Profiling Phytochemical Constituents and Antibacterial Efficacy of Ethanol Extract *of Anacardium occidentale* Linn (Cashew) Slender Branches

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Abstract: Antibiotic resistance in most bacterial infections remains a threat to humanity. This necessitates the search for natural sources of remedy from *Anacardium occidentale* Linn (cashew) slender branches. The plant slender branches were air dried for four weeks at 25^{o}C and process for extraction. Cold maceration method was used to obtain the extract using ethanol as menstruum. Antibacterial susceptibility test (AST) of the extract was carried out against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa* obtained from the department of Biological Science Laboratory Ciprofloxacin and dimethyl sulfoxide (DMSO) were used as positive and negative controls respectively. Ethanol extract at 200 mg/ml, shows 17.5±0.5 mm zone of inhibition against *P. aeruginosa*, 16±1.0 mm against *E. coli*, 14±0.0 mm against *P. mirabilis* and 11±1.0 mm against *S. aureus*. *Pseudomonas aeruginosa* was the most susceptible, while *S. aureus*, the most resistant to the extract. The findings of MIC and MBC revealed that the extract was bactericidal at 250 mg/ml. Profiling the phytochemical constituents revealed the presence of alkaloids, flavonoids, quinone, phenols, saponins, and carotenoids in the extracts. High Performance Liquid Chromatography shows the presence of quercetin a flavonoid, chlorogenic acids a phenol and testosterone which could be responsible for its antibacterial activity. This study reveals that *A. occidentale* Linn slender branch has antibacterial activity and could be use as precursor for drugs development.

Key word: Bacteria, extract, resistant, phytochemical

INTRODUCTION

The *Anacardium occidentale* Linn. (cashew), belong to the family anacardiaceae (Kannan *et al.*, 2009). It is a tree that grows up to 1.5 m in height with thick tortuous trunk and woody branches native to northeast Brazil with great economic and medicinal value (Rajesh *et al*., 2009), but is now cultivated extensively in all tropical areas, notably in India and East Africa. The main producing countries of cashews are India, Cote d'Ivoire and Vietnam (Salas-Salvadó and Pascual-Compte, 2023). *Anacardium occidentale* is commonly called Cashew in English, "Yazawa" in Hausa, "Okpokpo" in Igbo and Kaju in Yoruba (Arekemase *et al*., 2011). *Anacardium occidentale* (L) leaves is consumed fresh in some regions of Asian, American and African countries. The slender branches are used in some regions of Nigeria as tooth brush (Oviasogie *et al.*, 2016). It has been used in folk medicine to treat gastrointestinal disorders (acute gastritis, diarrhoea), mouth ulcers as well as throat problems according to Kudi *et al*. (1999). Sadiq *et al*. (2009) reported that *Anacardium occidentale* leaves, stems and bark extracts are used extensively for the treatment of diarrhoea, dysentery and colonic pain. Natural antimicrobials can be found in the ethanolic extracts of cashew leaf such as flavonoids, tannins, saponins, anthocyanins, and alkaloids. In *in vitro* experiments, tannins in cashew leaf has been reported to have antimicrobial and fungicidal substances (Arekemase *et al.*, 2011; Anand *et al*., 2015). Compounds such as flavonoids and quercetin in cashew leaves are also known as natural antimicrobials that can protect the body from pathogen attack (Ajileye *et al*., 2015). Hassan *et al*. (2019) concluded in their study that leaf extracts of *Anacardium occidentale* dissolved in distilled water and

ethanol had good potential for the development of antibacterial drugs for urinary tract pathogens like *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* after an *in vitro* cup-plate method of agar diffusion technique experiment.

Infectious diseases are responsible for 45% of deaths in low- income countries and 50% of premature death worldwide (Gangoue, 2007). In addition, among the death caused by microorganisms, bacterial infections account for 70% of cases (Antimicrobial Resistant Collaborators, 2022). To control these pathogens, antibiotics are frequently used but, unfortunately, the emergence of antibiotic-resistant bacteria has put an end to this wave of optimism (Adejuwon *et al*., 2011). About 64% of people infected with multidrug resistant *Staphylococcus aureus* (MRSA) infections are more likely to die than people infected with drug-sensitive species (WHO, 2022). On the contrary, mortality because of drug-resistant strains of *Pseudomonas aeruginosa* infections is ever-increasing, accounting for about 11% of hospital-acquired bacterial infections (Nwobodo *et al*., 2020). According to the WHO facts sheet on antimicrobial resistance, resistance to ciprofloxacin, an antibiotic often used to treat urinary tract infections, ranged from 8.4% to 92.9% for *Escherichia coli* (Yenehun *et al*., 2021). To face this increasing inefficiency observed with available antibacterials, it is essential to seek a novel broad spectrum action antibacterial substance with more effective action. Hence, this study seeks to investigate the phytochemical profile and antibacterial efficacy of *A. occidentale* Linn slender branches extract against *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa***.**

MATERIALS AND METHODS

Study area: This study was carried out in the Department of Biological Science Laboratory, Taraba State University, Jalingo, Taraba State, Nigeria. Jalingo is located on latitude 8°54' to 9°01'N and longitude 11°22' to 11°30'E (Garba *et al*., 2018).

Collection of plant materials: The plant materials were collected from Taraba State University farm, identified and authenticated by a botanist in the Department of Biological Sciences, Taraba State University, Taraba State Nigeria.

Preparation of plant extract: The authenticated slender branches of *A. occidentale* were air-dried for four weeks at 25ºC. The samples were ground using mortar and pestle into fine powder. Cold maceration extraction of the plant was made as described by Thomas *et al*. (2012) using ethanol as menstruum in a standard volumetric flask. Hundred grams of the plant sample was measured into a beaker containing ethanol. The set-up was left for 48-72 hours with frequent agitation. It was then filtered with a muslin cloth. The filtrate was evaporated to dryness in a rotary evaporator at 45ºC. The percentage yield of the extract (Y%) was calculated using the formula reported by Abbas *et al.* (2021):

$$Yield\ (\%) = \frac{Weight\ of\ solvent\ free\ extract}{Dried\ extract\ weight} \times 100$$

Sterility test of the dried extract: The slender branch extract was tested for the growth of contaminants. The extract was inoculated on nutrient ager and checked for sterility. The plate was incubated at 37ºC for 18-24 hours. The plates were observed for any sign of visible growth. No growth on plates indicates a sterile extract and successful extraction process.

Medium and test organisms: Pure isolates of *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*, were obtained from the Biological Science Laboratory's culture bank and authenticated using standard microbiological techniques according to the method of Efuntoye *et al*. (2010).

Antibacterial susceptibility testing: The antibacterial activity of the extract on the confirmed pure cultures of *Staphylococcus aureus, Proteus mirabilis, Escherichia coli,* and *Pseudomonas aeruginosa* was determined by the standard agar well

diffusion technique. The crude extract reconstituted using 20% DMSO to give the following concentration 200 mg/ml, 150 mg/ml, 100 mg/ml, and 50 mg/ml (Jesse *et al.,* 2021). Agar well diffusion method was carried out using nutrient agar and cork borer (8mm) to bore hole through the agar and appropriately label. The inoculum was manually adjusted to equal a 0.5 McFaland standard that was freshly prepared using barium sulphate and sulphuric acid. Incubation for 18-24 hours, the antibacterial efficiency of the slender branch-extract was determined by measuring the zone of inhibition formed around the well with a transparent metre rule (Cheesebrough, 2000; Caleb *et al.,* 2022).

Minimum inhibitory concentration and Minimum bactericidal concentration of extracts: The MIC of the extract was determined according to the micro broth dilution technique. Standardized suspensions of the test organism were inoculated into a series of sterile tubes of nutrient broth containing two-fold dilutions of leave extract and incubated at 37^{o}C for 24 hours. Alongside broth and extract control test tube were prepared. The MIC was read as the least concentration that inhibited the visible growth of the test organisms NCCLS (2000). The MBC was determined by selecting tubes that shows no visible growth during MIC determination and sub cultured onto nutrient agar plates using the spread plate technique and incubate for 18-24 hours at 37^{o}C. The least concentration, at which no growth was observed, was observed and recorded as the MBC (Ibekwe *et al*., 2001; Jesse *et al.,* 2021).

Phytochemical screening of A. occidentale slender branch extracts: The preliminary qualitative phytochemical screening was carried out using the method previously described by Trease and Evans, (1989) and Abalaka *et al*. (2010) with slight modification.

Detection of Alkaloids: Wagner's test: Wagner's reagent was added to the extraction if a brown-reddish formation is observed, and it indicates the presence of alkaloids (Kaur *et al.,* 2016).

Detection of Phenols: Add few drops of ferric chloride to 10mL of extract. A bluish-black colour indicates the presence of phenol (Shah *et al.,* 2015).

Detection of Flavonoids: Add few drops of sulphuric acid to the extracts, and the formation of orange colour indicates the presence of flavonoids (Roghini & Vijayalakshmi 2018).

Detection of Saponins: A 0.5 mg of the extract was mixed vigorously with 5 mL of distilled water. The formation of frothing indicates the presence of saponins (Mir *et al*., 2015)

Detection of Quinone: Take 1 gram of the extract and dissolve it in 5 ml of distilled water. Transfer 1 ml of this solution to a 5-milliliter test tube. Add 1 ml of concentrated sulfuric acid to the test tube. The formation of a red color indicates the presence of Quinone (Mir *et al*., 2015).

Detection of Carotenoid: Place 1 gram of the extract into a test tube. Add 1 ml of chloroform to the test tube and shake vigorously. Filter the mixture using Whatman filter paper. Add 85% sulfuric acid to the filtrate. The presence of a blue-colored precipitate at the interface indicates the presence of carotenoids (Roghini & Vijayalakshmi 2018).

Statistical analysis of data obtained: Data generated were subjected to statistical analysis. Results are expressed as mean values ± standard error (S.E.) of duplicate determinations. Data were analyzed using one-way Analysis of Variance (ANOVA) and Duncan's New Multiplier Range Test for mean separation at a 5% level of significance with Statistical Package for Social Sciences (SPSS) software version 21. Differences were considered significant at $p < 0.05$ (Jesse *et al.,* 2021).

RESULTS

Yield of extract *A. occidentale*

The percentage yield of ethanol extract obtained from *A. occidentale* Linn. slender branches extract to be 8.38%.

Antibacterial susceptibility of the isolates

The findings of the Antibacterial Susceptibility Test (AST) of *A. occidentale* Linn. slender branch ethanol extracts against *E. coli, S. aureus, Proteus mirabilis* and *Pseudomonas aeruginosa.* The concentrations of the extract used were 50, 100, 150 and 200 mg/ml. *Escherichia coli* had the following zone of inhibition 12.5±0.5, 14.0±0.5, 14.5±0.5 and 16.0±1.0 mm. *Staphylococcus aureus* was only susceptible at a concentration 200 mg/ml with 11.0±1.0 mm zone of inhibition. Zone of inhibition of 11.5±1.5, 12.5±0.5 and 14.0±0.0 mm was reported for *P. mirabilis*, while 13.0±0.0, 14.5±0.0, 15.0±0.0 and 17.5±0.5 mm zones of inhibition on *P. aeruginosa.* Ciprofloxacin exhibited 19.5±05, 20.0±0.0, 19.5±0.5 and 27.5±0.5 mm zones of inhibition against *E. coli, S. aureus, P. mirabilis* and *P. aeruginosa* respectively. No inhibition was observed for DMSO.

Minimum inhibitory concentration (MIC) and Minimum bacteriacidal concentration (MBC) of *A. occidentale* Linn. slender branches extract

The minimum inhibitory concentration on *E. coli, P. mirabilis* and *P. aeruginosa* was 125 mg/ml while for *S. aureus* it was 250 mg/ml. The four isolates had MBC at 250 mg/ml as revealed in Table 2.

Phytochemical compositions of the plant extracts Table 3, showed the results of the qualitative phytochemical screening of the plant extracts. It was observed that alkaloid, flavonoid, phenol, quinone, and saponins were the present while carotenoid and quinone were absent.

High performance liquid chromatography (HPLC) of *A. occidentale* Linn. slender branches ethanol extract

Figure 1 reveals the chromatogram of *A. occidentale* Linn. slender branches ethanol extract, quercetin, testosterone, and chlorogenic acids were confirmed present by HPLC analysis. For a gram of the slender branch extract, 6.0% of quercetin, 2.6% of testosterone, and 91.3% of chlorogenic acids were observed to be present.

Table 1: Antimicrobial susceptibility test (AST) of ethanolic extract of *A. occidentale* slender branch against some bacteria

Conc./Control (mg/ml)	Bacteria zones of inhibition (mm)			
	E. coli	*S. aureus*	*P. mirabilis*	*P. aeruginosa*
50	12.5±0.5	-	-	13.0±0.0
100	14.0±0.5	-	11.5±1.5	14.5±0.0
150	14.5±0.5	-	12.5±0.5	15.0±0.0
200	16.0±1.0	11.0±1.0	14.0±0.0	17.5±0.5
Cipro	19.5±0.5	20.0±0.0	19.5±0.5	27.5±0.5
DMSO	0±0.0	0±0.0	0±0.0	0±0.0

Key: - = No zone of inhibition, DMSO; Dimethyl sulfoxide (negative control), CIPRO; Ciprofloxacin (positive control), *zone of inhibition size mean ± standard error of mean of duplicate determination

Table 2. MIC and MBC of *Anacardium occidentale* slender branches extract

Bacteria	MIC (mg/ml)	MBC (mg/ml)
E. coli	125	250
S. aureus	250	250
P. mirabilis	125	250
P. aeruginosa	125	250

Key: MIC; minimum inhibitory concentration, MBC; minimum bactericidal concentration.

Table 3. Phytochemical profile of *Anacardium occidentale* slender branch crude extract

Phytochemical components	Availability (Present/Absent)
Alkaloid	++
Carotenoid	-
Flavonoid	+
Phenol	++
Quinone	-
Saponin	+

Key: + = Present, - = Absent

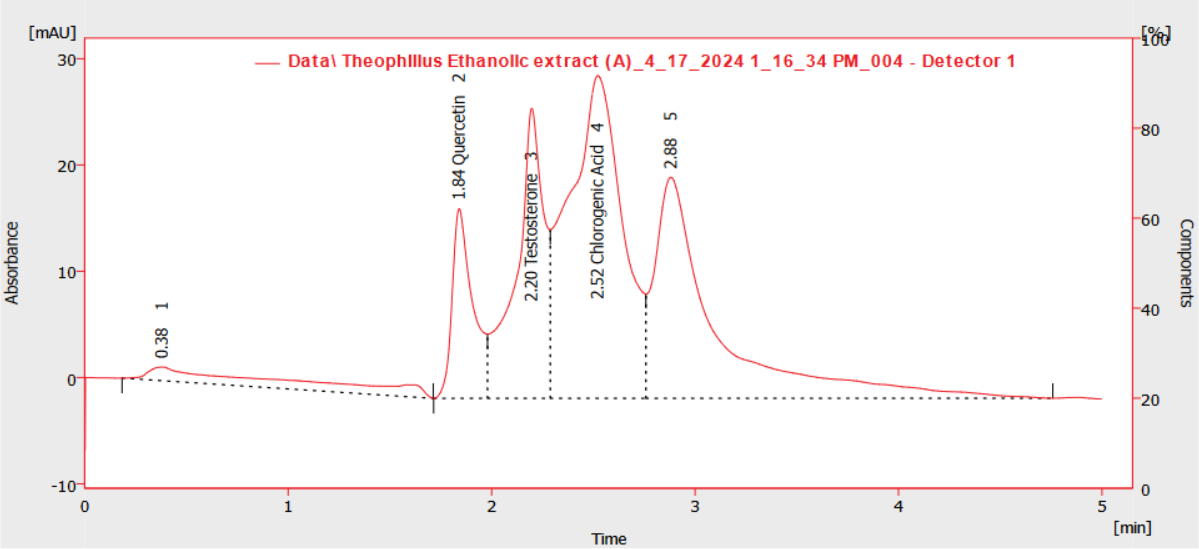

Figure 1: Chromatogram of *A. occidentale* Linn. slender branches ethanol extract

DISCUSSION

In this study, *A. occidentale* L. slender branches ethanol extract had 8.38% yield. The low percentage yield of the ethanol extract could be attributed to the high amount of husks in the plant slender branches. A contrasting findings was reported by Jesse *et al.* (2021) who observed 33.08% for petroleum ether and 30.31% yield for n-hexane extract of *A. occidentale* L. shell oil. The difference could be attributed to the type of menstruum used, plant part and method of extractions.

The findings of the antibacterial susceptibility test of the ethanol extract shows that *P. aeruginosa* (17.5±0.5 mm) was the most susceptible follow by *E. coli* (16±1.0 mm), *P. mirabilis* (14±0.0 mm) and then *S. aureus* (11±1.0 mm). This reveals that the extract possesses antibacterial substances, which agrees with the work of Sadiq *et al.* (2009) on the same plant. The extracts activity against *E. coli* justifies the traditional use of *A. occidentale* in the treatment of diarrhea. This is similar to the study of Ayepola and Ishola (2009), where the aqueous extract of the same plant have activity on the same test bacteria. Similarly, Chabi *et al.* (2014) and Anand *et al.* (2015) independently reported the ethanol leaf extract of *A. occidentale* to have antibacterial activity against the following bacteria pathogens *Enterococcus faecalis, S. aureus, Streptococcus mutans,* and *E. coli*. This could be due to certain compounds that could be responsible for their activity. Furthermore, the extract activity aligns with the work of Abalaka *et al.* (2009) and Oloninefa *et al.* (2018) on plants extracts.

Ciproflaxacin was use as positive control which give the greatest zone of inhibition as compare to the crude extract, this is no surprise because of the level of its purity. The crude extract might perform better than positive control if the extract is further refined and devoid of impurities (Parasa *et al.*, 2011). In the case of the negative control, DMSO gave the expected result because is know not to have any antimicrobial activity (Oloninefa *et al.*, 2016).

The MIC and MBC of the extract reveals that at certain concentration it is bacteriostatic and at higher concentration is bactericidal and this finding is at par with the studies of Akash *et al.* (2009). This shows that the extract can completely kill the test bacteria and effectively treat the infection cause by these pathogens (Ochei and Kolhatkat, 2010).

The phytochemical profile of the slender branches extract reveals the presence of

alkaloids, flavonoids, phenols and saponins. This was equally reported Goncalves *et al.* (2005). The presence of tannins, alkaloids, saponins, terpenes and flavonoids in *A. occidentale* leaves and the slight difference in phytoconstituents could be due to the season in which the plants materials were harvested, method of extraction and the phytochemical analysis that were carried out. Quercetin, testosterone and chlorogenic acids were confirmed in the extract by HPLC analysis. These bioactive compounds are known to have antimicrobial activities, alkaloids with anti-diarrhoeal effect, flavonoids inhibits *Vibrio cholerae*, *Streptococcus mutans*, S*higella* species, and viruses, and other phenolic compounds that are antibacterial and antifungal (Oloninafa *et al.,* 2018). Ajileye *et al*. (2015) reported that compounds such as quercetin and chlorogenic acids members of flavonoids and phenols family respectively in cashew leaves are also known as natural antimicrobials that can protect the body from pathogen attack. Thus, the antibacterial activity of the extracts on the test organisms may be due to the presence of these phytochemical components.

CONCLUSION

Anacardium occidentale Linn. slender branches extract has high (++) phytoconstituents profile. The High-Performance Liquid Chromatography (HPLC) shows that quercetin a flavonoid and chlorogenic acids such as phenol are present in large amount (++), hence its antibacterial activity. Further study needs to be done to purify and isolate the actual compound(s) that could be responsible for its activity.

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