

**Short Communication**

Activity of *Paullinia Pinnata* Leaf Fractions on Bacterial Isolates associated with Treatment-Failure Wounds

Oluwatoyin A. Adeyemo-Salami¹, Oluwole M. David², John O. Olanlokun¹¹Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria²Department of Microbiology, Ekiti State University, Ekiti State, Nigeria**OPEN ACCESS*****CORRESPONDENCE**Adeyemo-Salami, O.A.
soluwatoyin81@yahoo.com
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<https://doi.org/10.4314/njbmb.v39i1.4>**ABSTRACT**

The current surge of multidrug resistant infections has led to many cases of treatment failure in clinical practices. This has necessitated the search for new drugs from natural sources which could be used to curb this menace. *Paullinia pinnata* (Linn.) leaves are deployed traditionally for the treatment of various ailments such as malaria and the treatment of wounds. The essence of this study is to assess the antibacterial potential of *P. pinnata* leaf fractions on four antibiotic-resistant bacteria isolated from treatment failed wounds. The n-hexane and ethyl acetate fractions were obtained from the methanol extract of the leaves of *P. pinnata* by the one-solvent system fractionation. The antibacterial capacity of the fractions was determined by the agar well diffusion method and gentamicin (30 µg/mL) was the control. Clinical isolates of two Gram-positive (*Staphylococcus aureus* and *Bacillus cereus*) and two Gram-negative (*Klebsiella pneumoniae* and *Proteus vulgaris*) bacteria were used. The fractions showed no activity to moderate activity at lower concentrations. The ethyl acetate fraction showed bacteriostatic activity in a dose-dependent manner and was better than that of n-hexane, and could therefore be a good drug target for antimicrobial therapies, especially for wound treatment.

Keywords: *Paullinia pinnata*, Wound, Ethyl acetate fraction, n-hexane fraction, Bacteria**INTRODUCTION**

Bacteria are now becoming increasingly resistant to conventional antibiotics, and different diseases are emerging, although some are re-emerging. Most treatments fail because of antibiotic resistance in response to the development of strains of bacteria which are resistant to antibiotics. Overuse or misuse of antibiotics has been identified as a major cause of this phenomenon (Willey *et al.*, 2009). The use of antibiotics in meat production, the usage of wrong antibiotics, and taking an incomplete course of antibiotics as prescribed by a healthcare provider has put pressure on the bacterial

pathogen and has put the lives of farm animals and humans in danger (Haney and Hancock, 2022; Murray *et al.*, 2022). Alternative treatment options are needed in the current era of superbug emergence. The use of non-antibiotic therapies such as phytomedicine is a credible alternative in the management of failed bacterial infections that are now serious and potentially life-threatening globally (Chitemerere and Mukanganyama, 2014).

Paullinia pinnata Linn. is a climber that is being applied folklorically in the treatment and management of infectious and non-infectious diseases. This has been buttressed scientifically, including showing that it has antibacterial potential (Burkill, 2000; Olatujoye *et al.*, 2019; Adeyemo-Salami, 2020; Nyegue *et al.*, 2020; Afagnigni *et al.*, 2021). However, Adeyemo-Salami and

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Choudhary (2021) reported that when various bacterial species were exposed to different concentrations of methanol leaf extract, there was low inhibition. Sequel to this, we investigate the impact of fractions of the leaves of *P. pinnata* on specific human bacterial pathogens with the hypothesis that fractionation, will enhance the antibacterial activity. This study is therefore structured to assess the bactericidal efficacy of the n-hexane and ethyl acetate fractions of *P. pinnata* methanol leaf extract against bacteria isolated from wounds that have undergone prior treatment with diverse antibiotics.

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Paullinia pinnata (Linn). leaves were collected, authenticated and given the specimen voucher number FHI 106555 by the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. The leaves were processed to obtain the methanol extract as stated by Adeyemo-Salami and Farombi (2019).

Preparation of Fractions

Two hundred grams (200 g) of the methanol extract was then subjected to one-solvent system fractionation using 200 mL aliquots of n-hexane and then subsequently with 200 mL aliquots of ethyl acetate till no visible colour change was observed with each solvent. The fractions were then dried using a vacuum oven (Gallenham, England) at a temperature of 40 - 42°C. Thirteen grams (13 g) was realized for the n-hexane fraction and 10 g was realized for the ethyl acetate fraction (i.e. 6.5% and 5% yield, respectively).

Source of Bacteria

The test organisms used in this work were primarily isolated from treatment-failed wound samples in a tertiary health care facility in Ekiti State, Nigeria. The wound samples were selected after satisfying the criteria of Currie *et al.* (2014). The wounds were treated for 30 days with amoxicillin without any significant improvement. The bacteria were revived on Nutrient agar and incubated at 37°C for 24 hours after which they were inoculated and cultured on agar slant.

Determination of Antibacterial Activity of the Plant Extracts

The antibacterial activity of the each of the fraction was determined by the agar well diffusion method (Kivcak *et al.*, 2002; Das *et al.*, 2010). The fractions were tested against four clinically important bacteria. Two of the isolates, *Staphylococcus aureus* and *Bacillus cereus*, were Gram-positive, while *Klebsiella pneumoniae* and *Proteus vulgaris* were Gram-negative. Culture methods were used to confirm the identity of each of the isolates. The bacterial isolates were standardized as their turbidity was adjusted to 0.5 McFarland standards, which gives approximately 10⁶ cells/mL.

Each of the fractions was reconstituted with 5.0% Tween 80 to achieve five different concentrations which are 6.25, 12.50, 25, 50 and 100 mg/mL, and the antibacterial potency of these concentrations were determined. Gentamicin (30 µg/mL) was used as the control. The surface of the agar medium was seeded with the test organism earlier standardized, and allowed to dry. In the agar earlier seeded with a test organism, a 6 mm diameter well was aseptically bored using a sterile cork borer. A distance of at least 30 mm was ensured between wells. Each well was gently filled with different concentrations of the plant fraction, and the plate was carefully incubated aerobically at 37°C for 18 hours. The zone of inhibition around each of the wells was measured and recorded in millimeters. AAT Bioquest Tool (<https://www.aatbio.com/tools/ic50-calculator>) was used to calculate the concentration at which half maximal inhibitory activity (IC₅₀) was observed.

RESULTS

Table 1 reveals that upon exposure of *S. aureus* and *B. cereus* to the n-hexane fraction of *P. pinnata* leaves at 6.25 mg/mL and 12.5 mg/mL, there was no activity. The zone of inhibition with the n-hexane fraction was 8.50 mm at 100 mg/mL with *S. aureus*, and 7.20 mm and 8.50 mm with *B. cereus* at 50 mg/mL and 100 mg/mL, respectively. A dose-dependent zone of inhibition against *S. aureus* and *B. cereus* was observed with the ethyl acetate fraction. Gentamicin (the control) had zone of inhibition of 31.70 mm and 27.30 mm against *S. aureus* and *B. cereus*, respectively. The IC₅₀ for *S. aureus* with the n-hexane and ethyl acetate fractions are 85.86±0.02 mg/mL and 15.36±0.01mg/mL, respectively while that for *B. cereus* are 44.07±0.02 mg/mL with n-hexane fraction, and 11.21±0.01 mg/mL with ethyl acetate fraction.

The percentage inhibition of the n-hexane fraction on *S. aureus* in comparison with Gentamicin is 26.8 at 100 mg/mL while it was zero at 6.25, 12.5 and 50 mg/mL. The percentage inhibition of the ethyl acetate fraction on *S. aureus* in comparison with Gentamicin is 22.7 (at 6.25 mg/mL), 30.9 (at 12.5 mg/mL), 39.2 (at 25 mg/mL) and 47 (at 50 and 100 mg/mL). The percentage inhibition of the n-hexane fraction on *B. cereus* in comparison with Gentamicin is zero (at 6.25, 12.5 and 25 mg/mL), 26.4 (at 50 mg/mL) and 31.1 (at 100mg/mL). The percentage inhibition of the ethyl acetate fraction on *B. cereus* in comparison with Gentamicin is zero (at 6.25 mg/mL), 26.4 (at 12.5 mg/mL), 35.9 (at 25 and 50 mg/mL) and 45.4 (at 100 mg/mL) (Table 1).

Table 2 displays that upon exposure of *K. pneumoniae* and *P. vulgaris* at 6.25 mg/mL and 12.5 mg/mL to the n-hexane fraction of *P. pinnata* leaves, there was no activity. The zone of inhibition with the n-hexane fraction was 7.20 mm, 13.60 mm and 13.60 mm with *K. pneumoniae* at 25 mg/mL, 50 mg/mL and 100 mg/mL, respectively, and 9.80 mm, 11.10 mm, and 14.90 mm with *P. vulgaris* at 25 mg/mL, 50 mg/mL and 100 mg/mL, respectively. A dose-dependent zone of inhibition against *K. pneumoniae* and

P. vulgaris was observed with the ethyl acetate fraction. Gentamicin had zone of inhibition of 22.10 mm and 14.60 mm against *K. pneumoniae* and *P. vulgaris*, respectively. The IC₅₀ for *K. pneumoniae* with the n-hexane and ethyl acetate fractions are 44.07±0.01 mg/mL and 49.42±0.02 mg/mL, respectively while that for *P. vulgaris* are 21.10±0.02 mg/mL with n-hexane fraction, and 10.41±0.01 mg/mL with ethyl acetate fraction. The percentage inhibition of the n-hexane fraction on *K. pneumoniae* in comparison with Gentamicin is zero (at 6.25 and 12.5 mg/mL), 32.6 (at 25 mg/mL) and 61.5 (at 50 and 100 mg/mL). The percentage inhibition of the ethyl acetate fraction on *K. pneumoniae* in comparison with Gentamicin is zero (at 6.25 mg/mL), 32.6 (at 12.5 and 25 mg/mL), 44.3 (at 50 mg/mL) and 67.4 (at 100 mg/mL). The percentage inhibition of the n-hexane fraction on *P. vulgaris* in comparison with Gentamicin is zero (at 6.25 and 12.5 mg/mL), 67.1 (at 25 mg/mL), 76.0 (at 50 mg/mL) and 102.0 (at 100 mg/mL). The percentage inhibition of the ethyl acetate fraction on *P. vulgaris* in comparison with Gentamicin is zero (at 6.25 mg/mL), 67.1 (at 12.5 and 25 mg/mL), 76.0 (at 50 mg/mL) and 102.0 (at 100 mg/mL).

DISCUSSION

The study reveals that the n-hexane and ethyl acetate fractions of *Paullinia pinnata* methanol leaf extract had varying antibacterial capacities against *Staphylococcus aureus*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Proteus vulgaris*, with that against *P. vulgaris* being significant for both fractions at the highest concentration.

Staphylococcus aureus is a well-known bacterial human pathogen that is responsible for wound infection and colonization. The bacterium could cause either community-acquired or hospital-acquired infections. Multi-drug resistant strains, such as Methicillin resistant *Staphylococcus aureus* (MRSA), have been challenging in the treatment (Taylor and Unakal, 2022). The n-hexane fraction had no activity against *S. aureus* except at the concentration of 100 mg/mL. A dose-dependent activity, which ranged from low activity at 6.25 mg/mL (22.7%) to moderate activity at 100 mg/mL (47%), was observed with the ethyl acetate fraction. This seeks to support the findings of Imade *et al.* (2015) with the cold ethanol extract of the leaf which showed moderate activity against the bacteria. Moreover, it is in contrast to our previous finding with the crude methanol leaf extract, where we showed that it had no activity against *S. aureus* (Adeyemo-Salami and Choudhary, 2021). This confirms that fractionation can improve the activity of a plant extract.

Apart from having the ability to colonize wound, *Bacillus cereus* is a human food-borne pathogen which is one of the bacteria responsible for food- and water-borne food poisoning outbreaks worldwide (Dietrich *et al.*, 2021). It is responsible for two types of gastrointestinal disorders (emetic syndrome and diarrhoeal syndrome) as a result of ingestion of food contaminated with it. The toxins which they release and are responsible for these manifestations are the cyclic dodecadepsipeptide cereulide and the

proteinaceous enterotoxins hemolysin BL (*Hbl*) (which are responsible for the emetic syndrome), and nonhemolytic enterotoxin (Nhe) and cytotoxin K (*CytK*) (which result in the diarrhoeal syndrome) (Dietrich *et al.*, 2021).

The n-hexane fraction had no activity against *B. cereus* except at 50 mg/mL (26.4%) and 100 mg/mL (31.1%), which were low compared to Gentamicin. A dose-dependent activity, that ranged from no activity at the concentration of 6.25 mg/mL, to low activity at 12.5 mg/mL (26.4%) and moderate activity at 100 mg/mL (45.4%), was observed with the ethyl acetate fraction. This is a slight improvement on our finding which showed that the crude methanol leaf extract has low activity against *B. subtilis* (Adeyemo-Salami and Choudhary, 2021). Again, this confirms that fractionation can improve the activity of a plant extract.

Klebsiella pneumoniae is a human pathogen that is found (amongst other habitats) inhabiting mucosal surfaces such as the upper respiratory tract and the gut. However, infection ensues when there is a compromise in the immune system of an individual. This occurs in chronic diseases like diabetes (Chang *et al.*, 2021). Moreover, it is known to cause wound infections. The n-hexane fraction had no activity against *K. pneumoniae* at 6.25 mg/mL and 12.5 mg/mL but had low activity at 25 mg/mL (32.6%) and good activity at 50 mg/mL and 100 mg/mL (61.5%). Again, a dose-dependent activity against *K. pneumoniae* was displayed with the ethyl acetate fraction. There was no activity at the concentration of 6.25 mg/mL. At 12.50 mg/mL and 25.00 mg/mL, it showed low activity (32.6%). This was also observed at 50 mg/mL (44.3%). However, at 100 mg/mL, it had a good activity (67.4%) compared to Gentamicin. This is akin to the discovery of Adeosun *et al.* (2022) which demonstrated that phytol (a phytochemical) inhibited the features of *K. pneumoniae* which were exopolysaccharide production, virulence, initial cell attachment, hypermucoviscosity, biofilm formation and curli expression.

Proteus vulgaris is a human pathogen found in the gastrointestinal tract and it causes nosocomial infections such as urinary tract infections, sepsis as well as respiratory tract infections. In addition, it has been shown to colonize wounds and cause wound infections. It has been shown in recent times to be resistant to beta-lactams (Armbruster and Mobley, 2017). The n-hexane fraction had no activity against *P. vulgaris* at 6.25 mg/mL and 12.5 mg/mL. At 50 mg/mL it had a good activity (67.1%), and at 25 mg/mL (76.0%) and 100 mg/mL (102.0%) it had very good activity compared to Gentamicin. Again, a dose-dependent activity was observed with the ethyl acetate fraction. It had no activity at 6.25 mg/mL, good activity at 12.5 mg/mL and 25 mg/mL (67.1%) and very good activity at 50 mg/mL (76.0%) and 100 mg/mL (102.0%). This seeks to resonate with the findings of Salem *et al.* (2021) which revealed that *Citrus limon* extract had the highest anti *Proteus vulgaris* activity amidst eight other medicinal plant extracts.

Moreover, it is similar to the observation of Cock and van Vuuren (2014) who showed that *Lippia javanica* and *Terminalia sericea* methanol leaf extract had significant inhibitory activity against *P. vulgaris*.

Compared to the n-hexane fraction, the ethyl acetate fraction had moderate activity at higher concentrations

against all the pathogens and was even better than Gentamicin when *P. vulgaris* was exposed to it. Moreover, the IC₅₀ values generally reflect the potency of the ethyl acetate fraction compared to the n-hexane fraction against the pathogens. The results also serve to validate the hypothesis.

Table 1. Antibacterial Activity of *P. pinnata* Leaf Fractions on Gram-Positive Bacterial Pathogens

Fractions	Concentration (mg/mL)	<i>Staphylococcus aureus</i>			<i>Bacillus cereus</i>		
		Z.I (mm)	% Inhibition	IC ₅₀ (mg/mL)	Z.I (mm)	% Inhibition	IC ₅₀ (mg/mL)
N-hexane	100	8.50	26.8	85.86±0.02	8.50	31.1	44.07±0.02
	50	0.00	0		7.20	26.4	
	25	0.00	0		0.00	0	
	12.50	0.00	0		0.00	0	
	6.25	0.00	0		0.00	0	
Ethyl acetate	100	14.90	47.0	15.36±0.01	12.40	45.4	11.21±0.01
	50	14.90	47.0		9.80	35.9	
	25	12.40	39.2		9.80	35.9	
	12.50	9.80	30.9		7.20	26.4	
	6.25	7.20	22.7		0.00	0	
Control [Gentamicin (30 µg/mL)]		31.70			27.30		

Note: Z.I. - Zone of inhibition

Table 2. Antibacterial Activity of *P. pinnata* Leaf Fractions on Gram-Negative Bacterial Pathogens

Fractions	Concentration (mg/mL)	<i>Klebsiella pneumoniae</i>			<i>Proteus vulgaris</i>		
		Z.I (mm)	% Inhibition	IC ₅₀ (mg/mL)	Z.I (mm)	% Inhibition	IC ₅₀ (mg/mL)
N-hexane	100	13.60	61.5	44.07±0.01	14.90	102.0	21.10±0.02
	50	13.60	61.5		11.10	76.0	
	25	7.20	32.6		9.80	67.1	
	12.50	0.00	0		0.00	0	
	6.25	0.00	0		0.00	0	
Ethyl acetate	100	14.90	67.4	49.42±0.02	14.90	102.0	10.41±0.01
	50	9.80	44.3		11.10	76.0	
	25	7.20	32.6		9.80	67.1	
	12.50	7.20	32.6		9.80	67.1	
	6.25	0.00	0		0.00	0	
Control [Gentamicin (30 µg/mL)]		22.10			14.60		

Note: Z.I. - Zone of inhibition

CONCLUSION

Taken together, the ethyl acetate fraction showed moderate to significant antibacterial potential against the pathogens and would therefore be a good target for drug discovery against human pathogens, especially those associated with wounds.

AUTHORS' CONTRIBUTIONS

Conceptualization, OAA and JOO; **methodology**, OAA and OMD; **validation**, OAA, JOO and OMD; **formal analysis**, OMD; **investigation**, OMD; **resources**, OAA and OMD.; data

curation, OMD; **writing—original draft preparation**, OAA; **writing—review and editing**, OAA and OMD; **supervision**, OMD; **project administration**, OAA, JOO and OMD; **funding acquisition**, OAA and OMD. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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