Antibacterial Activity of *Anacardium occidentale* (Cashew) Stem Bark against Bacterial Isolates

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Abstract: The aim of this study was to investigate the relative antibacterial activity and phytochemical properties of ethanolic and water extracts of *Anacardium occidentale* (cashew) stem bark. The phytochemicals were screened using qualitative methods. Qualitatively analyzed phytochemical constituents in the stem bark extracts includes carbohydrates, alkaloids, flavonoids, saponins, tannins, sterols, anthraquinone, terpens and phenol. The microorganisms assayed for the antibacterial activities using the agar well diffusion were *Escherichia coli, Salmonella Typhi, Bacillus subtilis* and *Staphylococcus aureus*, studies on the susceptibility pattern and the zones of inhibition exhibited by the extracts shows a certain degree of inhibitory effects against the test organisms. Ethanolic extract of *A. occidentale* stem bark was effective against *B.subtilis* at concentration of 100mg/ml and 50 mg/ml only, *S. aureus* and *S. Typhi* at concentration of 100 mg/ml only, and *E. coli* at concentration of 100 mg/ml only while in aqueous extract of *A. occidentale* stem bark, there was effect against *S. aureus* at concentration of 100 mg/ml and 50 mg/ml, followed by *B. subtilis* at concentration of 100 mg/ml and 50 mg/ml, *S. Typhi* and lastly *E.coli* both at the concentration of 100 mg/ml. Considering the diameter of the zone of inhibition, it was noticed that there was little or no difference between the diameters of both extracts. Although, these results suggest an important ethno-pharmaceutical potential of *A. occidentale*as a source of compounds with broad-spectrum antimicrobial activity that can be used in the pharmaceutical industry its low activity may be due to low concentration of the extracts.

Key word: Antibacterial activity, *Anacardium occidentale*, bacteria

INTRODUCTION

The basis for using plants as medications is their chemical constituents' capacity to induce biochemical and physiological effects in living systems, these molecules, also known as phytochemicals or secondary metabolites, have become more popular in the domains of biochemistry, pharmacology, medical sciences, and microbiology (Ujowundu *et al*., 2010). Many plant species and their parts, like the fruits, leaves, roots, seeds, barks, and flowers, contain a variety of bioactive compounds that may have a range of medicinal benefits. Natural products play a significant role in the development of medications in the pharmaceutical sector and make up more than 50% of all modern clinical pharmaceuticals (Sushmita *et al*., 2012). Traditional medicinal plants are capable of producing a broad variety of chemical compounds that are required for the administration of therapeutic treatments in basic healthcare. They also provide less expensive alternatives to costly, produced Western medications that could have negative side effects (Dhankhar *et al*., 2011). Extracts from various plant components, particularly the stems, roots, fruits, and leaves, have all been utilized to treat inflammatory conditions, oxidative stress-related illnesses, and infectious diseases (Olajide *et al*., 2019). Synthetic antibiotics cause microbial resistance over time, which renders bacteria resistant to medication actions. As a result, using plant-based compounds may be a viable option (Manay and Shadaksharaswamy, 2017). In the 16th century, cashews were first planted in Agege, Lagos State, by Portuguese merchants who brought them to Nigeria. By human-transferred nuts, it spread to a few other

areas of the nation. Before their introduction, cashew trees were mostly used for apples, with the nuts having little value (Aliyu, 2012). Brazil, Benin Republic, Ghana, Cote d'Ivoire, Guinea Bissau, India, Mozambique, Philippines, Nigeria, Sri Lanka, Vietnam, and Tanzania are among the third-world nations that depend on the cashew tree as a vital crop (Adeigbe *et al*., 2015). Due to their economic value, evergreen cashew trees are also farmed for their wood, apples, and nuts. The bottom of the conical cashew apple has a c-shaped nut dangling from it. The kernel, a component that may be swallowed, is often eaten as a snack, nut for the dessert, or as an ingredient in bakeries and confectioneries (Ogunsina and Bangboye, 2014; Ogunwolu *et al*., 2015).Cashews are a concentrated food item that is rich in nutrients and full of energy. You may consume the cashew nut kernel raw, salted, or sugar-sweetened, and it has a great taste (Manay and Shadaksharaswamy, 2017). Since it is widely used in many various applications, it also provides a large source of diet-related invisible fat. Several temperate nations are seeing a surge in cashew demand as a result of this increased acceptability. Particularly in India and East Africa, the tree blossomed and naturally spread. The cashew industry presently relies mostly on the descendants of these wild cashews for their raw materials. The plant is now farmed for its shell oil and kernel, despite not originally being designed to do so.In Brazil, psoriasis, eczema, genital issues, scrofula, dyspepsia, and venereal illnesses are all treated with cashew leaves and stem bark, along with skin conditions associated to leishmaniasis, bronchitis, cough, intestinal coli, and syphilis (Godfrey *et al*., 2017). In the past, it has been used in Nigeria to treat cardiovascular issues. Many biological features, including those with antibacterial, antioxidant, anti-ulcerogenic, and anti-inflammatory actions, have been documented (Bahare *et al*., 2020).

MATERIALS AND METHODS

***Gathering and identifying plant sample*:** *Anarcadium occidentale* (Cashew) shrub bark was obtained and collected at Suleja, Niger state. The plant was recognized in the National Institute for Pharmaceutical Research and Development Abuja's Herbarium, which houses the department responsible for researching medicinal plants and traditional medicine. The voucher specimen was given a herbarium number when it was deposited. The plant part was thoroughly cleaned under running water before being let to air dry for two weeks in a well-ventilated area. The ground-up plant sample was held until required in a secure container.

***Collection of test organisms*:** *Escherichia coli, Salmonella Typhi, Bacillus subtilis,* and *Staphylococcus aureus* were included in the clinical sample given by the Department of Microbiology and Biotechnology at the National Institute for Pharmaceutical Research and Development in Abuja.

***Preparation of ethanol extracts*:** The ethanol-aqueous extract was made using ethanol that was 70% ethanol. A 500 g dry plant sample and 2500 ml of ethanol were mixed and left at room temperature for 48 hours. The extract was dried by evaporation after filtering. The plant extracts were stored for future use in an airtight container.

***Preparation of water extracts*:** With 800 ml of distilled water, 500 g of crushed plant material was steeped and heated for 20 minutes. After cooling to room temperature, the extract's supernatant was decanted and centrifuged for ten minutes. Using glass microfibre filter paper, the supernatant was filtered before being dried at 45 °C for a certain period of time until all the water had evaporated. A bottle that was airtight was used to prepare the plant extract for use.

***Screening of extracts for phytochemicals*:** Nine parameters were examined during phytochemical screening, including carbohydrates, alkaloids, steroids, phenols, flavonoids, terpenoids, anthraquinones, tannin, and saponin.

***Determination of the carbohydrates*:** Three grams of each plant extract were heated for 3 minutes in a water bath with 50ml of distilled water added. After filtering the mixtures while they were still hot, the cool filtrates were collected, and these were used in the subsequent experiments. Using 2 ml of the aforementioned plant samples and 3–4 drops of Molisch's reagent, the Molisch's test was conducted. After concentrated sulfuric acid was added in little amounts, a lower layer formed. A purple color ring in the liquid's interphase indicates the presence of carbohydrates. After shaking, the mixtures were allowed two minutes to stand. It was thinned down with 5 mls of water. When a purple precipitate appears, carbohydrates are present.

***Determination of alkaloids*:** Three grams of the powdered ingredients and 50 ml of methanol were combined, macerated, and evaporated to dryness. The leftovers and 10 ml of 1% aqueous hydrochloric acid were mixed in a water bath. A 1 ml portion of each from the mixture was treated with Mayer's reagent and Dragendorff's reagent. By checking for turbidity or precipitation, the reagents were employed to evaluate if an extract contained alkaloids.

***Determination of anthraquinone derivatives*:** Borntrager's Test: About 0.5 g of the powdered plant samples were placed in a test tube along with 10 ml of chloroform, and the mixture was violently agitated for 5 minutes. The extracts were filtered, and the filtrate was agitated before being mixed with an equivalent amount of ammonia solution. It was believed that the bright pink colour in the top aqueous layer was a marker of free anthraquinones.

***Determination of sterols and terpens*:** About 5 g of the plant samples were dissolved in 10 ml of anhydrous chloroform, filtered, and the filtrates were divided into two parts for further analysis.

Lieberman-Burchard Test: The two plant samples' initial portions of the chloroform solutions were mixed with 1 ml of acetic anhydride after 1 ml of concentrated sulphuric acid was added down the test tube walls to generate a lower layer. Steroids were detected by the formation of a reddish-violet tint at the liquid interface and a green color in the chloroform layer.

Salkowski's Test: Carefully mixing 2 ml of concentrated sulphuric acid into the second part of the solutions of the two plant sample samples caused the acid to create a lower layer. A sign that terpenoids were present was the development of a reddish-brown color during the interphase.

***Determination of the saponins*:** Foam test: A 20 ml solution of the extracts diluted to a 1 ml concentration in distilled water was agitated in a cylinder for 15 minutes. Saponins are present when stable foam develops.

***Determination of tannins*:** A beaker containing 3 g of each plant's sample was combined with 50 ml of distilled water and heated for 3 minutes. Filtration was performed on the hot mixtures while they were still hot, and the test was conducted using the cooled filtrates.

Test for ferric chloride: The cooled filtrates were mixed with a few drops of 10 % iron III chloride ($FeCl_3$). Blue black or blue green colour was thought to be a sign of tannin content.

***Determination of flavonoids*:** Five grams of the materials were fully detanned in acetone. Warm water was used to wash away the leftovers after the acetone evaporated on a water bath. The mixtures were filtered, and the filtrates were then applied to the tests that came next.

Test with lead acetate: To a 10 % lead acetate solution, 5 ml of detanned water extract was added. The presence of flavonoids is indicated by reddish brown bulky precipitate.

***Determination of phenol*:** The materials were dissolved in a solution of water, ethanol, and a few drops of neutral ferric chloride solution—a solution made by mixing de-ionized water with ferric chloride. After a stable brown precipitate had developed, sodium hydroxide was added to the mixture. There is phenol present when red or blue coloration appears.

***Microbiological media used for the test*:** Mueller Hinton Agar and Mueller Hinton Broth were utilized as the study's medium. The manufacturer's instructions were followed in the preparation of all the media.

***Preparation of inocula*:** On nutrient agar slopes, stock cultures were maintained. The active cultures for the research were created by transferring a loopful of cells from the stock cultures into test tubes filled with Mueller-Hinton broth (MHB) and allowing them to grow for a whole night at 37°C. When cultures were diluted with new MHB and the findings were compared to 0.5 McFarland standards, values corresponding to 1.5 x 10^8 colony forming units of bacteria were discovered.

***Preparation of stock solution and serial dilution of the extract*:** About 0.4 g of the extracts and 4 ml of Mueller-Hinton broth were weighed into sterile vials using sterile Pasteur pipettes to produce a concentration of 100 mg/ml. A vortex shaker was used to blend the liquid, and it was given time to thoroughly dissolve. Two millilitres of the original stock solution was serially diluted into four bottles containing 2 ml of Mueller hinton broth in order to reach concentrations of 50 mg/ml, 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml.

***In-vitro antimicrobial susceptibility assay of the extract*:** Mueller-Hinton agar (MHA) was used for the test of antibacterial activity. Standardized cultures of each microbe, equivalent to 0.5 McFarland standards, were dispensed into 20 ml of sterilized MHA kept at 45 ° C, poured into Petri dishes, and gently swirled to ensure a uniform dispersion of the organisms under aseptic conditions. The mixture was then allowed to gel for an hour. For each plate with bacterial isolates, an 8mm-diameter well was made with the requisite labels using a sterile metallic cork borer. The bottoms of the wells were sealed with 10 µL of MHA. After that, 100 µL of varied extract concentrations were carefully pipetted into each well using a sterile micropipette. The wells were then placed in the safety hood for optimal agar diffusion before being incubated at 37°C for 24 hours.

Inhibitory zones could be observed on the plates, and their dimensions were measured in millimeters using a transparent meter ruler (mm). On duplicates, experiments were conducted. Further work included organism viability control (OVC) and medium sterility control (MSC) (OVC). Chloramphenicol 10 µg was used as the standard medication in the control setting.

RESULTS

Anacardium occidentale (cashew) stem bark ethanol and aqueous extract results of preliminary phytochemical screening are shown in Table 1. *Anacardium occidentale*'s ethanol extract was found to include a carbohydrate, anthraquinone, sterol, terpene, tannin, flavonoids, and phenol, but no alkaloids or saponin. The aqueous etract of *Anacardium occidentale*, which also included carbohydrate, alkaloids, anthraquinone, saponin, tannin, terpen, flavonoids, and phenol, however did not contain sterol.

Table 1: Phytochemical screening of ethanolic and aqueous extracts of *Anacardium occidentale* stem bark

Phytochemicals	ESBE	WSBE
Carbohydrates	+	+
Alkaloids	-	+
Anthraquinone	+	+
Sterols	+	-
Terpens	+	+
Saponin	-	+
Tanin	+	+
Flavonoids	+	+
Phenol	+	+

Key:+ =present, - =absent, ESBE=ethanol stem bark extract, WSBE=water stem bark extract

The antibacterial activity of an ethanol extract of *A. occidentale* is shown in Table 2, and all test species are inhibited by the extract at a dose of 100 mg/ml. All test organisms show resistance at concentrations of 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml, respectively, whereas only *Bacillus subtilis* was inhibited at a dose of 50 mg/ml. The zone of inhibition for the control (chloramphenicol) was the largest for all of the test organisms. The findings of the aqueous extract of *A. occidentale*'s antibacterial activity are shown in Table 3. At a dose of 100 mg/ml, the extract inhibited every test organism. It inhibits *Bacillus subtilis* and *Staphylococcus aureus* at a dosage of 50 mg/ml, but all test organisms show resistance at concentrations of 25 mg/ml, 12.5 mg/ml, and 6.25 mg/ml, respectively. For all of the test species, the control's zone of inhibition was larger (chloramphenicol).

Table 2: Antibacterial activities of ethanolic extract of *Anacardium occidentale* (cashew) stem bark

Concentrations (mg/ml)/Zone of inhibition (mm)						
Organisms	100	50	25	12.5	6.25	Control
E. coli	2	-	-	-	-	29
S. Typhi	3	-	-	-	-	31
B. subtilis	4	2	-	-	-	32
S. aureus	3	-	-	-	-	28

Key: - = no activity

Table 3: Antibacterial activities of aqueous extract of *Anacardium occidentale* (cashew) stem bark

Concentrations (mg/ml)/Zone of inhibition (mm)						
Organisms	100	50	25	12.5	6.25	Control
E. coli	2	-	-	-	-	29
S. Typhi	3	-	-	-	-	31
B. subtilis	3	2	-	-	-	32
S. aureus	4	2	-	-	-	28

Key: - = no activity

DISCUSSION

Many plant extracts have antimicrobial properties, and medicines use them as natural substitutes to treat a variety of diseases. Scientific research on plants used as medicines has shown promising phytochemicals that may be produced for the treatment of infectious and non-infectious disorders (Baba *et al*., 2018). As a consequence of the rising interest in the search for antimicrobial agents from natural sources, compounds that may serve as appropriate antimicrobial agents to replace synthetic ones have been discovered and developed (Alvarez-Martnez *et al*., 2020). These compounds are far less toxic and offer a wide range of therapeutic applications for viruses, bacteria, fungus, and other human disorders. This has led to the use of medicinal plants in drugs, dietary supplements, and nutraceuticals (Alvarez-Martnez *et al*., 2020). During the qualitative phytochemical screening of *Anacardium occidentale* stem bark in this research, anthraquinones, terpenes, tannins, flavonoids, and phenols were identified in both the ethanol and aqueous extracts. All other metabolites that were examined, with the exception of sterols were present in the aqueous extract of the *Anacardium occidentale* stem bark that was used in this study. Alkaloids and saponins, however, could not be discovered in the ethanol extract. Several studies have identified the occurrence of many metabolites, including phenols, flavonoids, glycosides, tanins,

alkaloids, and anthroquinones (Okey-Ndeche *et al*., 2020; Alvarez-Martnez, 2020). According to Da Silva *et al*. (2016) the following substances were discovered: flavonoids, tannins, organic acids, alkaloids, saponins, terpenes, and organic acids. The findings of this study's qualitative phytochemical contents are similarly compatible with those of Desai *et al*. (2017). Amira *et al.* (2020) studied on the nuts and leaves of *A. occidentale*, the nuts only have resins, flavonoids, phenols, carbohydrates, and alkaloids whereas the leaves also included tannins, resins, saponins, phlobatanins, flavonoids, sterols, and phenols.The study plant is commonly used in traditional medicine to cure a range of illnesses (Ajileye *et al*., 2015). The ethanol or aqueous extracts of *A. occidentale* had no discernible impact on the clinical bacteria under study. The antibacterial activity shown in this study may be caused by the phytochemical extract components that regulate the bioactivity of the extracts (Goncalves and Gobbo, 2012). For example, it has been shown that flavonoids may dissolve the bacterial cell wall, which affects the complete functioning of microbial cells (Catherine and Anoze, 2018). However, the ethanol extract of *A. occidentale* stem bark is only effective against *B. subtilis* at concentrations of 100 mg/ml and 50 mg/ml, *S. aureus* and *S. Typhi* at concentrations of 100 mg/ml only, and *E. coli* at concentrations of 100 mg/ml only. In contrast, the aqueous extract of *A. occidentale* stem bark was effective against *S. aureus* at concentrations of 100 mg/ml and 50mg/ml, followed by *B. subtilis* at concentration of 100 mg/ml, and 50 mg/ml, *S. Typhi* and lastly *E. coli* both at the concentration of 100 mg/ml. In a research by Chabi *et al*. (2014) it was discovered that ethanol and ethyl acetate-based extracts of *A. occidentale* leaf and bark inhibited the development of several microorganisms. Given the comparatively low activity of the extracts seen in the present study compared to that reported by Ngari *et al*. (2013), we can hypothesize that the difference may be caused by the greater concentration of extracts (200 mg/ml) used in their work. The ethanol and aqueous fruit extracts of the *A. occidentale* fruit's antimicrobial activities mean zone width of inhibition for *S. aureus* was reported by Okey-Ndeche *et al*. (2020). Between the sizes of 11mm and 28mm, *S. aureus* was found on several extracts, whereas *E. coli* ranged from 17 to 29 mm in size. In a different investigation, it was discovered that ethanol and aqueous stem-bark extracts from *A. occidentale* have antibacterial effects of on *S. aureus* and *E. coli* (Agedah *et al*., 2010). Our study demonstrates that *B. subtilis* in ethanol and aqueos extracts and *S. aureus* in aqueous extracts, at doses of 100 mg/ml, were more effective. However at 50 mg/ml, these two species showed a decrease in activity. Nevertheless, Arekemase *et al*. (2011) discovered a significant difference between the diameters of the inhibition at a dose of 200 mg/ml. While comparing the zones of diameters, it was shown that there was little to no difference between the diameters of the two extracts. A cold and hot water extract of *A. occidentale* was shown by Aderiye and David (2014) to have a substantial antibacterial effect against methicillin-resistant *S. aureus*, and *E. coli* O167:H7. Onuh *et al*. (2017) study on the ethanol extract of *A. occidentale* In terms of antibacterial activity was very effective against *E. coli, S. multans, B. cereus, S. Typhi* and also *C. albicans.* Rajash *et al*. (2015) research indicates that *A. occidentale* at a concentration of 500 mg/ml, has antibacterial effects on *S. aureus, B. cereus, E. coli, Serratia marcescens, and Zymomonas mobilis*. Moreover, a raw ethanol extract of *A. occidentale* was examined by Da Silva *et al.* (2016) against a variety of pathogenic organisms and found that all of the species, including *Helicobacter pylori* and Methicilin Resistant *Staphylococcus aureus*, are susceptible to the extract. Based on the Shobha *et al*. (2018) research, ethanol leaf extract of *A. occidentale* had activity against *S. aureus, E. fecalis, K. pneumoniae*, aqueous extract

alone shown efficacy against *C. albicans.* In the cashew stem bark ethanolic extract, *B. subtilis* also had the highest zone of inhibition of 31 mm), followed by *E. coli* and *S. Typhi* having (29 mm), and *S. aureus* (23 mm) having the least zone of inhibition. The control, chloramphenicol, had the highest zones of inhibition (32 mm) against *B. subtilis*, followed by *S. Typhi* having (31mm), *E. coli* (29 mm), and *S. aureus.*

CONCLUSION

Anacardium extracts of the stem bark of occidentale (Cashew) were highly concentrated in a number of secondary metabolites, both in ethanol and water. Anthraquinolones, carbonate, tannins, terpenes, and flavonoids were all present in both extracts. Unquestionably, this is what gives the extract of *A. occidentale* its antibacterial effects (cashew) against a wide range of pathogenic bacteria, as revealed by prior study and also by this one, but at very low activity. The ethanolic and aqueous extracts could possess antibacterial properties that are useful against pathogenic microbes. Secondary metabolites were present, which helped antibacterial properties of extracts of the stem bark of *A. occidentale*, which supports the plant's long history of usage in traditional folk medicine to treat a range of illnesses. Together, these results indicate that *A. occidentale* in the pharmaceutical industry, has great potential as a source of compounds with broad-spectrum antibacterial action.

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