

Original Research Article

Phytochemical Screening and Antibacterial Evaluation of *Anacardium occidentale* Root collected in Agbani, Eastern Nigeria

Abstract

Background: Different parts of *Anacardium occidentale* (Cashew) are used in folk medicine to treat dysentery, diarrhea piles, toothache and sore gums. Evaluation of phytoconstituents or novel compounds from the plant is highly needed to authenticate these claims.

Aim: The aim of the research was to conduct phytochemical analysis and antibacterial evaluations of extract and fractions of root of *Anacardium occidentale* from Agbani,

Methods: The pulverized material was extracted with methanol using cold maceration method. Solvent fractions were obtained using separating funnel based on liquid-liquid extraction technique starting with non-polar to high polar solvent. The cup-plate agar diffusion was used to conduct preliminary antibacterial evaluation of the extract and fractions. Agar dilution and streaking on the solid agar techniques were used to evaluate the test extract and fractions against some selected bacteria for minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) respectively.

Results: The phytochemical screening of extract and fractions showed that terpenoids, saponins, glycosides, fat and oil, alkaloids, flavonoids and tannins were present. The preliminary evaluations of the agent showed promising activity by producing inhibition zone diameter ranging from 10 ± 0.66 to 20 ± 0.98 mm. The MIC and MBC of the test extract and fractions against the test bacteria - *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus spp*, *Escherichia coli*, *Klebsiella spp*, *Salm typhi*, *Bacillus subtilis*, *Salmonella. spp* and *Proteus. mirabilis* – ranged from 0.0025-0.040 mg/mL and 0.020 – 0.080 mg/mL respectively

Conclusion: Our study showed that the extract and fractions of the cashew root possess promising antimicrobial activity. This suggests that the agents may provide novel compounds for antibacterial drug development research.

Keywords: *Anacardium occidentale*, Phytochemicals, Antibacterial, MIC, Agbani and Extraction

Introduction

Globally, the prevalence of multi-drug resistant pathogens in the developing and underdeveloped countries are causing serious public health concerns. This is made worse by the inaccessibility, adverse effects and high cost of orthodox drugs. Consequently, greater number of people in the rural areas of the developing and underdeveloped countries depends on medicinal plants in the prevention and treatment of diseases. Taking into consideration the rate of drug resistance and its serious threat to the health of people in developing and underdeveloped countries, there is an urgent need to search and develop new drugs to treat such infections caused by drug resistant organisms. Nowadays, 80% of people living in Sub-Saharan Africa are almost completely dependent on folk medical practices for their primary healthcare needs, and higher plants are

known to play a crucial role in traditional medicine [1]. In Nigeria, there is a strong dependence on plants as medicines, particularly in the rural areas [2] like Agbani, Enugu. Medicinal plants-based antimicrobial agents are very important in reducing the burden of multi-drug resistant pathogenic microbes of public health interest as there are fewer effective antimicrobial agents available for treating infections [3, 4]. Despite tremendous progress in human medicines, infectious diseases caused by microbes are still a major threat to public health and particularly in developing countries due to relative unavailability of medicine and the emergence of widespread drug resistance [5]. The affordability, accessibility, wider acceptability among the population and low toxic profile of medicinal plants has negated the numerous problems associated with synthetic therapeutics [6]. As a result prompting the present research on the root of *Anacardium occidentale* to evaluate its bioactive metabolites and antibacterial activity.

Anacardium occidentale R. (cashew) of the family Anacardiaceae, is a small-sized tree with a dome-shaped crown. The flowers are whitish turning pinkish-red. Fruits are a kidney-shaped nut attached to the distal end of an enlarged pear-shaped receptacle called the cashew apple [7]. The young and tender leaves of *A. occidentale* are a popular herb consumed raw and sometimes blanched to reduce their stringent taste. In traditional medicine, leaves are used for treating dysentery, diarrhoea and piles, and an infusion of bark and leaves are applied to relief toothache and sore gums [8]. Other uses of the leaf includes remedy for rheumatism and hypertension [9]. *A. occidentale* plant parts have been reported to possess pharmacological property, especially the leaves and stem bark [10,11]. They are rich in therapeutic bioactive metabolites which contribute to its significant antimicrobial activity [12,13]. There is paucity of information on the phytochemical constituent and the antimicrobial potentials of the root of this plant in Nigeria. Besides, due to variation in phytoconstituents of plants' parts vis-à-vis edaphic factor and geographical locations, their antimicrobial potentials vary. Currently, there is no documented work done on Agbani Cashew plant parts with respect to phytochemical screening and antimicrobial activity. As a result, there is need to search, develop and document potential plant based-drugs based on the location. This research, therefore, was designed to screen the extract and solvent fractions of root of *A. occidentalis* from Agbani for phytochemical constituents and antibacterial activity.

Material and method

Plant materials

Fresh roots of *Anacardium occidentale* were collected in January, 2022 at Agbani, Nkanu West Local Government Area of Enugu State, Nigeria. The plant was authenticated by the Taxonomist in the Department of Pharmacognosy, Enugu State University of Science and Technology Enugu. The fresh roots

were collected, washed, cut into pieces and air dried for two (2) weeks. The dried roots ground into powder using mechanical grinder and then stored in containers for further use.

Preparation of plant extract

A 500 g of the powdered sample was weighed out and added into container with lid. A 4 litre of methanol was 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 1000 ml beakers and kept under room temperature to evaporate to dryness with aid of fan. The extract was subsequently stored in the refrigerator for further analysis.

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). A 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. A 200 ml 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.

Phytochemical screening

The extract and fractions were screened for bioactive metabolites- alkaloids, flavonoids, tannins, saponin, terpenoids, steroids, cardiac glycosides and fats and oils -based on standard procedure [15].

Test organisms.

The organisms used were clinical isolates of *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella* spp *Proteus mirabilis*, *Streptococcus sp.* and *Bacillus subtilis*. The test organisms were obtained from Adonai Medical Diagnostic and Research Laboratory centre Nsukka, Enugu State.

Cup-plate agar diffusion

The antibacterial activities of *Anarcadium occidental root* and the standard drug- levofloxacin- were determined using the agar-well diffusion method [16]. A 200 mg each of the samples was dissolved in dimethylsulphoxide (DMSO) and then double-diluted serially to obtain the following concentrations; 0.2, 0.1, 0.05, 0.025mg/ml. A 20 ml sterile nutrient agar was aseptically poured into each sterile Petri dish, then, seeded with a 0.1 ml fresh organism (standardized by adjusting to 0.5 McFarland Standard) and allowed to set. Using a sterile cork-borer of 6 mm in diameter, equidistant wells were made in the agar. Using a micrometer pipette, 60 µl each of each dilution of the extracts and fractions was carefully introduced into the cup. As a procedural control, a 0.05 mg/ml of levofloxacin 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 37°C for 24 h for the bacterial isolates. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule.

Minimum Inhibitory Concentration (MIC)

The MIC of the extract and fractions against the test micro-organisms were determined using agar dilution method (17). 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 0.00125, 0.0025, 0.005, 0.01 and 0.020 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 (four different strains of the isolates per plate) 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 [18].

Minimum Bactericidal Concentration (MBC)

After taking the MIC readings, the lines of streaking on the agar plate with no visible colonies were streaked again and sub-cultured on freshly prepared Mueller–Hinton agar. The culture media were incubated appropriately for 48 h and then observed for growth. After 48 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) [19].

Data analysis:

Data resulting from the study were analyzed and evaluated on the basis of averages and percentage values. Tables were used for the presentation of results. Statistically, a descriptive analysis (one way anova) was performed, and variables were analyzed with the statistical package for social sciences (SPSS) version 23.0 for windows. The differences in data were considered statistically significant at $p < 0.05$.

Results

The percentage yield

The methanol extract gave a good yield of approximately 75 %. Butanol had the best fraction yield (8.7 %) followed by aqueous fraction (6.9 %). The n-hexane fraction produced the least percentage yield of about 3.6%.

Table 1: The percentage yield of extract and fractions

Samples	Mass of powder / MEAO (grams)	Yield of extract / fractions (grams)	% yield
Pulverized material	500	-	-
MEAO	-	44.77	74.95
HFAO	20	2.15	3.60
EFAO	20	3.48	5.82
BFAO	20	5.20	8.70
AFAO	20	4.13	6.91

Key: MEAO= methanol extract of *A. occidentale*, HFAO= n-hexane fraction of *A. occidentale*, EFAO= ethyl acetate fraction of *A. occidentale*, BFAO= n-butanol fraction of *A. occidentale* and AFAO= aqueous fraction of *A. occidentale*

The qualitative phytochemical screening

The results of the phytochemical analysis of methanol extract of *Anarcadium occidentale* root bark showed that terpenes, tannins, saponins, alkaloids, fat and oil, flavonoids and glycosides were present in copious amount. The ethyl acetate fraction contained more of the phytoconstituents compared to the polar fractions. Importantly, the hexane fraction showed the presence of terpenoids, and fat and oil only.

Table 2: The qualitative phytochemical constituents of extract and fractions of *A. occidentale* root

Sample	Alkaloids	Flavonoids	Terpenoids	Saponins	Tannins	Steroids	Fats&oils	Cardiac glycosides
MEAO	+	+	+	+	+	-	+	+
HFAO	-	-	+	-	-	-	+	-
EFAO	+	+	+	+	+	-	+	+
BFAO	-	+	-	+	+	-	-	-
AFAO	-	+	-	+	+	-	-	-

Key: MEAO= methanol extract of *A. occidentale*, HFAO= n-hexane fraction of *A. occidentale*, EFAO= ethyl acetate fraction of *A. occidentale*, BFAO= n-butanol fraction of *A. occidentale* and AFAO= aqueous fraction of *A. occidentale*. (+ present and - absent)

Antibacterial evaluation

For the preliminary antimicrobial sensitivity test, the results are shown in Table 3. At 0.2 mg/ml the methanol extract (MEAO) and all the fractions of *A. occidentale* inhibited the growth of almost all the test bacteria except for few bacteria that showed high level resistance. The inhibition zone diameter (IZD) produced by MEAO against the sensitive bacteria ranges from 06 ± 0.72 to 17 ± 0.18 mm depending on the species of test bacteria and the concentrations used whereas the *Klebsiella spp* showed resistance against the agent (IZD of MEAO = 0 mm). Other agents - n-hexane fraction of *A. occidentale* (HFAO), ethyl acetate fraction of *A. occidentale* (EFAO), n-butanol fraction of *A. occidentale* (BFAO) and aqueous fraction of *A. occidentale* (AFAO) – also showed concentration dependent activity against the test bacteria as shown in Table 3.

Table 3: Inhibition zone diameter (mm) of extract and fraction of *A. occidentale* against test organisms

Samples	C.(mg/ml)	<i>S.a.</i>	<i>E.f</i>	<i>Ps.a</i>	<i>P. m</i>	<i>S. sp</i>	<i>K. spp</i>	<i>E. c</i>	<i>S. t</i>	<i>B. s</i>
MEAO	0.200	14± 0.76	17± 0.18	14± 0.20	13± 0.06	16± 0.91	00	10±0.29	15±.69	15± .44
	0.100	13± 0.06	10± 0.04	11± 0.60	12± 0.11	14± 0.18	00	08±0.22	13±.67	10±0.36
	0.050	10± 0.66	10± 0.61	09± 0.66	08± 0.44	13± 0.92	00	08± .67	09±.29	10±0.42
	0.025	09± 0.46	00	08± 0.01	06± 0.09	10± 0.22	00	06± .72	00	00±0.12
HFAO	0.200	10± 0.28	10± 0.77	00	17± 0.37	18± 0.76	00	16±0.66	00	08±0.66
	0.100	00± 0.	00	00	16± 0.35	15± 0.33	00	00	00	00
	0.050	00± 0	00	00	00± 0.00	15± 0.71	00	00	00	00
	0.025	00± 0.	00	00	00± 0.00	15± 0.91	00	00	00	00
EFAO	0.200	15± 0.82	17± 0.71	15± 0.66	18± 0.96	17± 0.66	20±66	15± 0.08	19±.16	19± 0.39
	0.100	14± 0.76	13± 0.70	13± 0.76	15± 0.62	13± 0.36	12±.0	14± 0.06	13±.60	14± 0.32
	0.050	11± 0.60	10± 0.29	12± 0.76	15± 0.06	10± 0.38	12± .0	11± 0.22	10±.33	13± 0.03
	0.025	09± 0.36	00± 0.0	07± 0.46	14± 0.77	08± 0.33	08± .1	09± 0.06	10±.39	10± 0.42
BFAO	0.200	14± 0.54	15± 0.20	13± 0.63	12± 0.72	14± 0.03	10± .0	10± 0.73	15±.60	16± 0.34
	0.100	14± 0.60	12± 0.12	12± 0.73	11± 0.63	12± 0.63	08± .2	07± 0.39	13±.54	13± 0.23
	0.050	10± 0.03	12± 0.32	07± 0.33	08± 0.00	09± 0.66	08±.0	05± 0.33	13±.35	10± 0.06
	0.025	00	10± 0.73	00	07± 0.67	09± 0.68	00	00	10±.23	10± 0.10
AFAO	0.200	14± 0.66	14± 0.36	12± 0.96	10± 0.56	14± 0.48	00	12± 0.66	09± 0.96	12± 0.36
	0.100	09± 0.63	09± 0.68	00	08± 0.46	09± 0.77	00	10		10± 0.84
	0.050	00± 0.	07± 0.33	00	00	08± 0.36	00	08	08± 0.36	12± 0.06
	0.025	00	00	00	00	00	00	00	00	07± 0.00
LV	0.050	18± 0.06	24± 0.00	22± 0.01	24± 0.00	32± 0.01	10± .0	25± 0.99	24± .06	32± 0.51

Key: C= concentration of agents (mg/ml), MEAO= methanol extract of *A. occidentale*, HFAO= n-hexane fraction of *A. occidentale*, EFAO= ethyl acetate fraction of *A. occidentale*, BFAO= n-butanol fraction of *A. occidentale* and AFAO= aqueous fraction of *A. occidentale* and LV= levofloxacin. *Sa*, *Staphylococcus aureus*, *Ef*, *Enterococcus faecalis*, *Ps. a*, *Pseudomonas aeruginosa*, *Pm* *Proteus mirabilis*. *S.spp* *Streptococcus spp*, *Ec* *Escherichia coli*, *K.spp* *Klebsiella spp*, *S.t*, *Salm typhi*, *B.s* *Bacillus subtilis*

Minimum inhibitions and minimum bactericidal concentrations

At 0.05 mg/ml, the standard drug produced inhibition zone diameter ranging from 10 ± 0.0 to 32 ± 0.51 mm. Statistically, there was no significant difference ($p < 0.05$) existing among the antibacterial activities of MEAO, EFAO, BFAO and AFAO. However, there exists a significant difference ($p < 0.05$) between a pair of the following agents: MEAO and HFAO, EFAO and HFAO, BFAO AND HFAO, and AFAO and HFAO. The minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) of the agents against the test bacteria are shown in Tables 4 and 5 respectively. The values of the MIC and MBC of the agents ranged from 0.0025 to 0.04 mg/ml and 0.005 to 0.08 mg/ml respectively.

Table 4: MIC of the extract and fractions of *A. occidentale* against test bacteria

Test sample	MIC of the test sample (mg/ml) against test bacteria									
	<i>Sa,</i>	<i>Ef,</i>	<i>Ps. a,</i>	<i>Pm.</i>	<i>S.spp</i>	<i>St</i>	<i>K.spp</i>	<i>Ec</i>	<i>S.t,</i>	<i>B.s</i>
MEAO	0.04	0.02	0.04	0.02	0.02	0.02	NA	0.040	0.02	0.02
HFAO	NA	NA	NA	0.01	0.01	NA	NA	0.04	NA	NA
EFAO	0.01	0.005	0.01	0.005	0.01	0.02	0.0025	0.01	0.0025	0.0025
BFAO	0.02	0.01	0.01	0.01	0.01	0.02	0.02	0.04	0.010	0.01
AFAO	0.02	0.01	0.01	0.04	0.04	NA	0.04	0.04	NA	0.04

Key: MEAO= methanol extract of *A. occidentale*, HFAO= n-hexane fraction of *A. occidentale*, EFAO= ethyl acetate fraction of *A. occidentale*, BFAO= n-butanol fraction of *A. occidentale* and AFAO= aqueous fraction of *A. occidentale*. *Sa*, *Staphylococcus aureus*, *Ef*, *Enterococcus faecalis*, *Ps. a*, *Pseudomonas aeruginosa*, *Pm* *Proteus mirabilis*. *S.spp* *Streptococcus spp*, *Ec* *Escherichia coli*, *K.spp* *Klebsiella spp*, *S.t*, *Salm typhi*, *B.s* *Bacillus subtilis*. NA = No Activity

Table 5: MBC of the extract and fractions of *A. occidentale* against test bacteria

MIC of the test sample (mg/ml) against test bacteria

Test sample	<i>Sa</i> ,	<i>Ef</i> ,	<i>Ps. a</i> ,	<i>Pm</i> .	<i>S.spp</i>	<i>St</i>	<i>K.spp</i>	<i>Ec</i>	<i>S.t</i> ,	<i>B.s</i>
MEAO	0.08	0.04	0.04	0.04	0.04	0.04	NA	0.040	0.04	0.04
HFAO	NA	NA	NA	0.02	0.02	NA	NA	0.04	NA	NA
EFAO	0.02	0.005	0.02	0.005	0.02	0.04	0.02	0.04	0.01	0.01
BFAO	0.04	0.02	0.02	0.01	0.01	0.02	0.02	0.08	0.02	0.02
AFAO	0.04	0.02	0.02	0.08	0.08	NA	0.08	0.08	NA	0.08

Key: MEAO= methanol extract of *A. occidentale*, HFAO= n-hexane fraction of *A. occidentale*, EFAO= ethyl acetate fraction of *A. occidentale*, BFAO= n-butanol fraction of *A. occidentale* and AFAO= aqueous fraction of *A. occidentale*. *Sa*, *Staphylococcus aureus*, *Ef*, *Enterococcus faecalis*, *Ps. a*, *Pseudomonas aeruginosa*, *Pm* *Proteus mirabilis*. *S.spp* *Streptococcus spp*, *Ec* *Escherichia coli*, *K.spp* *Klebsiella spp*, *S.t*, *Salm typhi*, *B.s* *Bacillus subtilis*. NA = No Activity

Discussion

The methanol solvent used produced a very good yield of extract, suggesting a better solvent for the extraction and butanol had the best yield among the fractions indicating that most of the phytoconstituents are polar in nature. Findings from this study, indicated that the methanol extract and different fractions of root bark of *Anacardium occidentale* possesses promising antibacterial properties and this could be attributed to the presence of high concentration of bioactive metabolites such as terpenes, tannins, saponins, alkaloids, fat and oil, flavonoids and glycosides. Previous researchers have analyzed chemical constituents of medicinal plants and identified tannins, flavonoids, saponins, alkaloids and terpenoids which demonstrated reasonable antibacterial action [20-23]. These classes of compounds have been frequently found in plants of Anacardiaceae family [24]. Therefore, they might be responsible for the observed antibacterial properties [25]. This was corroborated by low MIC values obtained for some of the agents. This reveals that the root possess bioactive metabolite of therapeutic potentials just like the leaves and stem bark in agreement with the results of previous research work [26, 27]. This is also in agreement with previous work that reported antimicrobial activity of leaves and stem bark of *A. occidentale* [28]. Similarly, our ethylacetate fraction showed high antibacterial potency against all the bacteria tested, which is also in agreement with the results of the work previously reported [29]. Conversely, our test methanol extract showed promising activity against all the test bacteria except *Klebsiella spp*, which is not in agreement with the results of the investigations by other researchers [8], where *A. occidentale* bark methanol extract (60%)

exhibited antimicrobial activity against 13 out of 15 bacterial isolates, obtaining the activity against *Shigella dysenteriae* and *Klebsiella pneumoniae*. Secondly, Our n-hexane fraction exhibited good activity against *Proteus mirabilis* and *Streptococcus spp* but did not show good activity against *S. aureus*, *E. faecalis*, *P. aeruginosa*, *Escherichia coli*, *Salm typhi*, and *Bacillus subtilis*. This is not in line with the results of other works where the n-hexane extracts from *A. occidentale* aerial parts showed inhibitory effects against bacteria (*S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *Mycobacterium smegmatis*) with MICs ranging from 62.5 to 250 µg/mL [30]. The MIC and MBC of the test extract and fractions against the test bacteria - *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Streptococcus spp*, *Escherichia coli*, *Klebsiella spp*, *Salm typhi*, *Bacillus subtilis*, *Salmonella. spp* and *Proteus. mirabilis* -ranged from 0.0025-0.040 mg/mL and 0.020 – 0.080 mg/mL respectively. There exists a significant difference ($p < 0.05$) between the MIC and MBC values for most of the test bacteria. This suggests that the agents are both bacteriostatic and bacteriocidal in nature, but the latter effect manifests better at a concentration greater than those which shows inhibition. It is important to note that some authors consider MIC values of 0.250 mg/mL as strong antibacterial activity [31], whereas others use a stricter endpoint criteria [32], in which crude extracts with MIC values less than 0.1 mg/mL can be considered as active and are worthy for further studies. Taking into account the different criteria, extract and fractions with a MIC value ≤ 0.125 mg/mL were considered to be active in this study. As stated in Table 4, more than 80% of the extract and fractions were active against one or more bacteria. This has revealed a sparkle of hope as the test plant is endowed with many different classes of bioactive compounds that potentially inhibit the growth of bacterial pathogen, there by cause damage to the bacterial membrane, suppression of enzymes and toxins, and inhibition of bacterial biofilm formation [33]. The results obtained with Gram-negative species can be partially explained by the morphological differences observed in bacteria cell wall. In fact, Gram-negative bacteria have an extra outer membrane, highly hydrophobic, that acts as a permeability barrier to a large number of compounds, mainly of hydrophilic nature [34]. To the best of our knowledge, this is the first report on determination of MIC and MBC values for extract and fraction of this plant in the study area. Therefore, the results of this study support the use of root of the plant in traditional medicine, for the treatment of infectious diseases and further gives clue to the possible phytochemical constituents responsible for the significant antibacterial effects.

Conclusion

The extract and different fractions from the root of *Anarcadium Occidentale* plant contains phyto-constituents that may be responsible for the observed antibacterial activity against the test bacteria. The study corroborated the folkloric claims that the plant is used in treating infectious diseases. As a result, further scientific research is required to investigate and substantiate the bioactive metabolites embedded in the active fraction plant with the ultimate goal of isolating the compound(s) responsible for its antibacterial activities.

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