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

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 hence, the 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 *P. aeruginosa, E. coli* and *S. aureus* isolates from wound infection.
Methodology: The fresh leaves of both plants were extracted using sofowora method and the phytochemicals screened. Each concentrations and antibiotic and ethanol set up as control were tested against the isolates using disc diffusion techniques.
Results: Alkaloids, flavonoids, tannins, anthraquinone and fixed oils were present on 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 (3.96%) than *Bryophyllum pinnatum* (0.40%), flavonoids 3.12% and 2.78%, tannins 0.46% and 1.24%, alkaloids 1.69% and 0.43%, and steroids 0.58% and 0.40% respectively. The crude extract of *V. amygdalina* had the highest zone of inhibition with 10.1 mm at 0.75 mg/ml concentration 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, there was no significant difference in their zones of inhibition produce since (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 depends on the concentration of the extract and the solvent used for extraction.

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

1 INTRODUCTION

Wound could be defined as an opening or abrasion in the skin as a result of exposure of the subcutaneous layer of the skin that provides moist, warm and nutritious environmentt 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 [1]. Wound infection may occur due to contaminant that debase the cleaning effect of the host's immunity, colonizes and proliferate in the host [2].

Comment [R1]: REMOVE IT

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 upset, 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 *Bryophyllum pinnatum* and *Vernonia amygdalina* on *pseudomonas aeruginosa, Echerichia coli* and *Stapylococcus 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 45'N 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 [20].

2. 8.1 Biochemical Assays

The biochemical analysis to confirm the isolated bacterial were carried out according to the methods described in [21] [22] [23].The confirmatory tests performed include indole, catalase coaglase, oxidase, motility, citrate utilization and urease tests.

2.8.1.1 Indole Production

Tryptophan broth was inoculated at 1 drop of 24 hrs broth culture and incubated at 35°C in ambient air for 48 hrs. 0.5 ml Kovac's reagent was added to the broth culture. A pink wine colored ring at the top after adding the Kovac's reagent signifies a positive result.

2.81.2 Catalase

A drop of hydrogen peroxide was dispensed onto a clean grease free slide and the bacteria isolate was inoculated onto the slide immediately, the slide was examined for bubbles of gas proving catalase activity [23].

Comment [R2]: REMOVE IT OR INCLUDE THE TESTS NAME IN BIOCHEMICAL ASSAYS SECTION ITSELF.

2.8.1.3 Coagulase

Slide method

A colony of the organism (Staph species) was added onto a drop of distilled water on a clean grease free microscopic slide and a loopful of blood plasma was mixed. These were an immediate clumping on positive slide and no clumping on the other slide which serves as a negative control. The clumping on the positive slide indicates the direct conversion of fibrinogen to fibrin [22] [23].

2.8.1.4 Oxidase Test

Using a filter paper initially impregnated with 1% substrate (Tetramethyl-P-phenylenediamine dihydrochloride, a sterile wire loop was used to collect a small part of a 24 hrs bacteria colony from the pure cultures and smeared on the filter paper containing were observed for colour change of blue-purpose due to the oxidation of tetramethyl-p-phenylenediamine dihydrochloride within few seconds and no colour change signifies a negative result [20] [22] [23].

2.8.1.5 Citrate Utilization Test

A slop of the culture media in the bijou bottle was inoculated slightly with 24 hrs test organism and incubated for 48 hrs at 37°C, development of a bright blue colour indicates positive test and no colour change indicates a negative citrate test. The organisms were sub cultured onto nutrient and MacConkey agar and were maintained on nutrient agar slants at 4° for antimicrobial analysis [24].

Comment [R3]: INCLUDE IN BIOCHEMICAL TEST PART.

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. Ceftriazone, oflodazole, ciprofloxacin 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.

2.10 Data Analysis

Data from this study were analyzed using Statistical Package for Social Science (SPSS) version 23. *P*-values less than 0.05 ($p<0.05$) was considered statistically significant.

3.0 RESULTS

3.2 Phytochemical Analyses of the Plants Extracts

Table 1 shows the results of the preliminary phytochemical screening of the extracts of the *B. pinnatum* and *V. amygdalina* revealed the presence of alkaloids, flavonoids, triterpenoids and carbohydrates respectively as shown in table 2. The results revealed the presence of active compounds in the plants studied. From the table below, it was observed that alkaloids, flavonoids, tannins, triterpenoids and carbohydrates were present in both plants but cardenolide were absent only in the leaves of *V. amygdalina* as saponins were absent in *B. pinnatum* leaves while Anthraquinone were absent in the leaves of the both plants as stated in table 1 .

Table 2 indicates the quantification of some chemical components of *V. amygdalina* and *B. pinnatum* leaves and the results obtained revealed that the total tannins content of *B. pinnatum* and *V. amygdalina* were 0.46% and 1.24%, Flavonoids contents obtained were 3.12% and 2.78%, Saponins were 3.96% and 0.40%, Alkaloids were 1.69% and 0.43% and Steroids 0.58% and 0.40% respectively as stated in table 2.

Table 1: 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

Table 2: Quantification of some Chemical Components of *B. pinnatum* and *V. amygdalina* leaves extracts

Phytochemicals	*V. amygdalina* (%)	*B. pinnatum* (%)
Tannins	0.46	1.24
Flavonoids	3.12	2.78
Saponins	3.96	0.40
Alkanoids	1.69	0.43
Steroids	0.58	0.40

Table 3: The inhibitory effect of extracts of *Vernonia amygdalina* on *Pseudomonas aeruginosa, E. coil* and *S. aureus* at different concentrations (mg/ml) and zones of inhibition (mm).

Organisms	Aqueous extracts			Ethanol extracts			Crude extracts		
	25 mg/ml	50 mg/ml	75 mg/ml	25 mg/ml	50 mg/ml	75 mg/ml	25 mg/ml	50 mg/ml	75 mg/ml
P. aeruginosa	1.0	2.4	3.4	5.1	6.2	7.5	0.01	0.1	0.2
E.coli	2.0	3.5	4.1	0.01	0.1	1.0	R	R	R
S. aureus	3.1	5.1	6.2	4.1	6.2	7.5	7.1	8.2	10.1
	X^2 = 1.772, P = 0.99;			*X^2 = 7.263, P = 0. 51*			X^2 = 1.77, P= 0.78)		

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

Table 3 shows that the antimicrobial effect of crude extracts of *V. amygdalina* had zones of inhibition (mm) of 0.01, 0.1, and 0.2, on *Pseudomonas aeruginosa* at concentrations(mg/ml) of 25, 50, 75 respectively as shown in table 3. The crude extract of *V. amygdalina* had no effect on *E.coli* as compared to the antibiotics and alcohol which serves as control but had effect on *S. aureus* with zones of inhibition (mm) of 7.1, 8.2 and 10.1, at concentration (mg/ml) 25, 50, 75 respectively as shown in table 3 above. There was no significant difference (X^2 = 1.77, P= 0.78) in the zones of inhibition.

The ethanol extract of *V. amygdalina* had zones of inhibition (mm) of 5.1, 6.2 and 7.5 on *Pseudomonas aeruginosa* at concentrations (mg/ml) 25, 50, 75, respectively and 0.01, 0.1, 1.0, on *Escherichia coli* at concentrations (mg/ml) 25, 50 and 75, respectively but inhibits *Staphylococcus aureus* with zones of inhibition (mm) of 4.1, 6.2 and 7.5 at concentrations (mg/ml) 25, 50 and 75 respectively as shown in table 3. There was no significant difference (*X^2 = 7.263, P = 0.51)* in the zones of inhibition.

The aqueous extract of *V. amygdalina* had zones of inhibition (mm) of 1.0, 2.4, 3.4, on *Pseudomonas aeruginosa* at concentrations (mg/ml) 25, 50, 75, respectively. Moreover, it had zones of inhibition (mm) of 2.0, 3.5 and 4.1 on *Escherichia coli* at concentrations (mg/ml) 25, 50 and 75 respectively and zones of inhibition (mm) of 3.1, 5.1 and 6.2 on *Staphylococcus aureus* at concentrations (mg/ml) 25, 50 and 75 respectively. There was no significant difference (X^2 = 1.772, *P* = 0.99) in the zones of inhibition in the effect of aqueous extract and the orthodox drugs as shown in table 3.

Table 4. The inhibitory effect of extracts of *B. pinnatum* on *Pseudomonas aeruginosa, E. coil* and *S. aureus* at different concentrations (mg/ml).

Organisms	Aqueous extracts			Ethanol extracts			Crude extracts		
	25 mg/ml	50 mg/ml	75 mg/ml	25 mg/ml	50 mg/ml	75 mg/ml	25 mg/ml	50 mg/ml	75 mg/ml
P. aeruginosa	3.1	3.3	5.5	4.0	5.1	7.0	1.0	2.4	3.5
E. coil	1.0	1.7	3.0	3.1	3.9	5.0	2.0	2.5	4.4
S. aureus	1.1	1.5	2.8	1.0	2.0	3.3	1.0	3.2	4.3
	X^2=1.80, *P* = 0.77			X^2=1.55,*P* = 0.82			*X^2=1.28,P=0.87*		

Table 2 indicates that the antimicrobial activities of crude extracts of *Bryophyllum pinnatum* had zones of inhibition (mm) of 1.0, 2.4 and 3.5 on *Pseudomonas aeruginosa* at concentrations (mg/ml) 25, 50 and 75 respectively. *Escherichia coli* were inhibited with the zones of inhibitions (mm) of 2.0, 2.5 and 4.4 at concentrations (mg/ml) 25, 50 and 75 respectively and had zones of inhibition (mm) of 1.0, 3.2, 4. 3, on *Staphylococcus aureus* at concentrations (mg/ml) 25, 50 and 75 respectively. There was no significant difference *(X^2 = 1.28, P = 0.87) in the* zones of inhibition in the effect of crude extract of *Bryophyllum pinnatum*.

The antimicrobial effect of ethanol extracts of *Bryophyllum pinnatum* leaves had zones of inhibition (mm) of 4.0, 5.1 and 7.0 on *Pseudomonas aeruginosa* at concentrations (mg/ml) of 25, 50, 75, respectively. The effect of ethanol extracts of *Bryophyllum pinnatum* leaves on *E. coli* had zones of inhibition (mm) of 3.1, 3.9 and 5.0 at concentrations (mg/ml) of 25, 50, 75, respectively and zones of inhibition (mm) of 1.0, 2.0 and 3.3 on *Staphylococcus aureus* at concentrations (mg/ml) 25, 50, 75 respectively as shown in table 4 above. There was no significant difference (X^2 = 1.55, P = 0.82) in the zones of inhibition.

The aqueous extract of *Bryophyllum pinnatum* leaves had antimicrobial activities on *Pseudomonas aeruginosa with zones* of inhibition (mm) of 3.0, 3.3 and 5.5 at concentrations (mg/ml) of 25, 50 and 75 respectively and had *zones* of inhibition (mm) of 1.0, 1.7 and 3.0 on *E. coli* at concentrations (mg/ml) of 25, 50 and 75 respectively. It also had effect on *Staphylococcus aureus* with the zones of inhibitions (mm) of 1.1, 1.5 and 2.8 at concentrations (mg/ml) of 25, 50 and 75 respectively as shown in table 4 above. There was no significant difference (X^2 = 1.80, *P* = 0.77*)* in the zones of inhibition.

Table 5 The Antimicrobial Activity of Combined Leaves extracts *V. amygdalina* and *B. pinnatum* and the controls on the Test Isolates

Organisms	V.A +B.P 1:1 ratio	OFD Zones of	CPX Inhibition	CEF (mm)	H^2O	ETL
P. aeruginosa	**2.6**	**1.4**	**1.1**	**0.5**	**0.0**	**0.6**
E .coli	**1.1**	**1.0**	**1.0**	**0.1**	**0.0**	**0.8**
S. aureus	**0.5**	**5.0**	**2.0**	**0.5**	**0.0**	**1.0**

X^2 = 1.60, P = 0.45 **KEY:** V.A= Vernonia amygdalina, B.P= Bryophyllum pinnatum, OFD= Oflodazole,CPX= Ciprofloxacin, CEF= Ceftriazone,H2O= Water, ETL= Ethanol.

3. Entails the antimicrobial effects of combined leaves extract of *Vernonia amygdalina and Bryopyllum pinnatum* on *P. aeruginosa, Escherichia coli and Staphylococcus aureus. The mixed* extract had zones of inhibition (mm) of 2.6, 1.1 and 0.5 respectively as shown in the table above. There was no significant difference (X^2 = 1.60, *P* = 0.45) in the zones of inhibition between the mixed extract and the orthodox drugs.

4 DISCUSSION

The ethanolic extracts of the both plants showed higher inhibitory effects against all the tested organisms than the aqueous and the crude extracts. This could be due to the ability of the ethanol to extract some of the active components or compounds from the leave samples like Saponin*;* phenolic compounds, and other secondary metabolites that have antimicrobial properties [26],[27]. These findings agrees with [29][28] who have demonstrated that *Bryopyllum pinnatum* and *Vernonia amygdalina* leaves extracts have antimicrobial activity against many species of microorganisms. This study observed that the two plants have antibacterial activities against both gram positive and gram negative bacteria. The difference in the zones of inhibition between the gram positive and the gram negative bacteria to the different plant extracts may be due to their structural differences in their cell walls. The gram positive cell wall is thicker than the gram negative cell wall because of its high content of peptidoglycan with teichoic acids while the gram negative bacteria cell wall has a thin layer of peptidoglycan without teichoic acids [30].

The concentrations of the extracts also determine the extent or level of susceptibility as observed in this study. That is, the higher the concentration, the higher the inhibitory activities of the substance. This observation is in agreement with the observations of [31], [32]. Hence, as the concentration of the both extracts decreases, the antimicrobial activities on the tested isolates also decreased insignificantly (*P* > 0.05). However, the least concentrations of one of the crude extract of *V. amygdalina* at 25 mg/ml produced significant zone of inhibition of 7.1 mm on *S. aureus* except *P. aeruginosa* with zone inhibition of 1.01 mm and *E. coli* which showed resistance. The result in this study revealed a high level of resistance to the standard antibiotics by the isolates. The susceptibility of *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* were tested against some selected broad spectrum antibiotics which includes oflodazole, ciprofloxacin and ceftriazone. *S. aureus* was inhibited by oflodazole with the zone of inhibition of 5.0 mm, Ciprofloxacin = 2.0 mm, ceftriazone = 0.3 mm, *P. aeruniginosa* with 1.4, 1.1, 0.01 and *E. coli* with 1.01, 1.01 and 0.1 respectively. The result in this study revealed a high level of resistance to the standard antibiotics by the isolates. In comparison to the leave extracts of *V.*

The susceptibility of *Pseudomonas aeruginosa, Escherichia coli* and *Staphylococcus aureus* were tested against some selected broad spectrum antibiotics which includes oflodazole, ciprofloxacin and ceftriazone. *S. aureus* was inhibited by oflodazole with the zone of inhibition of 5.0 mm, Ciprofloxacin = 2.0 mm, ceftriazone = 0.3 mm, *P. aeruniginosa* with 1.4, 1.1, 0.01 and *E. coli* with 1.01, 1.01 and 0.1 respectively. The result in this study revealed a high level of resistance to the standard antibiotics by the isolates. In comparison to the leave extracts of *V.* The

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In comparison to the leave extracts of *V. amygdalina* and *B. pinnatum* moreover, the antimicrobial activities of the test isolates to both crude, ethanol and aqueous extracts of the both plants were slightly higher than to the antibiotics selected but the difference is insignificant (P>0.05). This study agrees with [33]. The combined extracts of the both plants had effect on both the gram negative and gram positive bacteria isolates tested in this study. It was observed that the both herbs when combined together can act synergistically on some bacteria isolates, proving the action of combined regimen of the research work by [34]. This study has shown that a combination of *B. pinnatum and V. amygdalina* extracts inhibits the growth of *P. aeruginosa, E. coli and S. aureus* at higher concentration. The assessment of the efficacy of the crude, ethanol and aqueous extracts of *B. pinnatum* and *V. amygdalina* on the selected bacteria isolated from wound showed no significant difference in the zones of inhibition observed by the isolates in respect to the graded concentrations (P> 0.05).The antimicrobial results of this study confirms the uses of *B. pinnatum and V. amygdalina* in the treatment of various infectious diseases as claimed by ethano medicinal professional (Herbalist). The assessment of the efficacy of the crude, ethanol and aqueous extracts of *B. pinnatum* and *V. amygdalina* on the selected bacteria isolated from wound showed no significant difference in the zones of inhibition observed by the isolates in respect to the graded concentrations (P> 0.05). The antimicrobial results of this study confirms the uses of *B. pinnatum and V. amygdalina* in the treatment of various infectious diseases as claimed by ethano medicinal professional (Herbalist). The assessment of the efficacy of the crude, ethanol and aqueous extracts of *B. pinnatum* and *V. amygdalina* on the selected bacteria isolated from wound showed no significant difference in the zones of inhibition observed by the isolates in respect to the graded concentration. Hence, comparing the antimicrobial activities of the various extract of the both plants on the wound isolates of *P. aeruginosa, E. coli and S. auerus* and the control antibiotics, there was no significant variation in the zones of inhibition produced since P > 0.05.

5 Conclusion

From the result, the zone of inhibition increases as the concentrations increases. Therefore, the antimicrobial effect of these plants depends on the concentration of the extract and the solvent used for extraction.

6 Recommendations

The use of these extract should be introduced in the tertiary health institution in Nigeria. Further studies should be done on these plants to determine the mode of action, biochemical targets on microorganisms and also the use of animal models to ascertain the safety for 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).Nutrients 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.