs. After 48 hrs, it was stirred once again and then the mixture was extracted first using a double layer muslin cloth and filtered through a Whatsman no.1 filter paper into a conical flask. The extract (filtrate) was evaporated (concentrated) with a rotary evaporator (England Lab Science Model: RE-52A) to separate the ethanol and concentrate the extract and then transferred to water bath (Techmel & Techmel USA, Model: TT-6) at 40^{o}c to obtain the dried extract and then stored in the refrigerator for antimicrobial use.

2. 7 Phytochemical Screening

The Phytochemical components of the *Vernonia amygdalina* and *Bryophyllum pinnatum* fresh leaves were analysed according to the methods described by [18][19]; for alkaloids, flavoids, tannins, anthraquinone, triterpenoid and steroids, carbohydrates, cardenolide, and cyanogenic glycosides (saponins).

2. 8 Isolation of the Microbes

The microbial isolates were characterized and identified based on their cultural characteristics using differential and selective media, Gram's staining and biochemical reactions as previously described [20, 21, 22, 23, 24].

2.9 Antimicrobial Activities of the Extracts

The leave extracts were tested for antimicrobial activity using disc diffusion techniques [24]. The method described by CLSI (clinical laboratory standard institute, [25] was employed. Few colonies from the nutrient agar slants (*Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* respectively) isolates were diluted with peptone water in the bijou bottle. About 3 ml each of the inoculated broth was placed onto the surface of a pre-dried nutrient agar plate and spread out evenly at the surface to ensure equal distribution of the organism on the agar. The plates were incubated for few minutes at room temperature for absorption of the inoculums. Then, a sterilized No 1 Whatsman filter paper of about 6 mm were prepared and impregnated with 25 mg/ml, 50 mg/ml and 75 mg/ml respectively. The impregnated discs were placed on the surface of the agar plate already seeded with the organism. The plates were allowed to stand for few minutes at room temperature for proper diffusion of the extracts on the seeded agar before incubation. Oflodazole (antibiotics), ethanol and water were set up as controls. The plates were then incubated at 37^{0c} for 24 hrs [24]. After 24 hrs incubation, the zone of inhibition were measured using a metre rule to the nearest millimeter (mm) and the p-values of the zone of inhibition were calculated. The *P*-value of $P \leq 0.05$ = significant and *P*> 0.05 and above = insignificant.

2.1.10 Data Analysis
Analysis

All experiments were conducted in triplicates and at least two independent occasions. Results from this study were presented as mean ± SD and statistical analyses were performed using an unpaired t test in which *P*- values was calculated (Graphpad Prism Version 5.03). Statistical significance was defined as a *P*-value of less than 0.05 at 95% confidence interval.

3.0 RESULTS

3.1 Isolation and Identification of Isolates

From the gram staining result, the gram negative organisms were red, rod in appearance, while the gram positive was purple cocci in clusters. The biochemical tests carried out on the microbial isolates confirms the ability of the isolates to utilize some compound and not others. *E. coli* showed indole,

lactose and nitrate positive, *P. aeruginosa,* oxidase positive and *S. aureus* coagulase and catalase positive and others as shown in Table 3.1.

3.2 Phytochemical Analysis of the Plants Extracts

The plant extracts were screened for the presence of phytochemical components. Table 3.2a presents the results of the preliminary qualitative phytochemical analysis of *V. amygdalina* and *B. pinnatum* leaves extracts. Out of eight (8) phytochemical analysed, only five (5) components were detected in both leave extracts: alkaloids, flavonoids, tannins, triterpenoids and carbohydrates. Cardenolides were absent only in *V. amygdalina* and saponins were absent in *B. pinnatum* qualitatively (Table 3.2a). The concentrations of five (5) of the detected phytochemical are shown in Table 3.2b.

Experiments were conducted in triplicate and mean ± SD are represented as bar on the chart (figure 3.1). Statistical significance is considered at $P<0.05$. Steroids has the P-value of 0.2879 which is statistically not significant ($P>0.05$) while others showed high level of significance at $P<0.05$. These results showed that the plant in this study has low levels of steroids as compared to other phytochemicals investigated. On the other hand, *V. amygdalina* has the highest levels of saponins and other phytochemicals compared to *B. pinnatum* leaves extract as shown in the chart (figure 3.1, Table 3.2b).

Table 3.1: Biochemical Analyses of the Selected Isolates

Organisms	Catalase	Nitrate	Oxidase	Growth at 42^0C	Indole	Oxidizes Lactose	Citrate	Coagulase	Motility
P. aeruginosa	-	+	+	+	-	-	+	-	+
E. coli	-	+	-	+	+	+	-	-	+
S. aureus	+	+	-	+	-	V	-	+	+

KEY: + = Positive, - = Absent, V = variable

Table 3.2a: Qualitative Phytochemical Analysis of *B. pinnatum* and *Vernonia amygdalina*

Plants	*B. pinnatum*	*Vernonia amygdalina*
Phytochemicals		
Alkaloid:		
Wagners	-	-
Dragendorff's test	+	+
Hager's test	+	-
Mayer's test	-	-
Flavonoids:		
Shinoda test	+	+
Lead acetate test	ND	ND
AlCl3 test	ND	ND
Tannins:		
$Fecl_3$ test	+	+
Phlobatannins	+	-
Gelatin test	ND	ND
Albumin test	ND	ND
Anthraquinone (test):		
Free anthraquinone	-	-
Combined anthraquinone	-	-
Triterpenoid/Steroids		
Liebermann –Buchard test	+	+
Salwoski test	-	+
Fixed Oils:		
Carbohydrates:		
Molisch test	+	+
Fehling's test	+	+
Cardenolide:		
Keller Killani Test	+	-
Kedde test	ND	ND
Cyanogenic glycosides:	ND	ND
Saponins:		
Frothing test	-	+
Haemolysis test	ND	ND
Emulsion test	-	+

Key: + = Present, - = Absent, ND = not determined

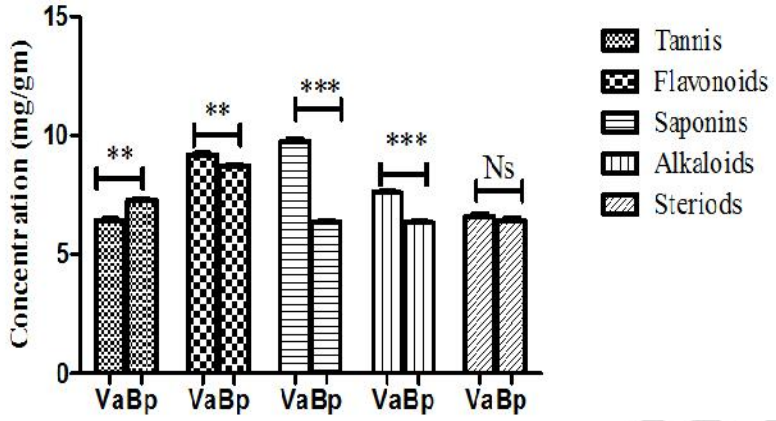

Figure 3.1: Quantification of Chemical Components of *B. pinnatum* and *V. amygdalina* Leaves Exracts. Experiments were conducted in triplicates and mean ± SD are represented in the chart. Statistical significance is considered at *P<0.05* shown by *. Key: Ns= not significant, Va = *V. amygdalina. Bp = B.pinnatum.*

Table 3.2b Comparison of the Levels

Phytochemicals	P—value
Tannins	0.0017
Flavonoids	0.0022
Saponins	<0.0001
Alkaloids	0.0001
Steroids	0.2879

3.3 The Antimicrobial Susceptibility of *V. amygdalina* Leaves Extracts on the Bacterial Isolates

Figure 3.2 compares the efficacies of crude extracts of *Vernonia amygdalina* and oflodazole (antibiotics) as control on three (3) microbial isolates (*P. aeruginosa*, *E. coli* and *S. aureus*). Experiments were conducted in triplicate and mean ± SD are represented as bar on the chart (Figure 3.2). The crude extract showed significantly lower zones of inhibition ($P<0.05$) when compared with the control (Oflodazole). It also showed significantly higher efficacies on *S. aureus* ($P=<0.0001$) compared to *P. aeruginosa* ($P = 0.0001$) and *E.coli* ($P = 0.0309$) isolates at the same concentrations (75 mg/ml) ($P<0.05$) (Table 3.3). Hence, the control antibiotics (Oflodazole) showed significantly highest efficacies compared to the *Vernonia amygdalina* crude extracts ($P<0.05$) (Figure 3.2).

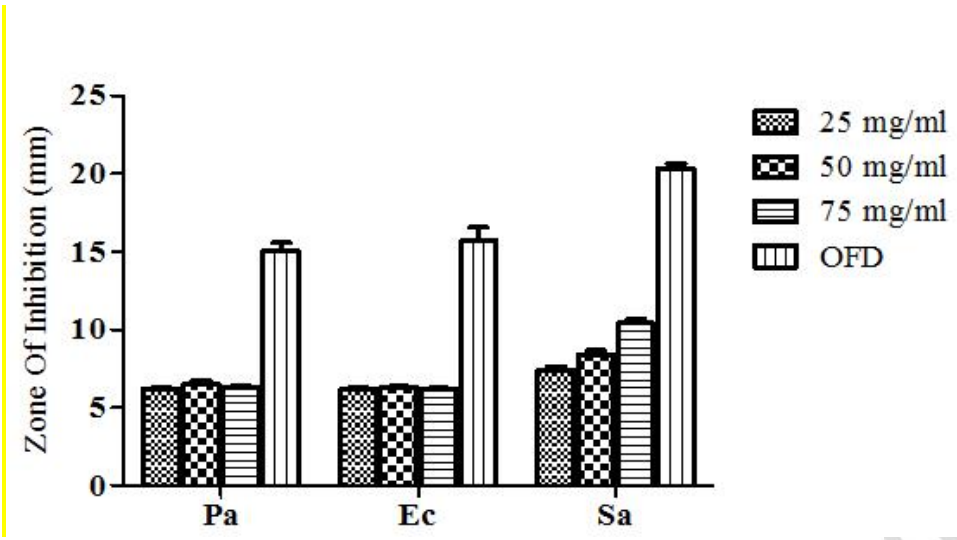

Figure 3.2: Antimicrobial Susceptibility of the Crude Extract of *Vernonia amygdalina* Leaves on the Bacterial Isolates. Experiments were conducted in triplicates and mean ± SD are represented in the chart. Statistical significance is considered at (P<0.05). Key: *Pa = P. aeruginosa, Ec = E. coli, Sa = S. aureus,* OFD = Oflodazole

Table 3.3 Comparison of Crude Extract of *Vernonia amygdalina* and Oflodazole (OFD) Efficacies

Organisms	OFD x 25 mg/ml	OFD x 50 mg/ml	OFD x 75 mg/ml
P. aeruginosa	0.0001	0.0002	0.0001
E. coli	0.0309	0.0324	0.0309
S. aureus	<0.0001	<0.0001	<0.0001

Figure 3.3 represents the effects of ethanolic extracts of *V. amygdalina* leaves at different concentrations (mg/ml) on three (3) bacterial isolates using oflodazole (OFD) and ethanol as controls. Experiments were conducted in triplicate and mean ± SD are represented as bar on the chart. Statistical significance is considered at P<0.05. It was observed that the ethanolic extract of *V. amygdalina* leave extract at 75 mg/ml concentration and the control antibiotics (Oflodazole) showed high significant variation (P<0.05) on *S. aureus* compared to *P. aeruginosa* and *E. coli* whereas at the same (75 mg/ml) concentration and the control (ethanol) did not show significant variation in the zones of inhibition on the isolates (*P. aeruginosa* and *S. aureus*) compared to the antibiotic control (oflodazole) (P>0.05) (Table 3.4).

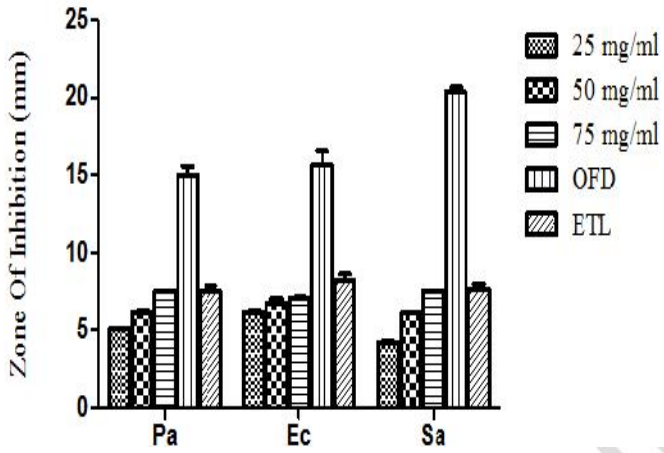

Figure 3.3: Effects of Ethanol Extracts of V. amygdalina Leaves on Bacterial Isolates. Experiments were conducted in triplicates and mean ± SD are represented as bar in the chart. Statistical significance is considered at *P*<0.05. Key: *Pa =P. aeruginosa, Ec = E. coli, Sa = S. aureus,* OFD = Oflodazole, ETL = ethanol.

Table 3.4 Comparison of Ethanol Extract of *V. amygdalina* with Oflodazole (OFD) Efficacies on the Bacterial Isolates

Organisms	OFD x 25 mg/ml	OFD x 50 mg/ml	OFD x 75 mg/ml	ETL x 25 mg/ml	ETL x 50 mg/ml	ETL x 75 mg/ml
P. aeruginosa	<0.0001	0.0001	0.0002	0.0035	0.0333	0.9372
E. coli	0.0004	0.0007	0.0006	0.0069	0.0362	0.0462
S. aureus	<0.0001	<0.0001	<0.0001	0.0005	0.0098	0.7109

These values are the respective P –values of comparisons

Figure 3.4 compares the effects of modern antibiotics (control) and aqueous extracts of *V. amygdalina* on three (3) bacterial isolates from wound infection. Low concentration of 25 mg/ml of the aqueous extract and Oflodazole (control) was used for antibiotics sensitivity studies and this showed significant difference in the zones of inhibition on all the test isolates with the control (antibiotics). More so, the isolates were treated with 50 mg/ml and 75 mg/ml concentrations of the aqueous extracts for 24 hrs at 37°C and were further exposed to oflodazole antibiotics disc from Oxoid, UK. The zones of inhibition were measured and the experiment repeated on two independent occasions. The aqueous extracts showed significantly lower levels (*P*<0.05) in the zones of inhibition on the isolates compared to the control (oflodazole) which showed higher significant variation in the zones of inhibitions (*P*<0.05) (Table 3.5) (figure 3.4). Nevertheless, extract has higher effect on S. aureus at graded concentration compared to *P. aeruginosa* and *E. coli*.

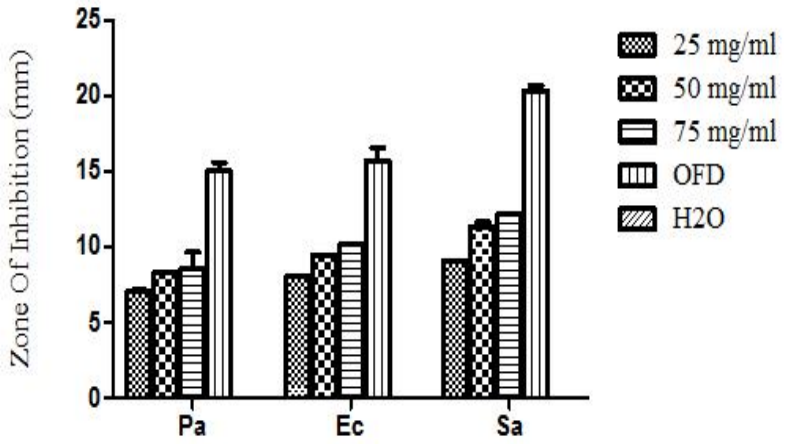

Figure 3.4: The Effects of Aqueous Extracts of *V. amygdalina* Leaves on the Bacterial Isolates. Experiments were conducted in triplicates and mean ± SD are represented in the chart. Significance is considered at $P<0.05$. Key: Pa = *P. aeruginosa, Ec =E. coli, Sa = S. aureus,* H_2O = Water, OFD = Oflodazole.

Table 3.5 Comparison of Aqueous Extracts of *V. amygdalina* with Oflodazole (OFD) Efficacies

Organisms	OFD x 25 mg/ml	OFD x 50 mg/ml	OFD x 75 mg/ml
P. aeruginosa	0.0002	0.0003	0.0069
E. coli	0.0010	0.0021	0.0033
S. aureus	<0.0001	<0.0001	<0.0001

The values of the respective P-values of comparisons

3.4 The Antimicrobial Susceptibility of *B. pinnatum* Leaves Extracts on the Test Isolates

The antimicrobial activities of crude extracts of *B. pinnatum* leave were investigated (Table 3.6, Figure 3.5). Comparison of the crude extract of *B. pinnatum* leaves with oflodazole antibiotics (control) showed significantly higher levels of inhibition to *S. aureus* at 75 mg/ml concentration as compared to *E. coli* and *P. aeruginosa* at the same concentration ($P<0.05$). The control (Oflodazole) showed more than 5-fold high mean ± SD with the plant extract (Figure 3.5).

Figure 3.6 depicts the efficacies of different concentrations of ethanol extracts of *B. pinnatum* leaves and controls (Oflodazole (OFD) and Ethanol (ETL)) on three (3) bacterial isolates. All concentrations of the extract showed significantly lower zones of inhibition ($P<0.05$) when compared with the control antibiotics (Oflodazole), (figure 3.6). However, the ethanol extract of *B. pinnatum* showed higher efficacies than the control (ethanol). Lower level of significance was observed on S.aureus compared to *P. aeruginosa* and *E. coli* with this plant ethanol extract and the control (Oflodazole) (Table 3.7, Figure 3.6). Moreover, all concentrations of the ethanol extracts of *B. pinnatum* leaves showed significantly lower variation ($P<0.05$) in the zones of inhibitions on *P. aeruginosa* compared to other isolates used in this study (Table 3.7).

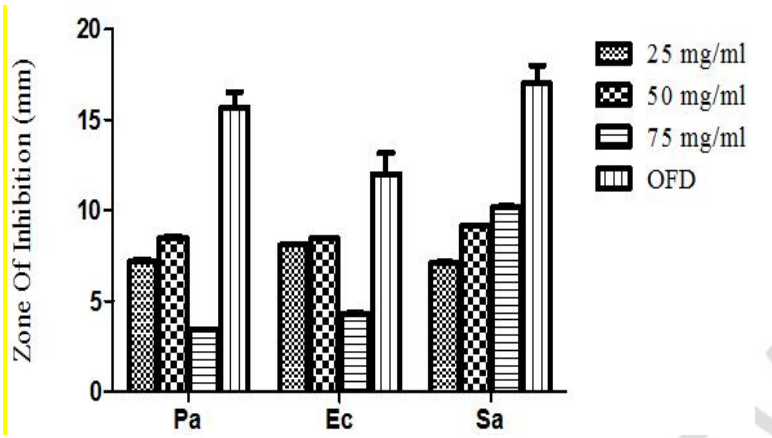

Figure 3.5: The Antimicrobial Activities of Crude Extracts of *B. pinnatum* Leaves on the Bacterial Isolates. Experiments were conducted in triplicates and mean ± SD are represented in the chart. Significance is considered at $P<0.05$. Key: Pa = *P. aeruginosa, Ec* = *E. coli, Sa* = *S. aureus,* H_2O = Water, OFD = Oflodazole.

Table 3.6 Comparison of Crude Extract of *B. pinnatum* Leaves with Oflodazole (OFD) Efficacies

Organisms	OFD x 25 mg/ml	OFD x 50 mg/ml	OFD x 75 mg/ml
P. aeruginosa	0.0005	0.0013	0.0002
E. coli	0.0280	0.0377	0.0026
S. aureus	0.0006	0.0014	0.0025

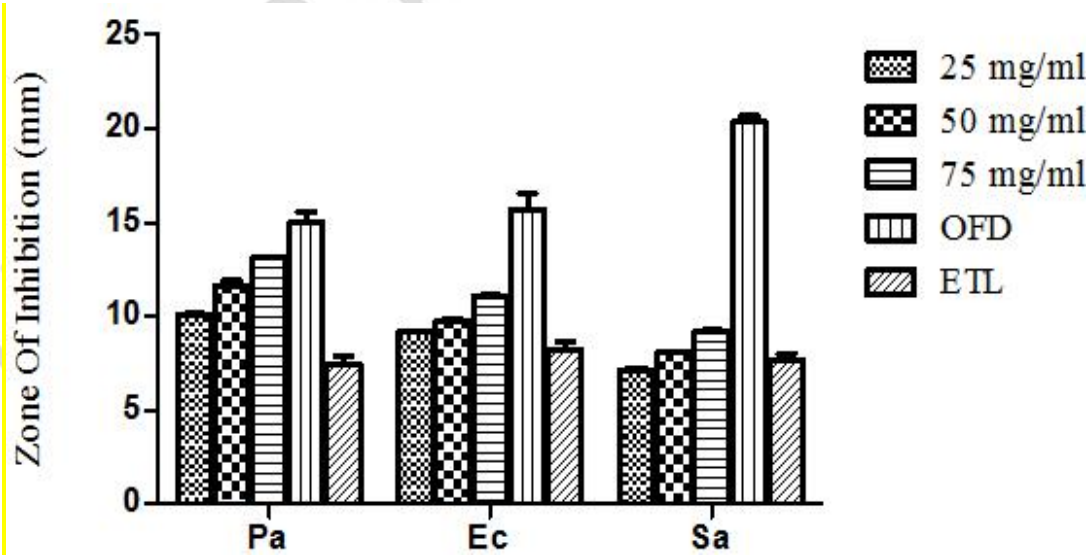

Figure 3.6: Antimicrobial Activities of Ethanol Extracts of *B. pinnatum* Leaves. Experiments were conducted in triplicates and mean ± SD are represented as bar on the chart. Statistical significance is considered at $P<0.05$. Key: Pa= *P. aeruginosa*, Ec= E. coli, Sa= S. aureus, OFD= Oflodazole, ETL= Ethanol.

Table 3.7 Comparison of Ethanol Extract of *B. pinnatum* Leaves with Oflodazole (OFD) and Ethanol Efficacies

Organisms	OFD x 25 mg/ml	OFD x 50 mg/ml	OFD x 75 mg/ml	ETL x 25 mg/ml	ETL x 50 mg/ml	ETL x 75 mg/ml
P. aeruginosa	0.0011	0.0061	0.0307	0.0027	0.0009	0.0001
E. coli	0.0018	0.0026	0.0065	0.0771	0.0190	0.0021
S. aureus	<0.0001	<0.0001	<0.0001	0.2206	0.3007	0.0106

The respective *P*-values of comparison

Figure 3.7 represents the efficacies of aqueous extracts of *B. pinnatum* on three (3) microbial wound isolates at graded concentrations. The aqueous extracts showed significantly lower variation on the zones of inhibitions on the test isolates when compared with the control (Oflodazole) ($P<0.05$) (Table 3.8). From the chart, it was observed that the efficacy of the aqueous extract of *B. pinnatum* leaves follows concentration gradient on the test isolates. The aqueous extract showed higher efficacy on *P. aeruginosa* compared to *E. coli* and *S. aureus* ($P<0.05$) but at 75 mg/ml concentration of the extract, *E. coli* showed insignificant variation on the zones of clearance with the control (Oflodazole) at $P>0.05$ (Table 3.8).

3.5 Antimicrobial Activities of the Combined Extracts of *V. amygdalina* and *B. pinnatum* on the Test Isolates

Figure 3.8 represents the efficacies combined extracts of *V. amygdalina* and *B. pinnatum* on microbial isolates. It was observed that *P. aeruginosa* had the highest zone of inhibition at all concentration (25, 50 and 75) mg/ml compared to *E. coli* and *S. aureus* at $P<0.05$.

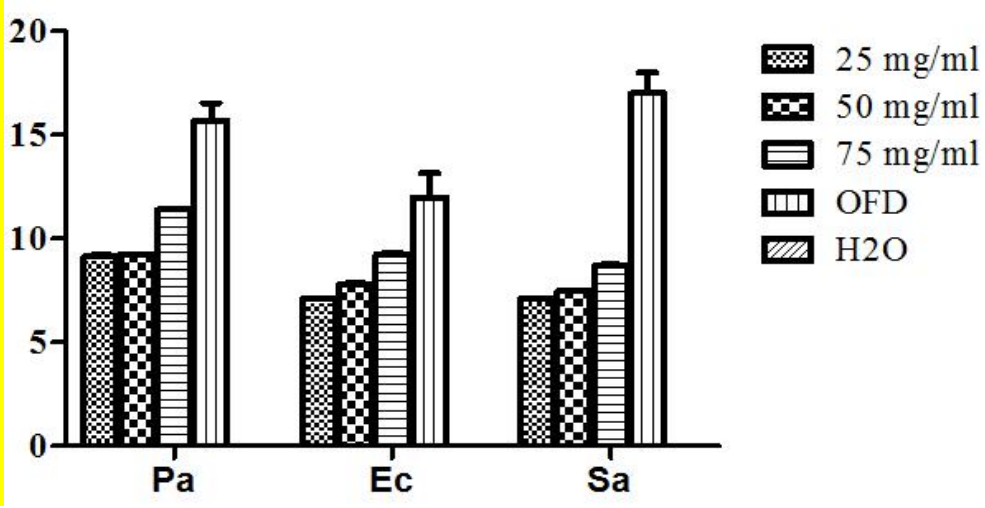

Figure 3.7: Antimicrobial Activities of Aqueous Extracts of *B. pinnatum* Leaves. Experiments were conducted in triplicates and mean ± SD are represented as bar in the chart. Statistical significance is considered at $P<0.05$. Key: *Pa = P. aeruginosa, Ec = E. coli, Sa = S. aureus*, H_2O = Water, OFD = Oflodazole.

Table 3.8 Comparison of Aqueous Extract of *B. pinnatum* Leaves with Oflodazole Efficacies

Organisms	OFD x 25 mg/ml	OFD x 50 mg/ml	OFD x 75 mg/ml
P. aeruginosa	0.0018	0.0019	0.0085
E. coli	0.0133	0.0221	0.0757
S. aureus	0.0006	0.0007	0.0012

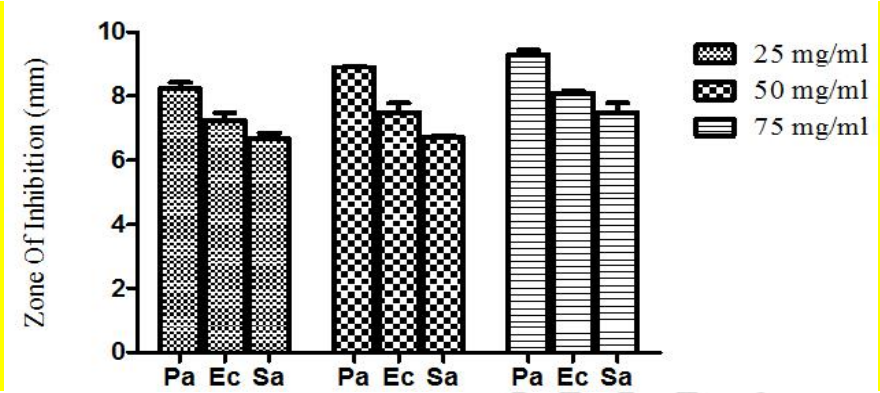

Figure 3.4: The Effects of Combined Extracts of *V. amygdalina* and *B. pinnatum* Leaves on the Bacterial Isolates. Experiments were conducted in triplicates and mean ± SD are represented in the chart. Significance is considered at $P<0.05$. Key: Pa = *P. aeruginosa, Ec =E. coli, Sa = S. aureus,* OFD = Oflodazole.

4.0 DISCUSSION

The phytochemical and antimicrobial activities of *B. pinnatum* and *V. amygdalina* leaves extracts against *P. aeruginosa*, *E. coli* and *S. aureus* isolated from wound infection were investigated. The trend of bacterial resistance to antibiotics is a serious challenge. Leaves and roots are useful therapeutic agents against some pathological infection [5]. Some researchers have found that microorganisms develop virulence properties and resistance due to antibiotics, antimicrobial and other endogenous molecule exposure [35, 36]. The result of this study indicated that the plants had some bioactive substances that have been recognized to have antimicrobial activities as reported by an earlier researcher [19]. These bioactive substances include tannins, Saponins, steroids, triterpenoids, alkaloids and others. They enhances the plants numerous functions in phytotherapeutic medicine [28, 37, 33].

The phytochemical results showed that the plants in this study have low levels of steroids as compared to other phytochemicals investigated. Quantitatively, steroids has the *P*-value of 0.2879 which is statistically not significant (P>0.05) as compared to other bioactive components of the plants which showed higher level of significance at *P<0.05.*On the other hand, *V. amygdalina* has the highest levels of saponins and other phytochemicals compared to *B. pinnatum* leaves extract as shown in the chart (figure 3.1, Table 3.2b). The present study disagrees with the observations of Effiong [38], who reported that flavonoids have the highest value followed by saponins. Ethanol and an antibiotic (Oflodazole) were used as control in comparison with the study plants extracts. Oflodazole (OFD) is very effective against gram negative and gram positive bacteria. It is a broad spectrum antibiotics made up of ofloxacin and ornidazole.

From the result, crude extract of *V. amygdalina* (figure 3.2), showed lower level of sensitivity to all the isolates except *S. aureus* which had higher level of efficiency when compared to the control (oflodazole) (P<0.05).The higher efficacy of the crude extract of *V. amygdalina* on *S. aureus* than *E. coli* and *P. aeruginosa* may be due to high content of bioactive constituents of the extract and the structural difference between the gram negative and gram positive bacterial isolates used in this study. This observation agrees with the research carried out by [28, 39].

The efficacy of ethanolic extract of *V. amygdalina* against *P. aeruginosa, E. coli* and *S. aureus* isolates (Figure 3.3, Table 3.4) showed high level of significance variation on the isolates than the control antibiotics (OFD) (P < 0.05). More so, the ethanol control and the extracts at 75 mg/ml concentration did not show significant variation on *P. aeruginosa* and *S. aureus* (P > 0.05) (Table 3. 4). The levels of sensitivity seen in ethanol extract of *V. amygdalina* in this study were moderately sensitive at high concentrations. This implies that the ethanol have the ability to extract the bioactive components such as saponins and other secondary metabolites that have antimicrobial properties.

The insignificant variation shown on *P. aeruginosa* and *S. aureus* (P> 0.05) compared to ethanol control in this study might be due to biofilm formation and virulence gene expression which has been shown in Monsi, et al. [46]. The ethanol extract of *Vernonia amygdalina* were reported to have shown some levels of inhibition on the test isolate growth which is in accordance with the results in this current study. Previous study have demonstrated antimicrobial effectiveness of methanol and ethanol extract of medicinal plants such as Aspilia Africa [27]; *Kalanchoe pinnata* [40]. Hence, ethanol could be a good solvent for extraction *Vernonia amygdalina* as herbal remedy for the treat of wound infection. The aqueous extract of *Vernonia amygdalina* leave showed significantly lowered levels of sensitivity (P<0.05) when compared to the control (Oflodazole) on all test isolates (*P. aeruginosa*, *E. coli* and *S. aureus*) in concentration dependent manner. Although, no significant variation in the zone of clearance was observed at 50 mg/ml and 75 mg/ml extract on *P. aeruginosa* when compared to that on *E. coli* and *S. aureus*. From the results (figure 3.4, Table 3.5) *S. aureus* isolates showed significantly higher levels of sensitivity (P = < 0.0001) at all concentrations of the extract (*V. amygdalina* leave) when compared to the significant level of sensitivity seen on *P. aeruginosa* and *E. coli*. The actions of aqueous extracts on *S. aureus* in this study agree with that of [41], [1]. The high level of sensitivity seen on *S. aureus* may be due to structural difference in their cell wall and water as solvent. The aqueous extract seemed to have more affinity to the cell wall of gram positive bacterial than the gram negative bacteria. The aqueous extract of the load of *P. berghei* in mice by 73% when given intra-peritoneally for 4 days [42]; [43] has also corroborated the effectiveness of the aqueous extract of *V. amygdalina* in managing malaria. The efficacy of the crude extract of *Bryophyllum pinnatum* on the test isolates showed higher significant level of sensitivity on *S. aureus* compared to *P. aeruginosa* and *E. coli* at three different concentrations (figure 3.5). Lower levels of sensitivities are seen with the crude extract compared to the control (Oflodazole) (figure 3.5) (P <0.05).

At higher concentration (75 mg/ml) of the crude extract of *Bryophyllum pinnatum*, the gram negative test isolates showed lower level of significant activity (figure 3.5). This difference in the efficacy of the crude extract of *B. pinnatum* leave on the gram positive and the gram negative bacterial isolates used in this study may be due to the high content of peptidoglycan with teichoic acids present in gram positive bacteria and or less penetration affinity of the gram negative bacteria cell wall to high concentration of *Bryophyllum pinnatum* crude extracts. Previous studies have shown antimicrobial effectiveness of crude extract such as *Garcinia kola* [44] but the crude extract of *B. pinnatum* in this present study contrast with that of [45] who stated that the higher the concentration of the extract the higher the activity of the substance. The efficacy of ethanol extract of *Bryophyllum pinnatum* leave as demonstrated in figure 3.6 showed maximum level of significant zone of inhibition on *P. aeruginosa* than *E. coli* and *S. aureus* respectively (P <0.05). Generally on the ethanolic extract of B.pinnatum, Maxium efficacy was shown on the gram negative isolates compared to the gram positive isolates (P<0.05). This difference in the efficacy might bedue to structural differences in their cell walls. The gram positive have thick layer of peptidogly can with techoic acid while the gram negative has a thin layer of the peptidoglycan. The present study agrees with that of [16] and [27]. The effectiveness of the extract also showed that the higher the concentration the higher the efficacy (figure 3.6).

Furthermore, the aqueous extract of *Bryophyllum pinnatum* antimicrobial activity followed concentration gradient and organism dependent. Figure 3.7 showed that the higher the concentration, the higher the efficacy of the extract. The significant level of variation between the extract and the control (oflodazole) was low on *E. coli* (P>0.05) (Table 3.8) at 75 mg/ml (Figure 3.7). This implies that at higher concentration of the aqueous extract of *B. pinnatum* there was no significant variation in the level of efficacy between the extract and the control on *E. coli*. However, the aqueous extract showed higher zone of inhibition on *P. aeruginosa* compared to *E. coli* and *S. aureus* isolates used in this study. These contradict the study of [41] and [1] that recorded *S. aureus* as the more sensitive isolate to aqueous extracts of *B. pinnatum* leaves. The experiment performed on the effect of combined extracts of *B. pinnatum* and *V. amygdalina* leaves had effect on all the test isolates but showed more efficacy on the gram negative bacteria isolates than the gram positive isolate. *P. aeruginosa* compared to *E. coli* and *S. aureus* showed the highest zone of clearance at all concentrations (mg/ml) (25, 50 &75). The findings of this present study revealed a uniform effect of both herbs when combined together on the test bacterial isolates used. At concentrations of 25 mg/ml, 50 mg/ml and 75 mg/ml combined extracts, *P. aeruginosa, E. coli* and *S. aureus* were effectively inhibited. Many authors like [32, 27] had previously documented the antimicrobial capabilities of each of these important herbs particularly when used as single therapy at high concentrations.

Using the concept of combined medication therapy has produced synergistic interaction that could assist the concentration capable of complete inhibition of the organisms. Consequently, as the concentration of the extract increased, the activities on the bacterial isolates also increased insignificantly (P>0.05), (Figure 3.8). Moreover, the least concentration used in this study still produce significant zone of clearance on the test isolates supporting the action of combined regimen of the research done by [34]. Determining the antimicrobial efficacy *B. pinnatum* and *V. amygdalina* leaves extract on the selected wound isolates, the study generally showed significant difference in the zones of inhibition produced by the isolates with respect to varying concentrations (P<0.05). Comparing the activities of the both plant extracts on the wound isolates of *P. aeruginosa, E. coli* and *S. aureus* to that on the control antibiotics, there was significant variation in the zone of inhibition produced (P<0.05). The antimicrobial results of this study confirm the uses of *B. pinnatum and V. amygdalina* in the treatment of various infectious diseases as claimed by ethano medicinal professional (Herbalist).

Nevertheless, the activities of both plant extracts may produce insignificant variation at (P >0.05) in the zones of inhibition of the extract with that of the control antibiotic at higher concentration which now implies that the action of the extracts against the isolates and the control (ofloadazole) is the same.

5 Conclusion

The study recorded the inhibitory effects of both extracts on the selected organisms. The ethanol extracts of both plants had more inhibitory effects on all the test isolates. Therefore, the antimicrobial effects of these plant extract depends on the concentration, pH, the organism and or the solvent used for extraction.

6 Recommendations

It is recommended that these plants should be introduced in the tertiary health institution in Nigeria were modern antibiotics resistance and further studies should be done on these plants to determine the mode of action of the extracts and its biochemical targets on the microorganisms and also using animal model to ascertain the safety of combined extract to human consumption.

REFERENCES

[1] Oshim, O. I., Desmon, O. C., Nwobu, U. A. R., Azugwu, M. U. & Urama, U. E. (2016).Kinetics of minimum fungicidal concentration of *Vernonia amygdalina* (Bitter leaf) on microorganisms isolated from wound infections. International Journal of Surgical Research, 5(1), 8-14.

[2] Prescott, L. M, Harley. J. P. & Klein, D.A. (2011). Microbiology, *McGraw Hill. Washington.* Pp 908-909.

[3] Torpy, J. M., Alison, B. & Richard, M. C. (2005). Surgical Wound Infections. Journal of America Medical Association, 294(2), 21-22.

[4] Cercenado, E. & Ruiz De Gopegui, E. (2008). Community-acquired Methicillin-resistant Staphylococcus Acquired Methicillin-resistant *Staphylococcus aureus*. Journal of Infection Clinical Microbiology, 13, 19-24.

[5] Iram, G., Mariam, S., Halima, S., Shahbaz, M. A & Amin, M. A. (2012). Effect of Garlic and ginger including Aqueous Extract and Ethanol extract had been assayed separately against Drug Resistant Strains. Animals of Clinical Microbiology and Antimicrobials 11(8), 11-18.

[6] Mboto, C. L., Eja, M. E., Adegoke, A. A., Iwatt, G. D., Abikong, B. E., Takon, I. & Udo, I. (2009). Evidence of accelerated healing of male circumcision wounds, Fresh wounds and chronic ulcers using combined therapy of *Garcinia kola*, *Vernonia amygdalina* extracts in honey. African Journal of Microbiology Research, 3(9), 557-559.

[7] World Health Organization (2008).The promotion and development of traditional medicine. Technical report series. 622 - 630.

[8] Lovet, T., Kigigha. R. & Ebubechkwu, O. (2015). Global use of medicinal plants. *Key* Journal of Microbiology Research, 3(4), 41 – 45.

[9] Pravi, C.T (2007). Medicinal plants: Traditional knowledge. International Put Limited New Delhi pp. 216

[10] Preethi, R. M., Devanthan, V. V. & Longathan, M. (2010). Antimicrobial and antioxidant efficacy of some medicinal plants against food borne pathogens. Advance in Biological Research, 4, 122 – 125.

[11] Akinjogunla, O.J., Yah, S.C., Eghafona, N. O & Ogbemudia, F.O. (2010). Antibacterial activity of leave extracts of *Nymphaea lotus* (Nymphaeceae) on Methicillin Resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) isolated from clinical samples 1(2), 17.

[12] Odugbemi, T. (2006). Outline of medicinal plants in Nigeria. 1st Edition. University of Lagos Press, Nigeria, pp. 77.

[13] Raymond, I. Ozolua, C., Eboka, J, Comfort, N. & Duru, D. (2010). Effect of aqueous leaf extract of *Bryophyllum pinnatum* on guinea pig tracheal ring contractility. Niger Journal of Physiological Science, 25, 149-157.

[14] Nwali, B. U., Okaka, A. N., Ibian, U. A. & Aja, P. M. (2012). Phytochemical Composition of *Bryophyllum pinnatum* leaves. International Journal of African Biological Research, 2(4), 614-616.

[15] Amabe, O.A., Kebe, E.O., Emem, S. E & Mokutima, A.E. (2014). The effect of ethanolic Extract of *Bryophyllum pinnatum* on the microanatomy of the tests of adult males wister rats. European Journal of Biology and Medical Science Research, 2(2), 37-44.

[16] Anibijuwon, I. I., Oladejo, B. O. Aletitun, D. O. & Kolawole, O. M. (2012). Antimicrobial activities of *Vernonia amygdalina* against oral microbes. Global Journal of Pharmacology, 6(3), 178-185.

[17] Cogen, A.L., Nizet, N. & Gallo, R.L. (2008). Skin microbiota: A source of disease of defence British Journal of Dermatology, 158 (3), 442-455.

[18] Evans, W. C. (2002). Trease & Evans pharmacognosy, 15th edition. Saunders London.

[19] Sofowora, A. I. (2008). Screening Plant for bioactive agents In: Medical plants and traditional medicine in Africa. 3rd edition 199-203.

[20] Cheesbrough, M (2006). Microbial identification and antimicrobial susceptivity. District Laboratory Practice in tropical Countries part *2*. Cambridge University Press, United Kingdom. Pp 157-178.

[21] Betty, A. F., Daniel, F. S & Alice, S. W. (2007). Pseudomonas, Burkhoderia and similar organisms. In: Bailey & Scott's Diagnostic Microbiology. 12th edition 24: 340-344.

[22] Ochei, J. & Kolhatkar, A. (2008). Isolation and identification bacteria. In: Medical Laboratory Science Theory and Practice. New McGraw-Hill Publishing Company Limited. Pp. 644-658.

[23] Baker, F. J., Silverton's, R. E. & Palister C. J. (2011). A generalized scheme for the isolation and identification of bacteria from pathological specimens. In: Introduction to Medical Laboratory Technology, 3(1), 307-315.

[24] Akinjogunla, O. J., Ekoi O. & Odeyemi, A. T. (2011). Phytochemical Screening and in-vitro antibacterial Assessment of Aqueous leaf Extracts of *Vernonia amygdalina* (Asteraceae) and *Ocimum gratissimum* (Lamiaceae) on Moxifloxacin resistant *Escherichia coli* Isolated from Clinical and Environmental Samples. Nature and Science, 9(7), 12-16.

[25] Clinical Laboratory Standard Institute (CLSI) (2012). *Performance Standard for Antimicrobial disc susceptibility tests; approved standard.* 11th Edition USA Wayne.

[26] Okwu, D.E. & Josiah, C. (2006). Evaluation of the chemical composition of two Nigerian medicinal plants. African Journal of Biotechnology, 5(4), 357-361.

[27] Azuonwu, O., Azuonwu, T.C & Ibulubo, D (2017). Antimicrobial activity of leave extracts of *Bryophyllum pinnatum* and *Aspilia Africana* on pathogenic wound isolates recovered from patients Admitted in University of Port Harcourt Teaching Hospital, Nigeria. *Annals of Clinical and Laboratory Research* Medical Public Journals. 5(3), 185-195.

[28] Okwu, D.E & Nnamdi; F.U. (2011). Two novel flavonoids from *Bryophyllum pinnatum* and their antimicrobial activities. *Journal of Pharmaceutical Chemistry,* 3(2), 1-10.

[29] Fred, O., Oboh, J., Anita, A., Monday,I. I & Derek, A. (2013). Nutrient Phytochemicals. Fungi flora and aflatoxin in fresh and salted *Vernonia amygdalina* leaves. African Journal of Biotechnology. 12(38), 5655-5661.

[30] Ochei, J. & Kolhatkar, A. (2008). Isolation and identification bacteria. In: Medical Laboratory Science Theory and Practice. New McGraw-Hill Publishing Company Limited. Pp. 644-658.

[31] Cock, T. (2008). The Invitro Antimicrobial Activities of the various Fractions against a range of micro organisms. Journal of Aloe Vera Study*,* 2, 12-34.

[32] Adetunji, C. O., Olaniyi, O. O & Ogunkunle, A. T. (2013). Bacterial activity of crude extracts of *Vernonia amygdalina* on clinical isolates. Journal of Microbiology and Antimicrobial.

[33] Etim, L. B., Obande, G.A., Aleruchi, C. & Bassey, V. E. (2016). Antimicrobial potential of Bryophyllum pinnatum leaf extracts on bacteria obtained from infected infant respiratory tract. British Journal of Pharmaceutical Research, 10(6), 1-8.

[34] Abubakar, A. A., Oladele, H. A., Adejumoke, A. A., & Kayode, J. F. (2014). Synergistic effect of Combined Extract of *Bryophyllum pinnatum* and *Aloe Barbadensis* Enhances Antimicrobial Activity in-Vitro. Global Advanced Research Journal of medicine and Medical Sciences, 3(1), 026-032.

[35] Freestone, P.P., Hirst, R., Sandrini, S., Sharaff, F., H. & Hyman, S. (2012b). Pseudomonas aeruginosa – catechlomine inotrope interactions: A contributory factor in the development of ventilator associated pneumonia? *Chest,* 142 (5), 1200-1210.

[36] Sandrini, S., Alghofaili, F., Freestone, P.P.E. & Yesikaya, a, H. (2014). Host stress Hormone norebiofilm formation and virulence gene expression. *Biomedical central Microbiology*, 14 (1),

[37] Imaga, M. O. A. & Bamigbetan, D. O. (2013).Invivo biochemical assessment of aqueous extracts of *Vernonia amygdalina* (bitter leaf). *International Journal of Nutrition and Metabolism,* 5(2), 22 – 27.

[38] Effiong, E. E. (2014). Phytochemical, proximate, vitamins and mineral composition of *Ocimum gratissmum* leave. *Journal of Physical and Chemical Sciences,* 114, 321 – 329.

[39] Arekemase, M. O. & Oyeyiola, K. I (2013). Assessment of *Vernonia amygdalina* on some selected pathogenic micro organisms from University of Ilorin teaching hospital. *Journal of Microbiology, Biotechnology and Food Sciences,* 2(5), 2360 – 2365.

[40] Nayak, B. S., Marchsall, J. R. & Isitor, G. (2010). Wound healing potential of ethanolic extract of *kalanchoe pinnata*. Leaf a preliminary study. *Indian Journal of Experimental Biology,* 48, 72-576.

[41] Nwanjo, H. (2005). Efficacy of aqueous leaf extract of *Vernonia amygdalina* on plasma lipoprotein and osicative status in diabetic rat models. *Journal of Physiological Science,* 20(1-2), 30 – 42.

[42] Njan, A. A., Adza B., Agaba A. G., Byamgaba D., Diaz-Liera S., & Bansberg D. R. (2008). The analgesic and antiplasmodial activities and toxicology of *Vernonia amygdalina. Journal of Medicinal Food,* 11, 574-581.

[43] Iwalokun, B. A., Efedede, B. U., Alabi-Sofunde, J. A., Oduala, T., Magbagveola, O. A. & Akinwande, A. I. (2008). Hepa-toprotective and anti-oxidant activities of *Vernonia amygdalina*on acetaminophen-induced heptic-damage in mice. *Journal of Medicinal Food,* 9(4), 524 – 530.

[44] Adegboye, M.F., Akinpelu, D.A & Okoh, A. (2008). The bioactive and phytochemical properties of garcirica kola (Hecked) seed extract on some pathogens. *African Journal of Biotechnology*, 7 (21), 3934-3938.

[45] Ijeh, I. & Adedokun, A. T. (2006). Effects of administration of ethanolic extract of *Vernonia amygdalina.* On kidney function of experimental rabbit model. *Research Journal of Biotechnology,* 1, 34 – 35.

[46] Monsi, T. P., Abbey, S. D., Wachukwu, C. K. & Wokem, G. N. (2018). Levels of biofilm expression in *Klebsiella pneumoniae* isolates exposed to herbal drugs. Journal of Advances in Microbiology, 12(1), 1–7.