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RESEARCH ARTICLE

Evaluation of antioxidant and bioactive phytochemical properties of *Achyranthes aspera* L.

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ABSTRACT

Archeranthes aspera, a member of the Amaranthaceae family, is now widely distributed. This species has become invasive in Nigeria. Despite the natural antioxidant defense and repair mechanisms present in humans and other organisms, continuous exposure to free radicals inevitably causes biological harm that cannot be entirely mitigated. In a recent study, extracts of *Achyranthes aspera* were examined for their secondary metabolites, antioxidant properties, and antibacterial effects against several strains including *Staphylococcus aureus* (NCIB 8588), *Bacillus cereus* (NCIB 6349), *Klebsiella pneumoniae* (NCIB 418), and *Pseudomonas aeruginosa* (NCIB). Phytochemical analysis of the plant leaf revealed the presence of alkaloids, tannins, general glycosides, and terpenoids. Quantitative analysis of secondary metabolites showed that the plant extract contained 7.0 ± 2.5 mg/100g alkaloids, 11.5 ± 2.0 mg/100g tannins, 15.0 ± 3.1 mg/100g general glycosides, and 14.0 ± 1.5 mg/100g terpenoids. The investigation revealed that the concentration of leaf extract enhanced DPPH scavenging activity, albeit to a lesser extent than ascorbic acid. At concentrations of 50 and 100µg/ml, ascorbic acid exhibited scavenging activities of 22.66 ± 2.34% and 40.55 ± 1.55%, respectively, whereas *Achyranthes aspera* leaf extract displayed activities of 6.33 ± 2.04% and 11.65 ± 6.18%. Furthermore, the study demonstrated that the plant extract inhibited the growth of bacteria, rendering them vulnerable. The minimum inhibitory concentration of *Achyranthes aspera* ethanol leaf extract was found to be lowest against *S. aureus* and highest against *B. cereus*. These findings suggest that the ingredients present in the plant leaf extract hold promise for potential use in chemotherapy.

Keywords: *Achyranthes aspera,* amaranthaceae, antioxidant, antibacterial, chemotherapy, DPPH activity.

INTRODUCTION

Medicinal plants are integral to Earth's biological heritage, offering properties akin to conventional drugs. Utilized in medicine since ancient times, these plants possess antimicrobial qualities that combat the proliferation of microorganisms (Crover et al., 2002; Pandian et al., 2020; Ashokkumar et al., 2021a & b; Ashokkumar et al., 2023; Banso and Ajayai 2023b). Antimicrobial agents play a critical role in treating infectious diseases by either preventing them or treating them at low concentrations without causing harm to the host. Given microbial resistance, the continuous search for novel antimicrobial compounds remains imperative.

Achyranthes aspera, commonly known as the chef flower and a member of the Amaranthaceous family, is widely distributed across tropical regions (He et al., 2017). It exists both as an introduced species and a prevalent weed in various locales, proving invasive in specific regions, including several Pacific Island environments. Various extracts of *Achyranthes aspera*, including those from seeds, ethyl acetate, stem, leaf, ethanol, and methanol, as well as aqueous flower extracts, have been shown to possess antibacterial efficacy. The plant exhibits antibacterial properties against Gram-positive bacteria commonly encountered in hospitals and is utilized as an herbal antibacterial finish for cotton fabrics in healthcare textiles (Dey, 2011).

The methanolic leaf extract of the plant has shown significant inhibitory effects against certain viruses in *in vitro* assessments (Chakraborty et al., 2002). Both human and rat sperm are susceptible to the spermicidal activity of the plant extracts. Moreover, the ethyl acetate extract of Achyranthes aspera has exhibited activity against the larvae of the cartel tick *Rhipicephalus microplus* (Acarilxodidae) and the sheep internal parasite Paramphistomum cervi (Khandagle et al., 2011; Kamalakannan et al., 2011).

Furthermore, the plant is recognized for its cancer chemopreventive and antitumor characteristics. The non-alcoholic fraction of the plant demonstrates significant antitumor properties, while methanol-extracted leaves display inhibitory activity against human pancreatic cancer cells, indicating potent anti-proliferative and anticancer effects (Subbarayan et al., 2010). Additionally, the methanol extract of the leaves exhibits antidepressant effects in mice and rats through forced swimming and tail suspension tests. This study aims to investigate the antimicrobial and antioxidant properties of *A. aspera*. It seeks to understand if plant compounds could provide safer or more effective alternatives to synthetic antimicrobials.

MATERIALS AND METHODS

Plant material collection

Fresh leaves of *A. aspera* were gathered from the botanical garden of the Federal Polytechnic in Bida, Niger State, Nigeria. The authenticity of the leaves was confirmed at the International Institute of Tropical Agriculture (IITA) in Ibadan, following the criteria set by the International Committee for Botanical Nomenclature (ICBN). The samples were carefully packaged in polythene bags, labelled accordingly, and stored for future use.

Extraction procedure

The leaves were air-dried in the shade at room temperature, following the method outlined by Banso et al. (2023) with slight modifications. Ten grams of plant powder were combined with 200 ml of methanol in a covered conical flask, sealed with aluminium foil, and subjected to rotary shaking for 24 hours at 35°C to ensure thorough homogenization. Subsequently, the extract was then filtered using Whatman No. 1 filter paper and refrigerated until use.

Qualitative phytochemical screening

Testing for chemical constituents in the leaf extract was conducted using various methods as follows,

Test for general glycosides: The one gramme of coarsely powdered leaf samples were divided into two beakers, with one receiving 5 ml of dilute sulfuric acid. After heating both beakers for 5 minutes, the contents were filtered into test tubes. The filtrate was then alkalized with 5% sodium hydroxide and heated with Fehling's solution, with the formation of a reddish-brown precipitate indicating the presence of general glycosides (Mann et al., 2008).

Test for Saponins: A 0.5-gram sample of the extract was dissolved in 10 ml of distilled water and warmed. The presence of foam indicated the presence of saponins.

Test for Alkaloids: A 15-mg sample of the leaf extract was dissolved in 6 ml of 1% HCl and stirred in a water bath. Dragendroff's, Mayer's, and Wagner's tests were performed to detect the presence of alkaloids.

Test for Steroids: The chloroform extract of the leaf sample was evaporated to dryness and subjected to

Liebermann-Buchard's reaction. The observation of blue-green hues indicated the presence of steroidal compounds (Banso et al., 2021).

Test for Terpenoids: Similar to the steroids test, the appearance of red, pink, or violet colouration indicated the presence of terpenoids.

Test for Tannins: The extract was dissolved in distilled water, and a few drops of 5% ferric chloride were added. The formation of a black or blue-green precipitate confirmed the presence of tannins.

Test for Sesquiterpenes: An aqueous extract of the leaf underwent a series of steps, and the emergence of a green-to-black colouration upon the addition of ferric chloride indicated the presence of sesquiterpenes (Banso et al., 2021). These tests provide insights into the chemical composition of the *A. aspera* leaf extract, helping to identify the presence of specific bioactive compounds such as saponins, alkaloids, steroids, terpenoids, tannins, and sesquiterpenes.

Quantitative analysis

Determination of total phenolic content

The Folin-Ciocalteu method and UV-Vis spectrophotometer measured the total phenolic content of leaf extract's. Following is the process: First, 1 ml of 1 mg/methanol leaf extract was quantified and placed in a 25-ml volumetric flask. Next, 9 ml of distilled water was added to the leaf extract flask. After adding 10 ml of 7% sodium carbonate solution to the flask, the mixture was violently mixed. The flask was incubated in the dark for a given time after adding 4 ml of distilled water to reach 25 ml. Meanwhile, gallic acid solutions (20, 40, 60, 80, and 100 µg/ml) were produced and incubated with the test sample for the specified duration. The leaf extract was replaced with one millilitre of distilled water to form a blank. A UV-Vis spectrophotometer evaluated the absorbance of the gallic acid solutions and test sample at 550 nm after incubation. The sample absorbance was compared to known gallic acid concentrations. This comparison allowed the leaf extract's phenolic content to be calculated using 550 nm absorbance values (Chakraborty et al., 2002; Geetha, 2010). This approach quantifies leaf extract phenolic components, revealing its antioxidant capability and total phenolic content.

Determination of total tannin content

The leaf extract's tannin content was measured using Folin-Ciocalteu and a UV spectrophotometer. The process is given below: One ml of 1 mg/ml ethanol leaf extract was measured and placed in a 10 ml volumetric flask. To dilute the extract, 7.5 ml distilled water was added to this flask. To achieve thorough blending, 0.5 ml of Folin-Ciocalteu reagent was added to the volumetric flask mixture and violently shaken. After 5 minutes, 1 ml of 35% sodium carbonate solution was added to the flask. Adjusting the solution volume to 10 ml. In a second flask, 1 ml of distilled water replaced the leaf extract to generate a blank specimen. We created gallic acid solutions at 20, 40, 80, and 100 µg/ml concentrations. The sample and gallic acid solutions incubated for 30 minutes. Following incubation, the absorbance of these solutions was measured at a wavelength of 725 nm using a UV-Vis spectrophotometer (Hullatti and Murthy, 2010). By comparing the absorbance readings of the sample solution with those of the gallic acid solutions of known concentrations, the tannin content in the leaf extract can be determined. This method offers valuable insights into the presence and concentration of tannins, contributing to the comprehensive analysis of the leaf extract's chemical composition.

Determination of total flavonoid content

To quantify leaf extract flavonoids, the following approach was used: 1 ml of a 1 mg/ml leaf extract solution was measured and placed in a 10 ml volumetric flask. Diluting the extract with 4 cc of distilled water in the flask. 0.3 ml of 5% sodium nitrate and 10% aluminium chloride were added to the diluted extract solution. To distribute the reagents, the flask was thoroughly stirred. Next, 2 ml of 1M sodium hydroxide was added to the mixture. The solution was well-mixed to aid the reaction. The solution was 10 ml after adding 2.4 ml of distilled water. A secondary flask was empty and filled with 1 cc of distilled water instead of extract. Standard solutions of quercetin were produced at concentrations of 20, 40, 60, 80, and 100 µl. After dissolving quercetin in methanol, standard solutions were made. The standard and sample solutions were incubated for 30 minutes to allow the reaction. After incubation, a UV-Vis spectrophotometer assessed the absorbance of the standard and test solutions at 510 nm (Akhtar and Iqbal, 1991; Malarvili and Ganathi, 2009). By comparing the absorbance readings of the sample solution with those of the standard solutions of known concentrations, the flavonoid content in the leaf extract can be determined. This method enables the quantification of flavonoids present in the extract, providing valuable information about its chemical composition and potential health benefits.

Antioxidant activity

The DPPH (2,2-diphenyl-1-1-picrylhydrazyl) radical scavenging assay was conducted as follows: Dissolve 2.4 mg of DPPH in 100 ml of methanol to create the DPPH stock solution. Prepare various concentrations (50, 100, 150, 200, and 250 µg/ml) of the plant extract in ethanol. Combine 3 ml of DPPH solution with 100 µl of each concentration of the leaf extract in a 25 ml volumetric flask. Additionally, prepare ascorbic acid solutions with the same concentrations in distilled water. Create a control sample by adding only DPPH. Use methanol as a reference solution. Incubate the solutions for 30 minutes to allow the reaction to take place. After the incubation period, measure the absorbance of both the standard (ascorbic acid) and test (leaf extract) solutions at 515nm using a UV-Vis spectrophotometer. The radical scavenging activity of the leaf extract was determined using the formula provided by Khanna et al. (1992) and Priya and Krishna (2007).

DPPH (%) = Ab(control) – Ab(sample) /Ab(control) × 100

Where Ab(control) is the absorbance of the control (DPPH only), and Ab(sample) is the absorbance of the test sample (leaf extract or ascorbic acid).

This assay assesses the ability of the plant extract to scavenge DPPH radicals, providing information about its antioxidant potential. The percentage of DPPH scavenging activity is indicative of the extract's ability to neutralize free radicals, with higher percentages suggesting stronger antioxidant properties.

Antimicrobial activity

Test organism

The study utilized specific strains of *Staphylococcus aureus* (NCIB 8588), *Bacillus cereus* (NCIB 6349), *Klebsiella pneumonia* (NCIB 418), and *Pseudomonas aeruginosa* (NCIB 950). The organisms were acquired from the National Institute of Pharmaceutical Research, Abuja.

Standardization of bacteria

McFarland standard was employed as a reference to adjust the turbidity of the bacteria suspension within the range of $1X10^6$ bacteria cell/ml (the equivalent of 0.5 McFarland standards) and was maintained throughout the study.

Antibacterial bioassay

The agar diffusion method, outlined by Banso and Banso (2023), was utilized to assess the impact of the leaf extract on the growth inhibition of *S. aureus*, *B. cereus*, *K. pneumoniae*, and *P. aeruginosa*. The procedure involved the following steps: Bacterial cells were cultured and evenly spread onto several nutrient agar plates using a sterilized glass spreader. A 50 mg/ml solution of the plant material extract, dissolved in DMSO, was prepared. Using a sterilized Pasteur pipette, 200 µl of the extract solution was introduced into wells on the agar surface of the culture plates. The plates were incubated at room temperature for one hour to facilitate the diffusion of substances before the growth of organisms. After the incubation period, the plates were transferred to an incubator set at 37°C and left to incubate for 24 hours. Zones of inhibition were quantified by measuring the diameter of the clear zones around the wells where the extract was introduced. Plates containing wells of antibiotics, such as Amoxicillin, were used as positive controls. The diameter of the inhibition zones caused by the plant extract was compared to that of the positive control to assess the antibacterial activity. This method allows for the evaluation of the antibacterial properties of the leaf extract by measuring its ability to inhibit the growth of specific bacterial strains. The size of the inhibition zones provides information about the effectiveness of the extract against the tested bacteria, aiding in the determination of its potential as an antibacterial agent.

Determination of minimum inhibitory concentration (MIC)

The MIC was determined using the broth microdilution method, following the guidelines recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Here's a breakdown of the procedure: Initially, the extract was dissolved in a solution containing 10% DMSO. Using a serial dilution method with a dilution factor of 1/2, the extract solution was diluted in a microtiter plate containing Mueller Hinton broth. Each well in the microtiter plate contained a concentration of 5x10^6 colony-forming units per millilitre (CFU/ml) for assessment. Amoxicillin, a standard medication, served as the positive control, with a final concentration of 10µg/ml in the wells. To prevent contamination, a sterile sealer was applied to the microtiter plate. The plate was then incubated at 37°C for 24 hours to facilitate bacterial growth. The MIC is defined as the lowest concentration of the extract that completely inhibits bacterial growth. A lower MIC value indicates higher activity of the extract against the tested bacteria. By observing the wells for the absence of bacterial growth after incubation, the MIC of the extract can be determined

(Islam et al., 2011). This method allows for the assessment of the extract's potency in inhibiting bacterial growth and provides valuable information for evaluating its antimicrobial efficacy.

Data analysis

Statistical analyses including analysis of variance and Tukey's multiple range tests were conducted using SPSS version 20.0 to determine significant differences between means. A p-value below 0.05 was considered statistically significant

RESULTS AND DISCUSSION

The phytochemical analysis of the plant leaf examined in this study revealed the presence of alkaloids, tannins, glycosides, and terpenoids in *A. aspera.* The plant leaf did not contain any saponins, steroids, or sesquiterpenes (Table 1).

Table 1. Phytochemical constituent of leaf extract of *Achyranthes aspera*

Active principle	*A. aspera* leaf extract
Alkaloids	+
Tannins	+
General glycoside	+
Saponins	-
Steroids	-
Terpenoids	+
Sesquiterpenes	-

(+) = Detected (+) = Not detected

Total secondary metabolite in leaf extract of A. aspera

The total alkaloid, tannins, general glycoside and terpenoids detected in the leaf extract of *Achyranthes aspera* were 7.0±2.5, 11.5±, 2.0 15.0±3.1 and 14.0±1.5 respectively (Table 2).

DPPH (2, 2-diphenyl-1-1-picrylhydraxyl) scavenging activity

The DPPH scavenging activity of ascorbic acid and the leaf extract of *A. aspera* is displayed in Table 3. The findings indicated that the DPPH scavenging activity is contingent upon the concentration. The values measured for ascorbic acid at concentrations of 50, 100, 150, 200, and 250µg/ml were 25.66±2.34, 40.55±1.55, 54.74±2.33, 84.32±1.98, and 90.41±1.33, respectively. Recorded values for Achyranthes aspera were 6.33±2.04, 11.65±6.18, 18.34±2.65, 22.50±3.22, and 33.85±1.66, respectively.

Table 2. Total secondary metabolites detected in ethanol leaf extract of *A. aspera*

Active principle	Quantity (mg/100g) ± SD
Alkaloids	7.0 ±2.5
Tannins	11.5± 2.0
General glycoside	15.0±3.1
Saponins	ND
Steroids	ND
Terpenoids	14.0±1.5
Sesquiterpenes	ND

ND = Not detected

Table 3. DPPH Scavenging activity of ascorbic acid and leaf extract of *Achyranthes aspera*

Concentration (µg/ml)	Percentage of DPPH scavenged by ascorbic acid ±SD	Percentage of DPPH scavenged by leaf extract ±SD
50	25.66±2.34	6.33±2.04
100	40.55±1.55	11.65±61.8
150	54.74±2.33	18.34±2.65
200	84.32±1.98	22.50±3.22
250	90.41±1.33	33.85±1.66

SD = Standard deviation

Antimicrobial property of leaf extract of *Achyranthes aspera*

The measured zones of inhibition against the test organisms ranged from 11.4±0.2 to 18.1±3.0 in diameter. The zones of inhibition against aqueous, methanol, and ethanol extracts were measured as 10.8±0.3, 14.5±1.2, and 12.5±0.4, respectively, when tested against S. aureus (Table 4). The values of 13.4±0.2, 18.1±3.0, 15.5±2.0, and 38.9±1.3 were measured for *K. pneumonia* when the extracts were tested against the organism. The aqueous, methanol, and ethanol extracts yielded 12.4±1.6, 16.0±2.1, and 16.5±1.0, respectively, when tested against *P. aeruginosa* (Table 4).

Table 4. Susceptibility pattern of leaf extracts of *Achyranthes aspera*

	Leaf Extract (50mg/ml)			Standard drug (10µg/ml)
	Diameter of zone of inhibition (mm) ±SD			
Bacterial strain	Aqueous Ex.	Methanol Ex.	Ethanol Ex.	Amoxillin
S. aureus (NCIB 8588)	10.8±0.3	14.5±1.2	12.5±0.4	29.6±2.2
B.cereus (NCIB 6349)	11.4±1.2	15.9±0.3	13.8±0.1	30.7±0.2
K. pneumoniae(NCIB418)	13.4±0.2	18.1±3.0	15.5±2.0	38.9±1.3
P. aeruginosa (NCIB 950)	12.4±1.6	16.0±2.1	16.5±1.0	32.5±0.3

SD = Standard deviation, Ex = extract

The result of the MIC indicates that the minimum inhibitory concentration of *Achyranthes aspera* methanolic leaf extract against *S. aureus* (NCIB 8588) was 20mg/ml; however, the minimum inhibitory concentration recorded against *B. cereus* (NCIB 6349) *K. pneumonia* (NCIB418), *P. aeruginosa* (NCIB 950) were 35, 25 and 30mg/ml respectively (Figure. 1).

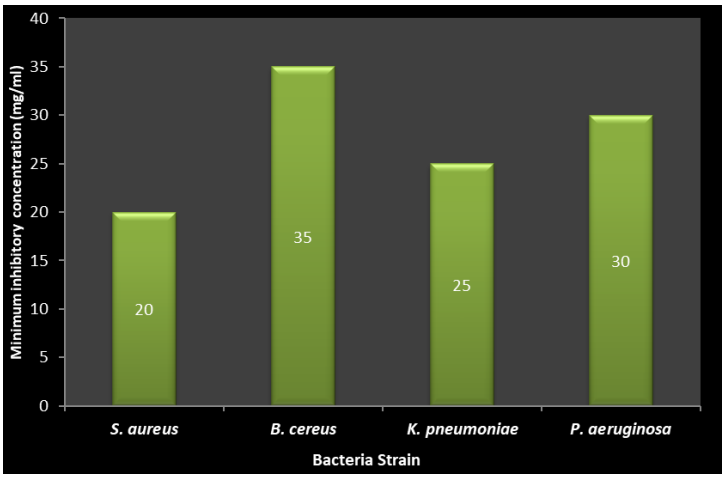

Figure 1. The minimum inhibitory concentration of methanol extract of *Achyranthes aspera* against test bacteria

DISCUSSION

The leaf extract of *Achyranthes aspera* was found to contain alkaloids, tannins, general glycosides, and terpenoids, consistent with the findings of Yang et al. (2018). It should be noted that specific secondary metabolites may be localized to particular areas of a plant, and environmental stress can influence the levels of different secondary metabolites in plants (Yang et al., 2018). Factors such as developmental stage, plant organs, fertilization, soil pH, light intensity, water availability, and genetics can influence the qualitative and quantitative production of secondary plant metabolites (Bjorkman et al., 2011; Vazquez-Leon et al., 2017).

In addition, this research revealed that the leaf extract of *A. aspera* exhibited increased DPPH scavenging activity with higher extract concentrations. However, the extract demonstrated lower antioxidant power compared to ascorbic acid. Antioxidants, including flavonoids and phenolics present in plants, play a crucial role in preventing the initiation and progression of oxidizing chain reactions. The presence of tannins and glycosides in the leaf extract of Achyranthes aspera may contribute to its antioxidant properties, as glycosides and tannins are examples of phenolic compounds known for their redox properties and ability to neutralize free radicals (Asmamaw and Yalemtsehay, 2017).

Furthermore, the study revealed that the leaf extracts of *Achyranthes aspera* demonstrated antibacterial activity against *S. aureus, B. cereus, K. pneumoniae*, and *P. aeruginosa*. The observed variations in the zones of inhibition among the test organisms indicate their susceptibility to the plant extract. The effect of the extract may vary depending on the target species and other factors such as initial population density, growth rate, and antimicrobial agent diffusion rate (Banso et al., 2020; Banso et al.,

2021). These variations underscore the potential of the plant extract for medicinal applications, possibly attributed to the presence of phytochemicals, which are recognized as valuable sources of pharmaceutical drugs (Mann et al., 2008).

This study showed that the lowest MIC value of the ethanol leaf extract of *Achyranthes aspera* was recorded against *Staphylococcus aureus*, while the highest value was recorded against *B. cereus*. Banso et al. (2020) suggest that antimicrobial agents with low activity exhibit higher minimum inhibitory concentration values, whereas highly effective agents display low values. This study proposes that the compounds in Achyranthes aspera leaf extract might have promising applications in chemotherapy.

CONCLUSION

Leaf extracts of Achyranthes aspera have been identified to contain alkaloids, tannins, general glycosides, and terpenoids. Moreover, the DPPH scavenging activity of the leaf extract increases with higher concentrations of the extracts. This suggests a potential for antioxidant properties within the extract, which could be beneficial for combating oxidative stress and related conditions. Additionally, the presence of tannins and glycosides in the Achyranthes aspera leaf extract is associated with its antimicrobial properties. These compounds have been recognized for their ability to inhibit the growth of microorganisms, highlighting the potential of Achyranthes aspera as a source of natural antimicrobial agents. The identified elements within the extract hold promise for utilization in the formulation of novel drugs with antimicrobial properties. Further research and exploration of the specific mechanisms and efficacy of these compounds could lead to the development of new pharmaceuticals for combating microbial infections and related ailments.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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