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## Antibacterial Efficacy of Crude and Partially Purified Extract of *Paullinia pinnata* linn. on Infected Human Wound

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### Abstract

This study investigated the antibacterial properties and mechanism of action of *Paullinia pinnata* extracts on wound isolates; determined the rate of killing with the view to harnessing it as base for synthesis of novel antimicrobial agent.

The leaf of *P. pinnata* was extracted in ethanol for four days with regular agitation. The filtrate was concentrated using rotary evaporator at 40°C and fractionated on column chromatography. The extracts were assayed on the test organisms by agar diffusion technique. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts were carried out by agar dilution and agar diffusion techniques, respectively. The rate of kill-assay of ethyl acetate and ampicillin against *Staphylococcus aureus* and *Proteus vulgaris* was determined.

The results showed that antibacterial activity of crude ethanolic extract of the leaf of *P. pinnata* varied greatly with the wound organisms. The crude extract at 35 mg/mL had the highest zone of inhibition (22.00 mm) against *Staphylococcus* spp (C1). The MIC and MBC of the crude extract were 2.19 and 17.50 mg/mL, respectively on the wound bacteria.

The study concluded that the leaf extract of *P. pinnata* were found to possess antibacterial activity which compared positively with ampicillin.

**Keywords:** Leaf extract; Antibacterial activity; Wound isolates; *Paullinia pinnata*

### Introduction

The medicinal importance of large number of plants has not been investigated. Globally, plants have served as the richest source of raw materials for traditional as well as modern medicine, particularly in Africa and Asia [1]. Many of today's modern drugs have their origin in traditional plant medicine [2]. The therapeutic efficacies of many indigenous plants for several disorders have been described by practitioners of traditional herbal medicines [3].

*Paullinia pinnata*, a climbing shrub with compound leaves and winged rachis, inflorescences stand axillary on long stalks, and bearing paired collected tendrils with white flowers. *P. pinnata* grows in evergreen and mixed forests up to an altitude of 1200 m. The plant is a perennial climber with a height of 2.5 to 8.0 m. The fruit is up to 2.5 cm long [4]. In traditional medicine, various parts of *P. pinnata* are used for treating various diseases. In South West Nigeria, the leaf juice of *P. pinnata* is used to treat sore throat [5]. An infusion from *P. pinnata* is used for fever while the roots are used for the treatment of jaundice, leprosy, snake bites vomiting and nausea [6,7]. The whole plant is used to treat dysentery in Ghana. The roots, mashed with seeds of *Piper guineense*, are applied as a styptic to cut veins and to treat leprosy [8]. The roots are also chewed for coughs and pulmonary diseases, gonorrhoea, fractures or abscesses or used on open sores. It is also used for the treatment of Aphrodisiac [9].

Wounds, resulting from microbial infection, are the most common public health problems [10]. The common wound pathogens includes bacteria, fungi, protozoa and viruses among which the most common are beta haemolytic *Streptococci* [*Streptococcus pyogenes* (*S. pyogenes*)], *Staphylococcus aureus* (*S. aureus*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Proteus* spp, *Escherichia coli* (*E. coli*) and *Enterococcus*, *Acinetobacter* spp, *Klebsiella* spp and Coliforms [11-13]. Although wounds may heal through the body's natural process of regenerating dermal and epidermal tissues, chronic ones cause apparent impact on human health and economic growth [14]. Current means of treating chronic wounds include

irrigation, debridement, antibiotics, tissue grafts and proteolytic enzymes, which come with major drawbacks and unwanted side effects on human [15].

In this study, we investigate the antibacterial activity of *P. pinnata* against wound bacterial isolates with the possibility of formulating of antimicrobial agents of natural origin which could be used as cheaper alternative for the therapeutic management of wound infections.

## Materials and Methods

### Materials

**Source of plant material and identification:** Fresh mature leaf of *Paullinia pinnata* used for this research was collected from the Teaching and Research Farm of Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria on the 22nd of March 2017 with latitude (7 51' North), longitude (3 56' East) and altitude 304 m. The leaf was green in colour with voucher specimen's number: FPI 2211.

**Source of microorganisms:** The test bacteria (Gram positive and Gram negative) used for the study were obtained from Microbiology and Parasitology Department of Obafemi Awolowo University Teaching Hospital. These organisms are Locally Isolated Organisms (LIO). The locally isolated organisms are clinical bacterial isolates from human infected wounds.

Gram positive bacteria include: *Staphylococcus aureus*, *Staphylococcus* spp (C1), *Staphylococcus* spp (C2), *Streptococcus canis* (LIO), *Staphylococcus massiliensis* (LIO), *Streptococcus pyogen* (LIO), *Streptococcus* spp (C3), *Streptococcus* spp (C4), *Micrococcus* spp (C5), *Micrococcus* spp (C6), *Globicatella* spp (C7) and *Salinicoccus* spp (C8). Gram negative bacteria include: *Klebsiella pneumoniae* (LIO), *Klebsiella ozanae* (LIO), *Enterobacter asburiae* (LIO), *Aerococcus suis* (LIO), *Salmonella enterica* (LIO), *Limnospira* spp (C9), *Citrobacter* spp (C10), *Limnospira* spp (C11), *Citrobacter* spp (C12), *Proteus* spp (C13), *Yersinia* spp (C14) and *Proteus vulgaris* (LIO).

### Culture media used

All the culture media used in this study were manufactured by Lab M Ltd, UK. Nutrient broth and nutrient agar were used to subculture the Bacterial isolates, the wound isolates were stocked in the refrigerator on nutrient agar slant at 4°C. Media were prepared and sterilized at 121°C for 15 minutes.

### Antimicrobial agents

Ampicillin (1 mg/mL each) was used as positive control in the antibacterial testing of the plant leaf extract and multiple antibiotic disc for Gram positive and Gram negative bacteria for antibiotic sensitivity testing of the test bacterial isolates.

## Methods

### Drying and extraction of the plant material

The leaf of *Paullinia pinnata* was collected and air-dried under the shade at room temperature (25°C ± 1°C) until constant weight was obtained and subsequently ground to powder at the Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife. The powdery form was weighed (1300 g). About 1300 g of the powdered leaf sample was soaked in 5 L of 70% ethanol for five days with regular agitation and then filtered. The extract was filtered out into a clean sterile conical flask using Whatman filter paper. The filtrate was concentrated in vacuum at low temperature (40°C) using a rotary evaporator. The yield (108 g) of crude extract was recovered. The yield obtained was dark in colour. The leaf extract was later stored into a glass petri dish.

### Preparation of concentrations of crude ethanolic *Paullinia pinnata* leaf extract

The standard concentration 35 mg/mL was prepared by dissolving 175 mg of crude leaf extract of *Paullinia pinnata* in 5 mL of DMSO/H<sub>2</sub>O (2:3). The other concentrations (17.5 mg/mL, 8.75 mg/mL, 4.38 mg/mL, 2.19 mg/mL) were prepared by double dilution of the standard solution.

### Determination of the antibacterial activity of crude ethanolic *Paullinia pinnata* leaf extract

The susceptibility testing of the test bacteria to the plant leaf extract was determined using agar-well diffusion method with some modifications. The bacterial isolates were first grown in nutrient broth for 18 h before use. Then 0.2 mL of the standardized test isolates (108 cfu/mL of 0.5 McFarland standards) was then sub-cultured on molten Mueller-Hinton agar. The medium was allowed to set and wells were then bored into the agar medium using a sterile 6 mm cork borer. The wells were then filled up with 1 mL of the prepared extract concentrations (8.75 mg/mL, 17.5 mg/mL and 35 mg/mL). The remaining two wells were filled with 1 mg/mL of ampicillin and 1 mL dissolving solvent (DMSO/H<sub>2</sub>O) which serves as positive and negative controls, respectively. The plates were allowed to stand on the laboratory bench upright for 1 h to allow proper diffusion of the solution into the medium before incubating the plates in an incubator at 37°C for 24 h. The plates were later observed for the zones of inhibition and measured using transparent metre rule to the nearest millimetre (mm). The effects of the leaf extract on bacterial isolates were compared with standard antibiotic (ampicillin) at a concentration of 1 mg/mL which served as a positive control.

### Antibiotic disc sensitivity test for wound isolates used

The antibiotic disc sensitivity test was carried out on the test isolates to determine multiple antibiotic resistant microorganisms among them. Antibiotic susceptibility of the

isolates was done using the Kirby-Bauer's disc diffusion method and interpreted according to the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2013).

Mueller-Hinton agar was prepared according to the manufacturer's instruction. The medium was allowed to cool to 45-50°C and poured into sterile petri dishes. The sterile petri dishes were allowed to set on a level surface to a depth of approximately 4 mm. When the agar had solidified, plates were allowed to dry before use. Then 18-24 h old broth cultures of the isolates were standardized by diluting to 0.5 Mcfarland's standard (108 cfu/mL). A sterile swab stick was inserted into the standardized isolate inocula, drained to remove excess inoculum load and inoculated by spreading on the surface of prepared Mueller-Hinton agar plate. After this, the inoculated Mueller-Hinton agar plate was allowed to dry for few minutes with the lid closed. Then antibiotic impregnated discs of known concentration were carefully applied on the inoculated Mueller-Hinton agar plates using sterile forceps.

The antibiotic discs used for Gram negative microorganisms were manufactured by ABTEK Biologicals Ltd. UK. These antibiotics include Gentamicin 10 µg (GEN), Cefuroxime 30 µg (CRX), Ceftazidime 30 µg (CAZ), Ciprofloxacin 5 µg (CPR), Nitrofurantoin 300 µg (NIT), Augmentin 30 µg (AUG), ofloxacin 5 µg (OFL) and cefixime 5 µg (CXM). The antibiotic discs used for Gram positive microorganisms were manufactured by MAXICARE MEDICAL LAB. These antibiotics include pefloxacin 10 µg (PEF), gentamycin 10 µg (CN), ampiclox 30 µg (APX), zinnacef 20 µg (Z), amoxicillin 30 µg (AM), rocephin 25 µg (R), ciprofloxacin 10 µg (CPX), streptomycin 30 µg (S), septrin 30 µg (SXT) and erythromycin 10 µg (E). The plates were then incubated at 37°C for 18-24 h. The diameters of the zones of inhibition were measured with a transparent calibrated ruler to the nearest millimeter and recorded. The results were recorded as Resistant (R) and Susceptible (S) according to the guideline of Clinical and Laboratory Standard Institute (CLSI, 2013).

## Statistical Analysis

The experiments were carried out in triplicates. The results were presented as mean Standard Deviation (SD). Student's t-test was used for comparison between the two means and a one-way analysis of variance (ANOVA) was used for comparison of more than two means.

## Results and Discussions

### Total yield of extract obtained from the leaf of *Paullinia pinnata*

The leaf extract collected was dark in colour and the yield was 108 g, which was 8.31% of the powdered leaf sample (1300 g) used. Fractions of 5.4 g of n-hexane, 4.5 g of ethyl acetate, 1.7 g of acetone and 0.9 g of ethanol were obtained.

**Diameter of zone of inhibition exhibited by crude ethanolic leaf extract of *P. pinnata* and ampicillin against the wound bacterial isolates:** Tables 1 and 2 shows the antibacterial effect of Crude ethanolic leaf extract of *P. pinnata* and standard antibiotic against test bacterial isolates. Out of the 24 test bacteria used, 16 was susceptible to the crude ethanolic leaf extract of *P. pinnata* at concentrations of 35 mg/mL, 17.5 mg/mL and 8.75 mg/mL, respectively. The zones of inhibition exhibited by the test bacteria at concentration of 35 mg/mL ranged between 5.00 mm and 20.00 mm, the lowest zone of inhibition of 5.00 mm was observed for *Micrococcus brucei*, while the highest zone of inhibition 22.00 mm was observed for *Staphylococcus muscae*. Twenty out of the 24 test bacteria screened were susceptible to ampicillin with the zones of inhibition ranging between 15.00 mm to 30.00 mm, while *Enterobacter asburiae* was resistant to this antibiotic.

**Table 1** The diameter of zone of inhibition of the crude ethanolic extract of *P. pinnata* leaf against the test wound bacterial isolates.

Test Bacterial	Zones of Inhibition (mm)**			
	Crude Extracts of ethanol			Ampicillin
	(8.75 mg/mL)	(17.5 mg/mL)	(35 mg/mL)	(1 mg/mL)
<i>Micrococcus spp</i> (C6)	0	0	5.00 ± 1.41	25.00 ± 1.22
<i>Aerococcus suis</i>	9.00 ± 1.41	10.00 ± 1.41	12.00 ± 1.41	24.00 ± 2.12
<i>Staphylococcus aureus</i>	8.00 ± 1.41	12.00 ± 1.41	15.00 ± 1.41	20.00 ± 1.50
<i>Citrobacter spp</i> (C12)	12.00 ± 0.71	11.00 ± 1.41	15.00 ± 0.71	21.00 ± 0.00
<i>Globicatella spp</i> (C7)	11.00 ± 1.41	17.00 ± 1.41	19.00 ± 0.85	22.00 ± 1.55
<i>Streptococcus canis</i>	5.00 ± 1.41	7.00 ± 1.41	11.00 ± 1.41	25.00 ± 1.88
<i>Staphylococcus spp</i> (C2)	10.00 ± 1.41	11.00 ± 1.41	16.00 ± 1.41	25.00 ± 1.05
<i>Citrobacter spp</i> (C10)	9.00 ± 1.41	11.00 ± 1.41	15.00 ± 1.27	25.00 ± 0.34

<i>Proteus vulgaris</i>	9.00 ± 0.71	12.00 ± 0.71	15.00 ± 1.84	26.00 ± 0.13
<i>Staphylococcus massiliensis</i>	0	13.00 ± 0.00	18.00 ± 1.41	19.00 ± 0.71
<i>Salmonella enterica</i>	0	0	0	25.00 ± 0.85
<i>Staphylococcus</i> spp (C1)	10.00 ± 0.14	14.00 ± 0.14	20.00 ± 1.41	24.00 ± 1.27
<i>Salinococcus</i> spp (C8)	8.00 ± 0.71	11.00 ± 1.41	15.00 ± 1.41	22.00 ± 0.87
<i>Streptococcus pyogenes</i>	6.00 ± 1.41	9.00 ± 1.41	12.00 ± 1.41	19.00 ± 2.44

**Table 2** The diameter of zone of inhibition of the crude ethanolic extract of *P. pinnata* leaf against the test wound bacteria continues.

Test Bacterial	Zone of Inhibition (mm)**			
	Crude Extract of ethanol			Ampicillin
	8.75 mg/mL	17.5 mg/mL	35 mg/mL	1 mg/mL
<i>Streptococcus</i> spp (C3)	5.00 ± 1.41	9.00 ± 1.41	13.00 ± 1.41	0
<i>Limnorea</i> spp (C9)	6.00 ± 1.41	10.00 ± 1.41	12.00 ± 1.41	17.00 ± 2.44
<i>Klebsiella ozanae</i>	0	0	0	19.00 ± 0.00
<i>Streptococcus</i> spp (C4)	0	0	0	13.50 ± 1.20
<i>Klebsiella pneumonia</i>	0	0	0	18.00 ± 2.22
<i>Enterobacter asburia</i>	0	0	0	0
<i>Proteus</i> spp (C13)	0	0	0	0
<i>Yersinia</i> spp (C14)	0	0	0	0
<i>Limnorea</i> spp (C11)	0	0	0	0
<i>Macrocooccus</i> spp (C5)	7.00 ± 1.88	15.00 ± 1.41	18.00 ± 1.41	30.00 ± 1.32

All organisms are clinical isolates, locally isolated from human infected wound. \*\*: mean of three replicates; SD: Standard Deviation; C1-C14: Organisms code.

**Antimicrobial activities exhibited by the fractions obtained from the crude leaf extract of *Paullinia pinnata* against the wound bacteria isolates:** Table 3 shows the results of antimicrobial activities of the extract fractions (partitioned crude) at a concentration of 10 mg/mL on wound isolates. N-hexane fraction inhibited the growth of 15 out of the 16 susceptible wound isolates with the zones of inhibition ranging between 6.00 mm and 22.00 mm. The highest zone of inhibition of 22.00 mm was observed against *Aerococcus suis*, while the lowest zone of inhibition of 6.00 mm was observed against *Citrobacter* spp (C5). Also, *Streptococcus* spp (C3) was

resistant to n-hexane fraction. On the other hand, the ethyl acetate fraction inhibited the growth of 14 out of the 16 susceptible wound isolates while *Streptococcus canis* and *S. pyogenes* were resistant to this fraction. The zones of inhibition exhibited by the ethyl acetate fraction against susceptible test isolates ranged between 7.00 mm and 28.00 mm. The highest zone of inhibition of 28.00 mm was observed against *Aerococcus suis* lowest zone of inhibition 7 mm was observed against *Macrocooccus* spp (C6). The organisms were resistance to acetone and ethanol fractions.

**Table 3** Antimicrobial activities exhibited by the *Paullinia pinnata* leaf fractions against the wound bacterial isolates.

Test Bacteria	Zones of Inhibition (mm)**			
	Ethanol (10 mg/mL)	Acetone (10 mg/mL)	Ethyl acetate (10 mg/mL)	N-hexane (10 mg/mL)
<i>Proteus vulgaris</i>	0	0	20.00 ± 1.84	16.00 ± 0.00
<i>Staphylococcus massiliensis</i>	0	0	24.00 ± 1.41	14.00 ± 1.41
<i>Staphylococcus aureus</i>	0	0	26.00 ± 1.27	16.00 ± 2.69
<i>Aerococcus suis</i>	0	0	28.00 ± 1.27	22.00 ± 1.41

<i>Macrococcus</i> spp (C5)	0	0	25.00 ± 1.4	17.00 ± 0.00
<i>Limnospira</i> spp (C9)	0	0	15.00 ± 1.41	8.00 ± 0.14
<i>Staphylococcus</i> spp (C2)	0	0	22.00 ± 0.85	17.00 ± 0.71
<i>Staphylococcus</i> spp (C1)	0	0	9.00 ± 1.41	18.00 ± 0.85
<i>Streptococcus canis</i>	0	0	0	8.00 ± 1.88
<i>Streptococcus pyogenes</i>	0	0	0	17.00 ± 1.41
<i>Citrobacter</i> spp (C12)	0	0	21.00 ± 1.88	15.00 ± 1.85
<i>Streptococcus</i> spp (C3)	0	0	9.00 ± 1.41	0
<i>Globicatella</i> spp (C7)	0	0	25.00 ± 1.85	18.00 ± 1.21
<i>Macrococcus</i> spp (C6)	0	0	7.00 ± 1.21	15.00 ± 1.41
<i>Citrobacter</i> spp (C10)	0	0	10.00 ± 1.22	6.00 ± 1.88
<i>Salinicoccus</i> spp (C8)	0	0	22.00 ± 1.41	15.00 ± 1.85

**The synergetic antibacterial effect of ethyl acetate/N-hexane fractions *P. pinnata* leaf extract against the wound bacterial isolates:** There was considerably increase in the antibacterial activity of the plant extract on the wound isolates resulting from the combined activities of two most active fractions (N-hexane/ethyl acetate). The zone of inhibition ranged from 12.00 mm to 26.00 mm. The Highest zone of

inhibition of 26 mm was observed against *Aerococcus suis* and the lowest zone of inhibition of 12.00 mm was observed against *Macrococcus* spp (C6). The combined fractions have activities on all the sixteen susceptible wound bacteria with a higher zone of inhibition as compared to each fraction singly applied. **Table 4** shows the sensitivity pattern of the effect of the combined fraction.

**Table 4** The synergetic antibacterial effect of ethyl acetate/n-hexane fractions of *P. pinnata* leaf extract against the wound bacterial isolates.

Wound Bacteria	Zones of Inhibition (mm)**
	Ethyl acetate/n-hexane (10 mg/mL)
<i>Proteus vulgaris</i>	20.00 ± 0.00
<i>Staphylococcus massiliensis</i>	23.00 ± 1.21
<i>Staphylococcus aureus</i>	24.00 ± 2.10
<i>Aerococcus suis</i>	26.5 ± 0.68
<i>Macrococcus</i> spp (C5)	22.00 ± 1.66
<i>Limnospira</i> spp (C9)	16.00 ± 1.41
<i>Staphylococcus</i> spp (C2)	14.00 ± 1.85
<i>Staphylococcus</i> spp (C1)	18.5.00 ± 1.21
<i>Streptococcus canis</i>	13.00 ± 1.14
<i>Streptococcus pyogenes</i>	24.00 ± 1.85
<i>Citrobacter</i> spp (C12)	14.00 ± 1.88
<i>Streptococcus</i> spp (C3)	18.00 ± 1.22
<i>Globicatella</i> spp (C7)	17.5.00 ± 1.41
<i>Macrococcus</i> spp (C6)	12.00 ± 2.44
<i>Citrobacter</i> spp (C10)	20.00 ± 1.22
<i>Salinicoccus</i> spp (C8)	22.00 ± 1.88

\*\*=mean of three replicates; SD: Standard Deviation

## Antibiotic susceptibility testing of the test bacteria

The sensitivity test was carried out on the 16 microorganisms that were susceptible to the leaf extract of *P. pinnata* or its fractions to determine the multiple antibiotic resistant isolates among them. The result shows that *Enterobacter asburiae* and *Klebsiella ozanae* which exhibited multiple antibiotic resistances were susceptible to n-hexane

fraction and n-hexane/ethyl acetate combined fractions of the plant leaf extract. Other wound isolates were not multiple antibiotics resistant. *Aerococcus suis* was resistant to Gentamicin, Cefuroxime, Augmentin and Nitrofurantoin but was susceptible to Ceftazidime, Cefixime, Ciprofloxacin, and Ofloxacin. *Proteus vulgaris* was resistant to Gentamicin, Ceftazidime, Ciprofloxacin, Augmentin and Cefixime but was susceptible to Cefuroxime, Nitrofurantoin and Ofloxacin (Table 5).

**Table 5** The antibiotic susceptibility profile of the test gram negative bacteria.

Microorganism	GEN	CRX	CAZ	CPR	NIT	AUG	OFL	CXM
	Diameter of Zone of Growth Inhibition (mm)							
<i>Enterobacter asburiae</i> (LIO)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
<i>Klebsiella ozanae</i> (LIO)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)
<i>Aerococcus suis</i> (LIO)	0 (R)	0 (R)	16 (S)	18 (S)	0 (R)	0 (R)	20 (S)	0 (R)
<i>Limnoretella</i> spp (C9)	12 (S)	10 (S)	0 (R)	20 (S)	18 (S)	0 (R)	18 (S)	0 (R)
<i>Proteus vulgaris</i> (LIO)	18 (S)	20 (S)	0 (R)	0 (R)	20 (S)	0 (R)	20 (S)	0 (R)
<i>Citrobacter</i> spp (C10)	28 (S)	14 (S)	24 (S)	0 (R)	20 (S)	30 (S)	26 (S)	20 (S)
<i>Citrobacter</i> spp (C12)	20 (S)	0 (R)	0 (R)	0 (R)	18 (S)	0 (R)	0 (R)	0 (R)

GEN: Gentamicin (10 µg); CRX: Cefuroxime (30 µg); CAZ: Ceftazidime (30 µg); CPR: Ciprofloxacin (5 µg); NIT: Nitrofurantoin (300 µg); AUG: Augmentin (30 µg); OFL: Ofloxacin (5 µg) and CXM=Cefixime (5 µg).

**Table 6** shows the antibiotic susceptibility profile test Gram positive bacteria. *Staphylococcus* spp (C2) was resistant to all the antibiotics used except Ciprofloxacin and Septrin. *Citrobacter* spp (C12) was only susceptible to Pefloxacin, Ampiclox and Erythromycin. *Streptococcus canis* was resistant to all antibiotics except Ampiclox and Zinnacef. *Streptococcus equi* was resistant to all antibiotics except Ampiclox, Zinnacef and Erythromycin. *Globicatella* spp (C7) was only susceptible

to Ampiclox and Zinnacef. *Staphylococcus massiliensis* was only resistant to Ampiclox but susceptible to all other antibiotics. *Macrocooccus* spp (C5), *Staphylococcus* spp (C2), *Staphylococcus* spp (C1) and *Staphylococcus aureus* were resistant to Ampiclox and Amoxicillin but susceptible to other antibiotics. *Macrocooccus* spp (C6) was susceptible to all the antibiotics except Ampiclox.

**Table 6** The antibiotic susceptibility profile of the test gram positive bacteria.

Microorganism	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
	Diameter of Zone of Growth Inhibition (mm)									
<i>Staphylococcus massiliensis</i> (LIO)	25 (S)	22 (S)	16 (S)	24 (S)	0 (R)	20 (S)	32 (S)	24 (S)	16 (S)	28 (S)
<i>Macrocooccus lamae</i> spp (C5)	34 (S)	24 (S)	0 (R)	12 (I)	0 (R)	22 (S)	32 (S)	30 (S)	30 (S)	26 (S)
<i>Staphylococcus aureus</i> (LIO)	0 (S)	0 (S)	0 (R)	20 (S)	0 (R)	20 (S)	0 (S)	20 (S)	18 (S)	0 (S)
<i>Macrocooccus</i> spp (C6)	20 (S)	22 (S)	0 (R)	24 (S)	16 (S)	20 (S)	26 (S)	30 (S)	16 (S)	24 (S)
<i>Salinicoccus</i> spp (C8)	30 (S)	20 (S)	0 (R)	22 (S)	0 (R)	18 (S)	32 (S)	30 (S)	16 (S)	28 (S)
<i>Staphylococcus</i> spp (C1)	30 (S)	28 (S)	0 (R)	22 (S)	0 (R)	18 (S)	30 (S)	30 (S)	16 (S)	22 (S)
<i>Staphylococcus</i> spp (C2)	0 (R)	22 (S)	0 (R)	30 (S)	0 (R)	0 (R)				
<i>Streptococcus canis</i> (LIO)	0 (R)	0 (R)	0 (R)	25 (S)	17 (S)	0 (R)				

<i>Streptococcus pyogenes</i> (L10)	0 (R)	21 (S)	16 (S)	0 (R)	0 (R)	0 (R)	22 (S)	0 (R)	14 (S)	20 (S)
<i>Streptococcus</i> spp (C3)	0 (R)	0 (R)	18 (S)	10 (S)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	16 (S)
<i>Globicatella</i> spp (C7)	0 (R)	0 (R)	0 (R)	25 (S)	17 (S)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)

PEF: Pefloxacin (10 g); CN: Gentamycin (10 g); APX: Ampiclox (30 g); Z: Zinnacef (20 g); AM: Amoxicillin (30 g); R: Rocephin (25 g); CPX: Ciprofloxacin (10 g); S: Streptomycin (30 g); SXT: Septrin (30 g) and E: Erythromycin (10 g)

The Minimum Inhibitory Concentrations (MICs) and the Minimum Bactericidal Concentrations (MBCs) exhibited by antibiotic, crude and fractions of *P. pinnata* leaf extract against test bacteria.

The minimum inhibitory concentrations and the minimum bactericidal concentrations exhibited by the ampicillin, crude leaf extract, ethyl acetate and n-hexane fractions against the wound isolates are shown in **Table 7**.

**Table 7** The minimum inhibitory concentrations and minimum bactericidal concentrations of the antibiotic, crude extract and fractions of *P. pinnata* leaf exhibited against the test bacteria.

Bacterial Isolates	Ampicillin antibiotic		Crude extract		Ethylene fraction		n-hexane fraction	
	MIC (µg/mL)	MBC (µg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
<i>Proteus vulgaris</i>	500.0	1000	8.75	8.75	4.38	8.75	2.19	4.38
<i>Staphylococcus massiliensis</i>	62.50	125.0	4.38	8.75	4.38	8.75	2.19	4.38
<i>Staphylococcus aureus</i>	62.50	125.0	8.75	8.75	4.38	8.75	2.19	4.38
<i>Aeromonas suis</i>	500.0	1000	8.75	17.50	4.38	8.75	4.38	8.75
<i>Staphylococcus</i> spp (C1)	125.0	250.0	4.38	8.75	2.19	4.38	2.19	4.38
<i>Macrocooccus</i> spp (C5)	500.0	1000	4.38	8.75	2.19	4.38	2.19	4.38
<i>Macrocooccus</i> spp (C6)	500.0	1000	4.38	8.75	4.38	8.75	2.19	4.38
<i>Staphylococcus</i> spp (C2)	125.0	250.00	4.38	8.75	4.38	8.75	2.19	4.38
<i>Salinicoccus</i> spp (C8)	125.0	250.00	4.38	8.75	4.38	8.75	2.19	4.38
<i>Streptococcus canis</i>	62.50	125.00	4.38	8.75	4.38	8.75	2.19	4.38
<i>Streptococcus pyogenes</i>	62.50	125.00	2.19	4.38	4.38	8.75	2.19	4.38
<i>Citrobacter</i> spp (C10)	500.0	1000	17.50	35.0	8.75	17.5	8.75	17.5
<i>Citrobacter</i> spp (C12)	500.0	1000	8.75	17.50	4.38	8.75	2.19	4.38
<i>Streptococcus</i> spp (C3)	125	250	17.50	20.0	4.38	8.75	4.38	17.5
<i>Limnospira</i> spp (C9)	500.0	1000	8.75	17.50	4.38	8.75	4.38	17.5
<i>Globicatella</i> spp (C7)	250	500	17.50	35.0	4.38	8.75	2.19	4.38

The MICs exhibited by ampicillin against the test isolates ranged between 62.50 µg/mL and 500.00 µg/mL. The lowest MIC of 62.50 µg/mL was observed against *S. massiliensis*, *S. aureus*, *S. canis*, *S. pyogenes* whereas the highest MIC of 500.00 µg/mL exhibited by ampicillin was observed *P. vulgaris*, *A. suis*, *Macrocooccus* spp (C5), *Macrocooccus* spp (C6), *Citrobacter* spp (C10), *Citrobacter* spp (C12), *Limnospira* spp (C9). The MBC exhibited by the ampicillin antibiotic against test isolates ranged between 125.00 µg/mL and 1000.00 µg/mL. The lowest MBC of 125.00 µg/mL was observed against *S. massiliensis*, *S. aureus*, *S. canis*, *S. pyogene* while the highest MBC of 1000.00 µg/mL was observed against *P. vulgaris*, *A.*

*suis*, *Macrocooccus* spp (C5), *Macrocooccus* spp (C6), *Citrobacter* spp (C10), *Citrobacter* spp (C12), *Limnospira* spp (C9). The MICs exhibited by the crude extract against the test organisms ranged between 2.19 mg/mL and 17.50 mg/mL. The lowest MIC of 2.19 mg/mL was observed against *S. pyogenes* whereas the highest MIC of 17.50 mg/mL exhibited by crude extract was observed against *Citrobacter* spp (C10), *Streptococcus* spp (C3). The MBC exhibited by the crude extract against test isolates ranged between 4.38 mg/mL and 17.50 mg/mL. The lowest MBC of 4.38 mg/mL was observed against *S. pyogenes* while the highest MBC of 17.50 mg/mL was observed against *Citrobacter* spp (C10).

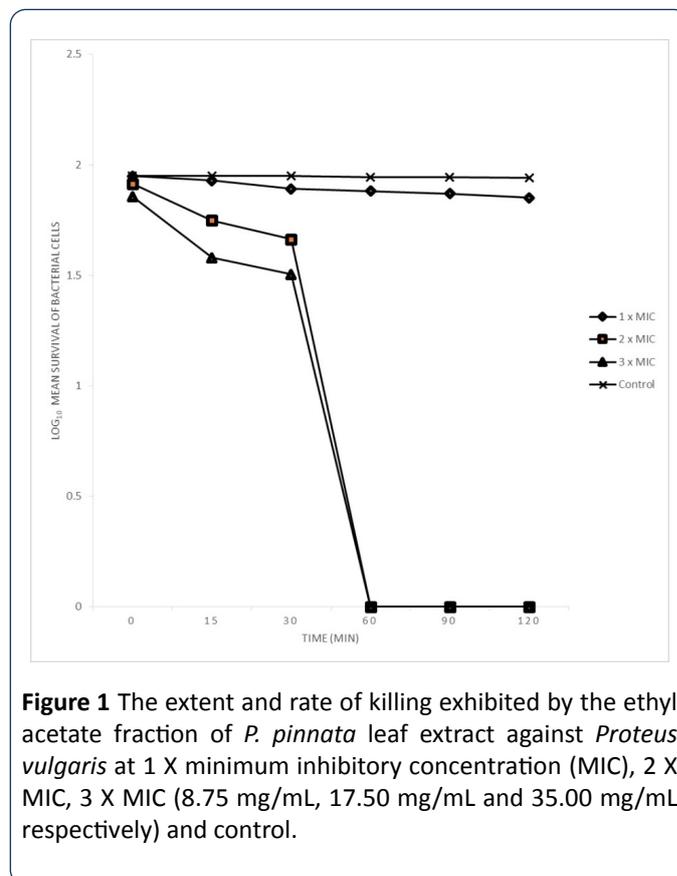
The MICs exhibited by n-hexane fraction against the test isolates used for this study ranged between 2.19 mg/mL and 8.75 mg/mL. The lowest MIC of 0.63 mg/mL was observed against *Staphylococcus* spp (C1), *Macrocooccus* spp (C5), while the highest MIC of 2.19 mg/mL was observed against *Citrobacter* spp (C10). The MBC exhibited by the n-hexane fraction against the test isolates ranged between 4.38 mg/mL and 17.5 mg/mL. The lowest MBC of 4.38 mg/mL was observed against *Staphylococcus* spp (C1), *Macrocooccus* spp (C5) and the highest MBC of 17.5 mg/mL was observed against *Citrobacter* spp (C10).

The MICs exhibited by ethyl acetate fraction against the test isolates ranged between 2.19 mg/mL and 8.75 mg/mL. The lowest MIC of 2.19 mg/mL was observed against *P. vulgaris*, *S. aureus*, *S. massiliensis*, *Staphylococcus* spp (C2), *Staphylococcus* spp (C1), *Macrocooccus* spp (C5), *Macrocooccus* spp (C6), *Salinicoccus* spp (C8), *S. pyogene*, *S. canis*, *Citrobacter* spp (C12) and the highest MIC of 8.75 was observed against *Citrobacter* spp (C10). The MBC exhibited by ethyl acetate fraction against test isolates ranged between 4.38 mg/mL and 17.5 mg/mL. The lowest MBC of 4.38 mg/mL was observed against *P. vulgaris*, *S. aureus*, *S. massiliensis*, *Staphylococcus* spp (C2), *Staphylococcus* spp (C1), *Macrocooccus* spp (C5), *Macrocooccus* spp (C6), *Salinicoccus* spp (C8), *S. pyogene*, *S. canis*, *Citrobacter* spp (C12), while the highest MBC of 17.5 mg/mL was observed against *Citrobacter* spp (C10) and *Staphylococcus* spp (C3).

The MBC exhibited by the n-hexane fraction against the test isolates ranged between 4.38 mg/mL and 17.5 mg/mL. The MICs exhibited by ethyl acetate fraction against the test isolates ranged between 2.19 mg/mL and 8.75 mg/mL.

**The extent and rate of killing exhibited by the most active (ethyl acetate) fraction of *P. pinnata* leaf extract against *Proteus vulgaris* and *Staphylococcus aureus*:** The extent and rate of killing of *Proteus vulgaris* exhibited by ethyl acetate fraction at 1 X MIC, 2 X MIC and 3 X MIC concentrations (8.75 mg/mL, 17.50 mg/mL and 35.00 mg/mL respectively) are shown in **Figure 1**. The percentage of the organisms killed by the extract at 1 X MIC in 0 minutes was 11% and at 15 minutes, it rose to 15%. After 30 minutes of contact time with this extract, the percentage of the organisms killed was 22%. When the contact time was 60 minutes, the percentage of the organisms killed was 24% and 26% at 90 minutes and this rose to 29% after 120 minutes of contact time. When the MIC was doubled, the percentage of organisms killed by the extract within 0 minutes contact time was 18% and at 15 minutes of contact time, the percentage of cells killed increased to 44% and with the increase of the contact time to 30 minutes, the percentage of organisms killed was 54%. When the contact time was 60 minutes, the percentage of the organisms killed was 100%, after 90 minutes of contact time, the percentage of the cells killed was 100% and at 120 minutes of the contact time interval, the percentage of the organisms killed has increased to 100%. The extent and rate of kill of the antibiotic at 3 X MIC after 0 minutes contact time was 28 and this increased to 62 after 15 minutes and also increased to 68% after 30 minutes of the contact time interval. Finally after 60,

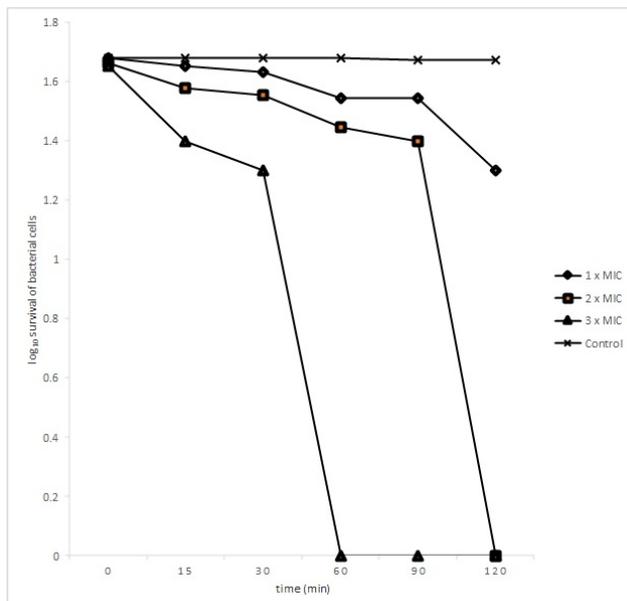
90 and 120 minutes of exposure of the organisms to the extract, the percentage of cells killed has increased to 100%.



**Figure 1** The extent and rate of killing exhibited by the ethyl acetate fraction of *P. pinnata* leaf extract against *Proteus vulgaris* at 1 X minimum inhibitory concentration (MIC), 2 X MIC, 3 X MIC (8.75 mg/mL, 17.50 mg/mL and 35.00 mg/mL respectively) and control.

Each designated point represents the mean log<sub>10</sub> survival of bacterial cells at a particular time interval in the presence of the extract.

The extent and rate of killing of *S. aureus* cells by ethyl acetate fraction *P. pinnata* at 1 X MIC, 2 X MIC and 3 x MIC are shown in **Figure 2**. The percentage of the organisms killed by the extract at 1X MIC in 0 minutes was 52%, while the percentage of organisms killed at 15 minutes rose to 55%, and after 30 minutes of contact time with this extract, the percentage of the organisms killed was 57% and eventually rose to 65% after 60 minutes. When the contact time was increased to 90 minutes, the percentage of the cells killed was still 65% and this rose to 80% after 120 minutes of contact time. When the MIC was doubled, the percentage of organisms killed by the extract within 0 minutes contact time was 54%, at 15 minutes of contact time, the percentage of cells killed increased to 62%, and with the increase of the contact time to 30 minutes, the percentage of organisms killed was 64%. When the contact time was 60 minutes, the percentage of the organisms killed was 72, after 90 minutes of contact time interval, the percentage of the organisms killed has increased to 75%, and increased sequentially to 100% at 120 minutes of contact time. The extent and rate of kill of the extract at 3 X MIC after 0 minutes contact time was 55% and this increased to 75% after 15 minutes and increased to 80% after 30 minutes of the contact time interval. Finally, after 60, 90, 120 minutes of exposure of the organisms to the extract, the percentage of cells killed has increased to 100%.



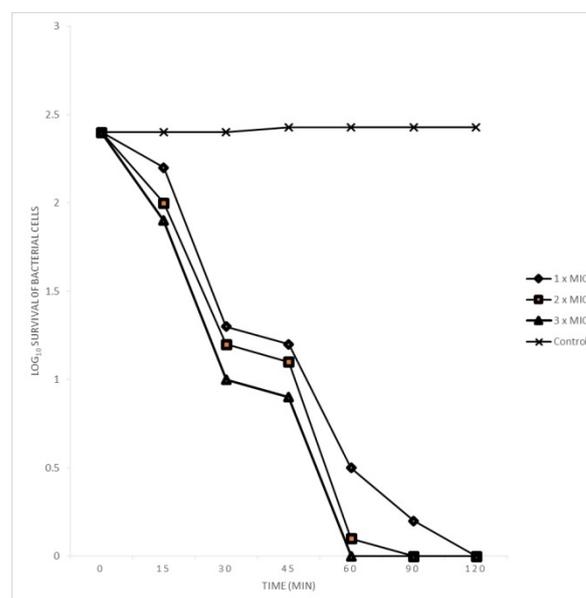
**Figure 2** The extent and rate of killing exhibited by the ethyl acetate fraction of *P. pinnata* leaf extract against *Staphylococcus aureus* at 1 X Minimum Inhibitory Concentration (MIC), 2 X MIC, 3 X MIC (8.75 mg/mL, 17.50 mg/mL and 35.00 mg/mL respectively) and control.

Each designated point represents the mean log<sub>10</sub> survival of bacterial cells at a particular time interval in the presence of the extract.

#### The extent and rate of killing exhibited by ampicillin antibiotic against *Proteus vulgaris* and *Staphylococcus aureus*:

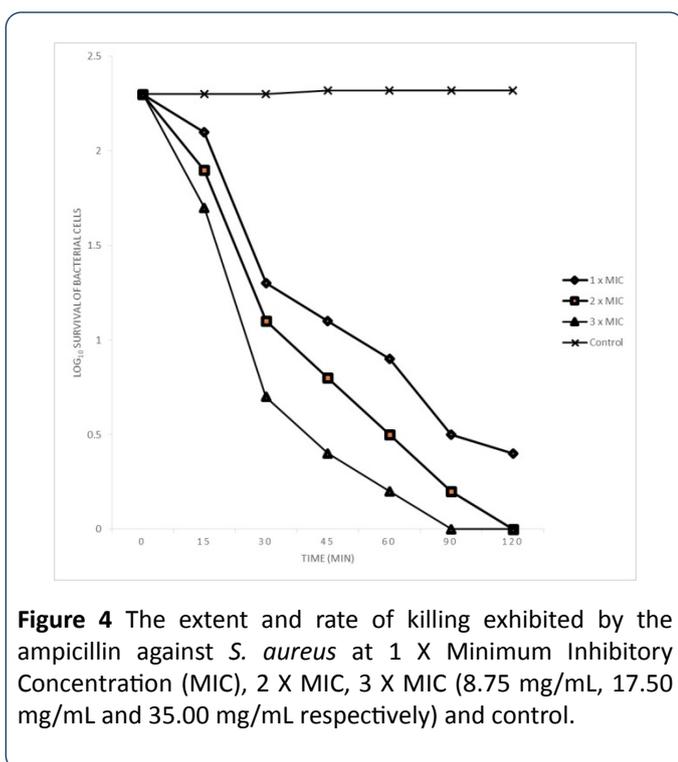
The extent and rate of killing of *Proteus vulgaris* exhibited by Ampicillin antibiotic at 1 X MIC, 2 X MIC and 3 X MIC concentrations (8.75 mg/mL, 17.50 mg/mL and 35.00 mg/mL respectively) are shown in **Figure 3**. The percentage of the organisms killed by the antibiotic at 1 X MIC in 0 minutes was 8.33%, while the percentage of organisms killed at 15 minutes rose to 46.28%, after 30 minutes of contact time with this antibiotic, the percentage of the organisms killed was 52.17%. At contact time of 60 minutes, the percentage of the organisms killed was 61.21% and at 90 minutes, it was 78.45% and eventually rose to 82.76% after 120 minutes of contact time. When the MIC was doubled, the percentage of organisms killed by the antibiotic within 0 minutes contact time was 17.39%, at 15 minutes of contact time, and increased to 52.17%; and with the increase of the contact time to 30 minutes, the percentage of organisms killed was 54.55%. At contact time of 60 minutes, the percentage of the organisms killed was 79.34% meanwhile at contact time of 90 minutes, the percentage of the cells killed was 91.74% and this rose to 100% after 120 minutes of contact time. When the MIC was doubled, the percentage of organisms killed by the antibiotic within 0 minutes contact time was 16.67%, at 15 minutes of contact time, the percentage of cells killed increased to 50.41%, and with the increase of the contact time to 30 minutes, the percentage of organisms killed was 54.55%. When the contact time was 60 minutes, the percentage of the organisms killed was 95.87%. After 90 and 120 minutes of the contact time interval, the percentage of the organisms killed has increased to 100%. The extent and rate of kill of the antibiotic at 3 X MIC after 0 minutes contact time was 20.83 and this increased to 58.68 after 15 minutes and also increased to 62.81 after 30 minutes of the contact time interval. After 60, 90 and 120 minutes of exposure of the

organisms to the antibiotic, the percentage of cells killed increased to 100%.



**Figure 3** The extent and rate of killing exhibited by the ampicillin against *Proteus vulgaris* at 1 X minimum inhibitory concentration (MIC), 2 X MIC, 3 X MIC (8.75 mg/mL, 17.50 mg/mL and 35.00 mg/mL respectively) and control.

The extent and rate of killing of *S. aureus* by Ampicillin antibiotic at 1 X MIC, 2 X MIC and 3 X MIC is shown in **Figure 4**. The percentage of the organisms killed by the antibiotic at 1 X MIC in 0 minutes was 8.70% while the percentage killed at 15 minutes rose to 43.48%, after 30 minutes of contact time with this antibiotic, the percentage of the organisms killed was 52.17%. At contact time of 60 minutes, the percentage of the organisms killed was 61.21% and at 90 minutes, it was 78.45% and eventually rose to 82.76% after 120 minutes of contact time. When the MIC was doubled, the percentage of organisms killed by the antibiotic within 0 minutes contact time was 17.39%, at 15 minutes of contact time, and increased to 52.17%; and with the increase of the contact time to 30 minutes, the percentage of organisms killed was 54.55%. At contact time of 60 minutes, the percentage of the organisms killed was 79.34% meanwhile at contact time of 90 minutes, the percentage of the cells killed was 91.74% and this rose to 100% after 120 minutes of contact time. When the MIC was doubled, the percentage of organisms killed by the antibiotic within 0 minutes contact time was 16.67%, at 15 minutes of contact time, the percentage of cells killed increased to 50.41%, and with the increase of the contact time to 30 minutes, the percentage of organisms killed was 54.55%. When the contact time was 60 minutes, the percentage of the organisms killed was 95.87%. After 90 and 120 minutes of the contact time interval, the percentage of the organisms killed has increased to 100%. The extent and rate of kill of the antibiotic at 3 X MIC after 0 minutes contact time was 20.83 and this increased to 58.68 after 15 minutes and also increased to 62.81 after 30 minutes of the contact time interval. After 60, 90 and 120 minutes of exposure of the organisms to the antibiotic, the percentage of cells killed increased to 100%.



**Figure 4** The extent and rate of killing exhibited by the ampicillin against *S. aureus* at 1 X Minimum Inhibitory Concentration (MIC), 2 X MIC, 3 X MIC (8.75 mg/mL, 17.50 mg/mL and 35.00 mg/mL respectively) and control.

Each designated point represents the mean log<sub>10</sub> survival of bacterial cells at a particular time interval in the presence of the extract.

Leaf extract of *Paullinia pinnata* was investigated to know its antimicrobial potency against some bacterial isolates comprising both Gram-positive and Gram-negative isolates. The crude extract at a concentration of 8.75 mg/mL, 17.5 mg/mL and 35 mg/mL and its fractions (10 mg/mL) inhibited the growth of 16 bacterial isolates out of the 24 isolates used for this study. It was therefore, observed that this plant extract compared favourably with the standard antibiotic ampicillin. Thus, the leaf extract of *P. pinnata* appears to be a potential source of antibacterial compounds that could be relevant in the treatment of infections caused by these microorganisms. The result shows that *Enterobacter asburie* and *Klebsiella ozanae* are multiple antibiotic resistant microorganisms but were susceptible to n-hexane fraction, ethyl acetate/n-hexane fraction of the plant extract. Thus, n-hexane and ethyl acetate may serve as the best solvents to extract the bioactive components of *P. pinnata*. The combined fraction of *P. pinnata* showed appreciable level of activity against most of the test isolates at a concentration of 10 mg/mL. Thus, these combined fractions (synergy) can also serve as base for the formulation of novel natural antimicrobial agents.

Time-kill assays are expressed as the rate of killing by a fixed concentration of an antimicrobial agent (*in vitro*) and it's one of the most reliable methods for determining tolerance of microorganisms to drugs. The killing rate of the most active fraction and antibiotic ampicillin was determined by using *Staphylococcus aureus* as Gram-positive representative and *Proteus vulgaris* as Gram-negative representative. It was observed that the rate at which the antibiotic and ethyl acetate fraction eliminated the cells increased with increase in

the concentrations of the antibiotic, fraction and contact time intervals. The results show a monophasic effects as reported by Akinyele et al. [12]. These fractions also kill the wound isolates at a shortest contact time. Pankey et al. [13] reported that the ability of plant leaf extract to eliminate or kill organisms at the shortest period of time is generally accepted definition of bactericidal activity in antibiotics and which is in supports of our findings.

The antibacterial activity of plant leaf extract is considered significant if the MIC of the extract is less than or equal to 200 mg/mL [14]. The MICs exhibited by the crude extract, the active fractions (n-hexane and ethyl acetate) and the combined fraction were below 200 mg/mL as observed in this study. This is an indication that the leaf extract of *P. pinnata* exhibited significant antibacterial activity against the test isolates and suggests it to be a veritable source of bioactive compounds that can serve as a base for the formulation of potent antibacterial especially against wound infection [15-18].

## Conclusion

The study concluded that the leaf extract of *P. pinnata* were found to possess antibacterial activity which compared positively with ampicillin. The mode of antibacterial action exhibited by the extract could be concluded to be by cytoplasmic membrane disruption which leads to the death of the test bacteria. The presence of secondary metabolites, bioactive compounds and membrane disruption properties of this plant may be responsible for its antibacterial potency.

The antibacterial potential of the crude leaf extract of *P. pinnata* against both Gram positive and Gram negative bacterial isolates at low concentration and minimal contact time established the effectiveness of the *Paullinia pinnata* leaf as a potential source of broad spectrum antimicrobial drugs in the treatment of multiple drug resistant pathogens. Antimicrobial drug formulated from this plant will assist in treating infections caused by test microorganisms and most importantly will aid in wound healing. *Paullinia pinnata* formulations in to antimicrobial agent could be more effective and cheaper compared to the expensive synthetic drugs.

These findings could be of help to Pharmaceutical Industries and Research Institutes in the formulation of new antimicrobial agents of natural origin for wound healing and to cater for the problem of multi-drug resistant bacteria. *Paullinia pinnata* if used to synthesize drug will reduce the rate of morbidity recorded from patients with high risk of wound infection.

## Recommendation

*Paullinia pinnata* is commonly sourced in most local environs. Its antibacterial potential against both Gram positive and Gram negative bacterial isolates at low concentration and minimal contact time established the effectiveness of the *Paullinia pinnata* leaf as a potential source of broad spectrum antimicrobial drugs in the treatment of multiple drug resistant

pathogens. Antimicrobial drug formulated from this plant will assist in treating infections caused by test microorganisms. The study has provided useful information on antibacterial properties of *P. pinnata* which could be harnessed for the development of cheaper alternative antibacterial agent capable of combating microorganisms implicated in human wound infections.

## Authors Contribution

All contributing authors have agreed to the submission of this manuscript for publication. AOO, FO conceived and designed the study, performed the experiments, analyzed data and interpreted the results JOA involved in analyzing the data, interpreted the results and wrote the paper. OOO monitored leaf extract production experiment, interpreted the results and edited the manuscripts.

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