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Phytochemical Screening and Antibacterial Activity of *Chrysophyllum albidum* Collected in Nsukka, South East Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Chrysophyllum albidum* is widely used by African people for the treatment of various types of diseases such as ear infection, sore throat, typhoid, cellulites, septicaemia, bactericemia, abscesses and tooth infections.
Aim: The study was conducted to investigate the chemical components and antibacterial activity of the extract and fractions from the root bark of *Chrysophyllum albidum* from Nsukka, South-east Nigeria.
Methodology: The fresh roots were collected, washed, cut into small pieces, air dried and pulverized to powder using mechanical grinder. Extraction and fractionation were done by cold

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maceration method and technique of liquid–liquid extraction respectively. The phytochemical analysis of the methanol extract and, n-hexane, butanol, aqueous and ethylacetate fractions of the plant part was carried out using standard method. The antibacterial activities were determined using cup-plate agar diffusion and agar dilution methods.

Results: The phytochemical screening of the extract revealed the presence of tannins, flavonoids, saponins, terpenoids, alkaloids, reducing sugar and cardiac glycosides. The inhibition zone diameter (IZD) produced by the agents against some selected Gram positive bacteria (GPB) and Gram negative bacteria (GNB) pathogens ranged from 6 – 25 mm and 6 – 12 mm respectively. The MIC and MBC values produced by the extract and fractions of the plant's part against the GPB ranged from 1.25 – 40 mg/ml and 5 – 80 mg/ml respectively Many of the GNB were not sensitive to the agents tested except *Pseudomonas aeruginosa* and *Klebsiella spp* that exhibited mild to moderate sensitivity to the agents.

Conclusion: These agents, therefore, exhibited a potent antibacterial activity against all the GPB and a few GNB pathogens tested due to their potent phytochemicals. The results of this work have corroborated the trado-medical use of root of *Chrysophyllum albidum* for treating ear infection, sore throat, typhoid, cellulites, septicaemia, bactericemia, boils and tooth infection/decay.

Keywords: Chrysophyllum albidum; phytochemicals; antibacterial; gram-positive; gram-negative; MIC; MBC.

1. INTRODUCTION

"Bacterial infections result in 17 million deaths worldwide annually, mostly in children and the elderly" [1]. "The morbidity and mortality associated with bacterial infections have remained significant, despite advances in antimicrobial chemotherapy. The situation has worsened with incessant increase in antibacterial resistance. Today, multiple drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Acinetobacter baumannii*, *Klebsiella pneumonia*, *Escherichia coli* and *Pseudomonas aeruginosa* pose a serious threat to human and animal health. There is also the emergence of bacterial pathogens with intrinsic resistance to antibiotics making the available antibiotics obsolete" [2]. In addition to this antibiotic resistance, there are other problems associated with the use various antibiotics, which include low efficacy, high cost, scarcity and adverse side effects which have led to the exploration for natural and potent antibacterial agents from medicinal plants. "Medicinal plants have been used for the treatment of various diseases as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. Medicinal plants are of great importance in drug development and humans have used them for different diseases from the beginning of human history" [3]. "The medicinal power of plants lies in phytochemical constituents that cause definite pharmacological actions on the human body" [4]. "According to WHO, 80% of the world's population depend on traditional medicine for treatment of many diseases" [5]

"*Chrysophyllum albidum* is one the medicinal forest fruit tree commonly found throughout tropical Africa. *Chrysophyllum albidum* and its related twin plant *Chrysophyllum africanum* (Family Sapotaceae) are often called the African star apple" [6]. "In Nigeria, *Chrysophyllum albidum* is given different names depending on the locality. In the western part, the Yorubas call it Agbalumo while it is called Udara in the eastern and southern parts of Nigeria. In the northern part, it is popularly known as Agbaluba. *Chrysophyllum albidum* is used in Nigeria for the treatment of different ailments" [6]. "The stem barks and root are used in urinary related infections" [7]. "The leaves are used as emollients and for the treatment of skin eruptions, diarrhoea and stomach ache" [8]. "The fruits are known as a natural source of antioxidants that can reduce free radical mediated diseases such as diabetes, cancer and coronary heart disease" [9].

"Phytoconstituents individually or in combination, determine the therapeutic value of a medicinal plant. Alkaloids, flavonoids, phenolics, tannins, saponins, steroids, glycosides, terpenes etc. are some of the important phytochemicals with diverse biological activities. The pharmacological activity of a plant can be predicted by the identification of the phytochemicals. The plant extracts from *Chrysophyllum albidum* possess

hepatoprotective activity" [10]. Ethanolic extract from *Chrysophyllum albidum* cotyledons collected from Benin City, Edo State, Nigeria.was found to possess antimicrobial activity against *P. aeruginosa, S. aureus and E.coli.* The questions that ignited this work were whether the roots of *Chrysophyllum albidum* gotten from Nsukka soil, Enugu State, Nigeria have similar phytochemical and antibacterial properties as the roots of the same plants found from other climes? Several researchers have carried out the in vitro inhibitory effects of extracts from the plants against bacterial isolates. Currently, there is paucity of information on the phytochemical constituents and antibacterial potentials of Nsukka *Chrysophyllum albidum* root extracts against bacterial pathogens. Therefore, this study was designed to evaluate the root bark of Nsukka *Chrysophyllum albidum* for phytochemical constituents and antibacterial activity.

2. MATERIALS AND METHODS

2.1 Materials

Study area: The roots of the plant were collected in Nsukka, South-east Nigeria. The laboratory work was done in the Microbiology Laboratory of Enugu State University of Science and Technology, Enugu, Nigeria.

2.2 Culture Media and Chemicals

All culture media and chemicals used were of analytical reagent grade. They were purchased from Oxoid (Cambridge,UK).

Plant material: The fresh roots of *Chrysophyllum albidum* collected from its natural habitat at Obollo Road in Nsukka Local Government Area of Enugu State, Nigeria, between December 2022 and January 2023 were used for the study. The identity of the plant was authenticated by a botanist - Mr. A .O .Ozioko- of the Bioresource Development and Conservation programme (BDCP) Nsukka, Enugu State and voucher specimen was deposited at the Herbarium of the same institution.

Test organisms: The organisms used were: *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, Klebsiella aerogenes, *Proteus mirabilis, Streptococcus pneumoniae.* and *Bacillus subtilis*. The test organisms were obtained from Pharmaceutical Microbiology Laboratory division of Enugu State University of Science and Technology, Enugu, Nigeria.

2.3 Methods

2.3.1 Collection and preparation of plant materials

Fresh roots of *Chrysophyllum albidium* were collected in January, 2023 in Obollo Road Nsukka Local Government Area, Enugu State, Nigeria. The fresh roots were collected, washed, cut into small pieces and air dried for two (2) weeks. The dried roots were pulverized into powder using mechanical grinder and then stored in containers for further use.

2.3.2 Extraction of the plant sample

The technique was carried out using cold maceration method as previously described [11]. A 210 g of the powdered sample was weighed out and added into container with lid. 4 L of methanol were added and the container was stoppered. The mixture was stirred thoroughly and kept at room temperature for the next 72 h with intermittent vigorous stirring at regular intervals. The mixture was filtered through a muslin cloth and secondly with a funnel fitted cotton wool at the base. The extract was poured through the set-up and a clearer filtrate was obtained. The extract was subdivided into smaller portions in 1000ml beakers and kept under room temperature to evaporate to dryness with aid of fan. The extract was subsequently stored in the refrigerator at 4°C.

2.3.3 Fractionation of the crude extract

The fractionation was carried out using the technique of liquid–liquid extraction. Three organic solvents, namely ethyl acetate, butanol and n-hexane were selected to partition the crude extract into individual fractions moving from the least polar solvent(lowest eluting power) to the most polar solvent(highest eluting power). 20 g of Crude extract was reconstituted in 100 ml of 5% aqueous methanol and added into the separation funnel. 200 ml of n-Hexane was added into the set-up, the separation funnel was corked and the mixture was shaken vigorously to ensure uniform mixing. The mixture was allowed to stand for 20 minutes partitioning into two immiscible layers of solvents, then the lower

layer (aqueous phase) was collected in a beaker and the upper phase (n-hexane phase) was as well collected in a separate beaker. The process was repeated two more times with 200 ml of n-Hexane each and on the third separation process the n-Hexane phase was clear indicating that nearly all n-Hexane soluble components has been extracted. The same process was repeated using ethyl acetate, a more polar solvent. On the third separation with 200 ml of ethyl acetate the ethyl acetate phase was clear indicating that all ethyl acetate soluble components have been extracted. The next solvent used was n-Butanol which was the most polar solvent. 200mls of the solvent was used and the process described above was repeated. The fourth separation process with n-Butanol phase was clear indicating that it contains more components of the extract. The solvent fractions were evaporated to dryness and weighed. The fractions were stored in a refrigerator at 4°C subject to more analytical investigations.

2.3.4 Phytochemical screening of plant extract

The quantitative chemical analysis of the crude extact was carried out for the presence of alkaloids, tannins, saponins, steroids, cardiac glycosides, flavonoids, and terpenoids using the method previously described [11].

2.3.5 Antimicrobial sensitivity test

The preliminary antibacterial activities of the methanolic extract and fractions (ethyl acetate, butanol, N-hexane and aqueous fraction) from the roots of *Chrysophyllum albidum* and the standard drug (ofloxacin) were determined using the agar well diffusion method as previously described [11]. All the extract and fractions were dissolved using Dimethylsulphoxide (DMSO) to produce the following concentrations; 100, 50, 25, 12.5 mg/ml and all the concetrations were tested against *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus p*neumoniae, *Klebsiella aerogenes., Escherichia coli*, *Salmonella typhi* and *Bacillus subtilis.* A 20 ml sterile nutrient agar was aseptically poured into each sterile Petri dish, then, seeded with a 0.1 ml of the fresh organism (standardized by adjusting to 0.5 McFarland Standard) and allowed to set. Using a sterile cork-borer of 8 mm in diameter, equidistant wells were made in the agar. Using a micrometer pipette, 60 ml each of each dilution of the extracts was carefully introduced into the cup. As a procedural control, a 0.05 mg/ml of ofloxacin was also introduced into one of the cups. The plates were allowed to stand on the bench for 1 h to allow diffusion of the extracts before incubation at 37ºC for 24 h for the bacterial isolates. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule.

2.4 Minimum Inhibitory Concentration (MIC)

The MIC of the extract and fractions against the test micro-organisms were determined using agar dilution method [12]. Five different dilutions of each of the extract and fractions in DMSO were prepared by two-fold dilution. The concentrations of the agents prepared for the test isolates were 1.25, 2.50, 5.0, 10, 20, 40 and 80 mg/ml. With an automatic micropipette, 1ml each of these different dilutions (one dilution per plate) was introduced into individual agar plates respectively. The molten agar and the diluted agent were mixed carefully and thoroughly and allowed to set. With the aid of a sterile wire loop the standardized test microorganisms were delivered on the agar surface of the plates containing different concentrations of the agent. This was done by streaking on the surface of the set agar. These inoculated agar plates were incubated at 37°C for 24 h. At the end of the incubations, the MICs were determined as previously reported [13].

2.5 Minimum Bactericidal Concentration (MBC)

After taking the MIC readings, the inoculated surface with no visible growth were sub-cultured on freshly prepared nutrient broth. The culture media were incubated appropriately for 24 h and then observed for growth. After 24 h, the lowest concentration from which the microorganisms did not recover and grow when transferred to the fresh media was recorded as the minimum bactericidal concentration (MBC) [14] .

2.6 Statistical Analysis

The antibacterial activities of extract and fractions from the roots of *Chrysophyllum albidium* were performed in triplicate independent experiments ($n = 3$). The calculated mean values are presented ± standard error (SE) and compared using the Duncan test

using spss software (Statistical Package for the Social Sciences, version 23.0, New York) and the difference was considered statistically significant ($P < 0{\cdot}05$).

3. RESULTS

3.1 Percentage Yield

The results of the percentage yield of the extract and fractions of *Chrysophyllum albidium* root back are presented in Table 1. The percentage yields of methanol crude extract, n-hexane fraction, ethylacetate fraction, n-butanol fraction and aqueous fraction were 69.56, 5.38, 10.16, 7.29 and 7.57 % respectively.

3.2 Phytochemical Screening of *Chrysophyllum albidium* Root

The results of the phytochemical analysis of the methanol extract and other fractions of *Chrysophyllum albidium root* are shown in Table 2. It is important to note that it is only terpenoid that was present in n-hexane fraction of the plant. Thus, saponins, flavonoids and tannins were present in copious amount in all the extract and fractions except the n-hexane fraction. Alkaloids were present in small amount in ethylacetate fraction and methanol crude extract of the plant part.

3.3 Antibacterial Sensitivity Test

The preliminary sensitivity tests and MIC/MBC determinations carried out on the extract and fractions of the plant's part under study are shown in Tables 3-7 and 8 respectively. The inhibition zone diameter (IZD) produced by the agents against Gram positive bacteria (GPB) and Gram negative bacteria (GNB) ranged from 6 – 25 mm and 6 – 12 mm respectively (Tables 3-7). Among the GNB tested, *Pseudomonas aeruginosa* and *Klebsiella spp* only showed sensitivity to some of the agents tested (Tables 3, 6 and 7). The MIC and MBC values produced by the test extract and fractions of the plant's part against the GPB ranged from 1.25 – 40 mg/ml and 5 – 80 mg/ml respectively (Table 8). Many of the GNB were not sensitive to the agents tested except *Pseudomonas aeruginosa* and *Klebsiella spp* that showed mild sensitivity to the agents (MIC and MBC values of 20 – 40 mg/ml and 40 - 80 mg/ml respectively.

Table 1. Percentage yield of the extracts of C*hrysophyllum albidium* root

Extract/Fractions	Mass of pulverized sample (gram)	Mass of extract / fractions recovered (gram)	% Yield
Extract (Methanol)	510	29.83	69.56
n-hexane	20	2.31	5.38
Ethyl acetate	20	4.36	10.16
n-butanol	20	3.13	7.29
Aqueous	20	3.25	7.57
Summation		42.88	100

Table 2. Phytochemical components of the root bark of *Chrysophyllum africanum*

S/N	Phytoconstituents	Methanol Extract	Hexane fraction	Ethyl acetate fraction	Butanol fraction	Aqueous fraction
1	Reducing sugar	+	-	-	+	+
2	Alkaloids	+	-	+	-	-
3	Saponins	+++	-	++	+++	+++
4	Tannins	+++	-	+++	+++	+++
5	Flavonoids	+++	-	+++	+++	+++
6	Steroids	-	-	-	-	-
7	Terpenoids	++	++	+	-	-
8	Cardiac Glycosides	+++	-	++	++	+++

Key; + Sparsely Present; ++ Moderately Present; +++ Copiously Present; - Absent. A = Methanol Extracts, B = n-Hexane fraction, C = Ethyl acetate fraction, D = Butanol fraction, E = Aqueous fraction

Table 3. Sensitivity of methanolic extract of *C. albidum* and ofloxacin against the bacteria tested

Bacteria	IZD (mm) ± SEM of different Concentration of methanolic extract of *C. albidum* (mg/ml)				Ofloxacin ug
	100	**50**	**25**	**12.5**	**5µg**
Sa	10.33 ±1.70	08.0 ± 1.30	06.10 ± 1.0	0	7.74 ± 1.21
Ef	12.35 ± 1.20	09.6 ± 0.93	6.88 ±0.55	0	6.7 ± 0.95
Pa	10.36 ± 1.54	09.00 ± 1.04	7.29 ± 0.98	0	7.57 ±0.63
Pm	0	0	0	0	7.2 ± 1.0
Stp	10.36 ± 1.54	11.00 ± 1.04	7.29 ± 0.98	0	8.57 ±0.63
Ka	10.36 ± 1.54	11.00 ± 1.04	7.29 ± 0.98	0	6.57 ±0.63
Ec	0	0	0	0	0
Sal	0	0	0	0	5.2 ± 1.0
Bs	22.80 ±1.07	20.23 ± 1.34	11.62 ± 0.85	4.0 ± 1.37	5.1 ± 1.6

Key; 0 = zero IZD, ie no activity, ofx = ofloxacin Sa. Staphylococcus aureus, Ef, Enterococcus faecalis, Pa, Pseudomonas aeruginosa Pm,Proteus mirabilis, Stp, Streptococcus pneumoniae., Ka, Klebsiella aerogenes., Ec Escherichia coli, Sal Salmonella typhi and Bs Bacillus subtilis

Table 4. Sensitivity of n-Hexane fraction of *C. albidum* and ofloxacin against the bacteria tested

Bacteria	IZD (mm) ± SEM of different Concentration of methanolic extract of *C. albidum* (mg/ml)				Ofloxacin ug
	100	**50**	**25**	**12.5**	**5µg**
Sa	0	0	0	0	7.74 ± 1.21
Ef	0	0	0	0	6.7 ± 0.95
Pa	0	0	0	0	7.57 ±0.63
Pm	0	0	0	0	7.2 ± 1.0
Stp	0	0	0	0	8.57 ±0.63
Ka	0	0	0	0	6.57 ±0.63
Ec	0	0	0	0	0
Sal	0	0	0	0	5.2 ± 1.0
Bs	11.00 ± 1.04	09.00 ± 1.04	7.29 ± 0.98	0	5.1 ± 1.6

Key; 0 = zero IZD, ie no activity, ofx = ofloxacin Sa. Staphylococcus aureus, Ef, Enterococcus faecalis, Pa, Pseudomonas aeruginosa Pm,Proteus mirabilis, Stp, Streptococcus pneumoniae., Ka, Klebsiella aerogenes., Ec Escherichia coli, Sal Salmonella typhi and Bs Bacillus subtilis

Table 5. Sensitivity of Ethylacetate fraction of *C. albidum* and ofloxacin against the bacteria tested

Bacteria	IZD (mm) ± SEM of different Concentration of methanolic extract of *C. albidum* (mg/ml)				Ofloxacin ug
	100	**50**	**25**	**12.5**	**5µg**
Sa	22.33 ±1.70	18.0 ± 1.30	16.10 ± 1.0	6.22 ±0.15	7.74 ± 1.21
Ef	10.35 ± 1.70	09.6 ± 0.493	6.02 ±0.45	0	6.7 ± 0.95
Pa	0	0	0	0	7.57 ±0.63
Pm	0	0	0	0	7.2 ± 1.0
Stp	20.36 ± 1.54	13.00 ± 1.04	7.29 ± 0.98	06.02 ±0.15	8.57 ±0.63
Ka	0	0	0	0	6.57 ±0.63
Ec	0	0	0	0	0
Sal	0	0	0	0	5.2 ± 1.0
Bs	24.82 ±1.07	19.26 ± 1.04	10.62 ± 0.80	6.0 ± 1.06	5.1 ± 1.6

Key; 0 = zero IZD, ie no activity, ofx = ofloxacin Sa. Staphylococcus aureus, Ef, Enterococcus faecalis, Pa, Pseudomonas aeruginosa Pm,Proteus mirabilis, Stp, Streptococcus pneumoniae., Ka, Klebsiella aerogenes., Ec Escherichia coli, Sal Salmonella typhi and Bs Bacillus subtilis

Table 6. Sensitivity of aqueous fraction of *C. albidum* and ofloxacin against the bacteria tested

Bacteria	IZD (mm) ± SEM of different Concentration of methanolic extract of *C. albidum* (mg/ml)				Ofloxacin ug
	100	50	25	12.5	µg
Sa	16.33 ±1.24	09.0 ± 0.39	06.10 ± 0.99	0	7.74 ± 1.21
Ef	14.05 ± 1.10	09.6 ± 0.73	06.88 ±0.55	0	6.7 ± 0.95
Pa	09.32 ± 1.54	06.00 ± 1.04	0	0	7.57 ±0.63
Pm	0	0	0	0	7.2 ± 1.0
Stp	10.31 ± 1.04	08.00 ± 1.04	06.29 ± 0.48	0	8.57 ±0.63
Ka	09.30 ± 1.54	07.00 ± 1.04	0	0	6.57 ±0.63
Ec	0	0	0	0	0
Sal	0	0	0	0	5.2 ± 1.0
Bs	20.80 ±1.07	18.23 ± 1.24	10.42 ± 0.85	0	5.1 ± 1.6

Key; 0 = zero IZD, ie no activity, ofx = ofloxacin Sa. Staphylococcus aureus, Ef, Enterococcus faecalis, Pa, Pseudomonas aeruginosa Pm,Proteus mirabilis, Stp, Streptococcus pneumoniae., Ka, Klebsiella aerogenes., Ec Escherichia coli, Sal Salmonella typhi and Bs Bacillus subtilis

Table 7. Sensitivity of Butanol fraction of *C. albidum* and ofloxacin against the bacteria tested

Bacteria	IZD (mm) ± SEM of different Concentration of methanolic extract of *C. albidum* (mg/ml)				Ofloxacin ug
	100	50	25	12.5	5µg
Sa	24.32 ±1.30	13.0 ± 1.31	10.10 ± 1.0	07.22 ± 0.68	7.74 ± 1.21
Ef	19.32 ± 1.20	16.6 ± 0.43	09.82 ±0.55	07.19 ± 0.98	6.7 ± 0.95
Pa	10.36 ± 1.24	09.00 ± 1.04	7.21 ± 0.38	06.24 ± 0.34	7.57 ±0.63
Pm	0	0	0	0	7.2 ± 1.0
Stp	20.36 ± 1.74	18.00 ± 1.24	10.29 ± 0.18	0	8.57 ±0.63
Ka	10.11 ± 1.54	08.00 ± 1.04	0	0	6.57 ±0.63
Ec	0	0	0	0	0
Sal	0	0	0	0	5.2 ± 1.0
Bs	23.85 ±1.22	21.23 ± 1.12	13.32 ± 0.65	8.0 ± 1.37	5.1 ± 1.6

Key; 0 = zero IZD, ie no activity, ofx = ofloxacin Sa. Staphylococcus aureus, Ef, Enterococcus faecalis, Pa, Pseudomonas aeruginosa Pm,Proteus mirabilis, Stp, Streptococcus pneumoniae., Ka, Klebsiella aerogenes., Ec Escherichia coli, Sal Salmonella typhi and Bs Bacillus subtilis

Table 8. The result of minimum inhibitory concentration, MIC and minimum bactericidal concentration MBC (mg/mL) of the *Chrysophyllum albidium* extract and fractions against the test bacteria

S/N	Organisms	Methanol Extract (mg/mL)		Aqueous Fraction (mg/mL)		Butanol Fraction (mg/mL)		Ethyl Acetate Fraction (mg/mL)	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
1	*Staphylococcus aureus*	20	40	+	+	2.5	5	5	5
2	*Enterococcus faecalis*	20	20	5	10	5	10	10	10
3	*Pseudomonas aeruginosa*	40	80	40	80	+	+	20	40
4	*Proteus mirabilis*	+	+	+	+	+	+	+	
5	*Streptococcus spp.*	10	20	+	+	5	10	5.0	10
6	*Klebsiella spp.*	40	80	+	+	40	80	40.	40
7	*Escherichia coli*	+	+	+	+	+	+	+	+
8	*Salmonella typhi*	+	+	+	+	+	+	+	+
9	*Bacillus subtilis*	5	5	2.5	5	1.25	5	5.0	5.0

KEY: A = Methanol Extracts, B = n-Hexane fraction, C = Ethyl acetate fraction
D = Butanol fraction, E = Aqueous fraction, NA = Not active against the organism tested

4. DISCUSSION

The results of the phytochemical analysis of methanol extract of *Chrysophyllum albidium* root showed the presence of terpenes, tannins, saponins, alkaloids, reducing sugar, flavonoids and glycosides but absence of steroids. (Table 2). Similarlly, the fractions revealed the presence of tannins, alkaloids, flavonoids, glycosides, saponins and terpenoids. This is in agreement with the findings of previous researchers on the presence of phytochemicals in the crude extracts of *Chrysophyllum albidum* [15]. The results of the phytochemical screening of the plant part under study revealed the following phytoconstituents in the descending order of their occurrence-flavonoids, tannins, saponins, alkaloids, glycosides, terpenoids, alkaloid and reducing sugars. This agrees with the previous phytochemical analysis of the leaves, stem bark and root extracts of *Chrysophyllum albidum* which revealed large quantities of flavonoids, saponins, tannins and Cardiac glycosides [15,16]. However, our findings that Nsukka *Chrysophyllum albidum* root bark contain small amount of alkaloid and reducing sugar, and no steroids disagree with the results of the previous researches [16]. Moreover, study by Okoli, [17] on the phytochemistry of *Chrysophyllum albidum* stem slash, seed cotyledon, leaves and root revealed the presence of alkaloids, tannins, phenols and flavonoids except cardiac glycosides in the root which is not in agreement with our findings in which glycosides are found in copious amount in the root of the plant. These phytoconstituents are known to be biologically active and contribute to the antimicrobial and antioxidant activities of medicinal plants [18]. These phytochemicals could be responsible for the antibacterial activities of the plant extract against test isolates used for this study [19]. There are different mechanisms through which these plant compounds exert antibacterial activity, for instance, post-translational inhibition by tannins which is achieved by the formation of irreversible complexes with proline-rich proteins [20]. Alkaloids have been found to be antineoplastic and antimicrobial [21]. Again, saponins have been demonstrated to exhibit significant antibacterial activities *in vitro* and *in vivo,* and also increase the immune function by enhancing the activities of lysozyme [22]. Flavonoids occur as a C3–C6 unit linked to a phenolic ring that is also hydroxylated. The antimicrobial properties of flavonoids are probably because they form complexes with both extracellular and soluble proteins, as well as bacterial cell wall. They could also disrupt cell membranes if lipophilic enough [23].

The results of the preliminary antibacterial screening conducted on the extract and fractions of the plant as shown by the IZD produced against the test organisms showed that all GPB tested were sensitive to the agents. The plant's root extract and fractions showed a promising activity against *S. aureus, E. faecalis, Streptococcus pneumonia* and *Bacillus subtilis* producing large inhibition zone diameter. The IZD values gotten against a greater percentage of the GPB were high (> 20 mm). This agrees with the previous findings which showed that *Chrysophyllum albidium* root extract has antibacterial activity against bacteria [19]. Many GNB tested showed resistance to the test agents. Methanolic extract, butanol and aqueous fractions of the sample showed promising activity against all GPB tested. *Proteus mirabilis, Escherichia coli* and *Salmonella typhi* were resistant to all the extract and fractions tested. Our findings agree with the results of the work done by Idowu (2016), which reported that the ethyl acetate fraction was active against all the test organisms with Gram-positive bacteria being more susceptible, while crude ethanolic extract, n-butanol and aqueous fractions had no activity against *E. coli* [15]. However, our findings disagree with the report from Okoli, [18] who demonstrated that Chrysophyllum albidum elicit anti-bacterial activity against *Escherichia coli*, but agrees with his report of high activity of the extacts from this plant against *Staphylococcus aureus, Bacillus subtilis and Pseudomonas aeruginosa*, Similarly, the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of the *C. albidum* root bark extract and fractions were also investigated. The MIC and MBC values produced by these agents against the GPB ranged from 1.25 - 40 mg/ml and 5 – 80 mg/ml respectively (Table 4). Many of the GNB were not sensitive to the agents tested, however, *Pseudomonas aeruginosa* and *Klebsiella spp* showed sensitivity to the agents with MIC and MBC values of 20 – 40 mg/ml and 20 – 80 mg/ml respectively. The results of the MIC and MBC gotten from this work showed that the agents have a good antibacterial activity against many of the bacteria tested, GPB exhibiting more sensitivity than GNB to the agents tested. Similar observations were made in the results of the work done by Odewade and Odewade [19]. Medicinal plant extract with very low MIC and MBC is known to possess significant antimicrobial potentials [24] The

antibacterial activity of plant extract is considered significant if the MIC of the extract is less than or equal to 200 mg/mL [25]. As observed in this study, the MIC values exhibited by the root extract were below 200 mg/mL. Therefore, active antibacterials compounds could be developed from this plant for the treatment of infections caused by GPB and some GNB.

5. CONCLUSION

The extract and fractions from the root bark of *Chrysophyllum albidum* exhibited a potent antibacterial activity against all the GPB and a few GNB pathogens tested. The significant effects produced by the agents were due to the bioactive phytoconstituents of the root bark of the plant. The results of this work have corroborated the trado-medical use of root of *Chrysophyllum albidum* for treating ear infection, sore throat, typhoid, cellulites, septicaemia, bactericemia, boils (abscesses) and tooth decay. Therefore, further work on the plant is highly advised to isolate, characterise and standardize the active compounds for the treatment of bacterial infections.

CONSENT AND ETHICAL APPROVAL

It is not appilicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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