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Antimicrobial Activity, Antioxidant Property, and Acute Toxicity Profile of Methanol Extract of *Alchornea cordifolia* leaf

Nestor Tordzagla[1]*, Ronald Bakang[1], and Stephen Antwi [2]

[1]*Department of Pharmaceutical Chemistry, School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana.*

[2]*Department of Pharmacology, Centre for Plant Medicine Research, Mampong-Akwapim, Ghana.*

Abstract

Introduction: The increasing rate of antimicrobial resistance has necessitated the discovery of new therapeutic agents to fight this infectious disease and disorders associated with oxidative stress. *Alchornea cordifolia* is used traditionally to treat many microbial infections in Ghana. Hence, this study investigated phytochemical composition, antimicrobial activity, antioxidant property, and acute toxicity of the methanol extract of *Alchornea cordifolia* leaf. **Methods:** Phytochemical composition was determined in accordance with reference experimental protocols. The antimicrobial assays were done using eleven bacterial strains and a fungal strain. The assays were agar well diffusion, minimum inhibition concentrations (MIC), minimum bactericidal concentrations (MBC), and minimum fungicidal concentrations. Antioxidant property was evaluated using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The safety profile was obtained by an acute toxicity model using rats in five groups (n=6). **Results and Discussion**: Phytochemical screening of the extract revealed the presence of important secondary metabolites. The MIC and MBC values ranged from 3.125 mg/mL to 12.5 mg/mL and 50 mg/mL to 100 mg/mL respectively. The extract exhibited significant antioxidant activity with the scavenging activity comparable to ascorbic acid at higher concentrations and the highest value of 93.59±0.27 % recorded at a concentration of 500 µg/mL of the extract. Acute toxicity studies confirmed the safety of the extract up to 5000 mg/kg. **Conclusion**: *Alchornea cordifolia* leaf extract can be used as both antimicrobial and antioxidant agents, the extract showed no toxicity in rats. Nevertheless, further investigation may be done to identify the exact compounds responsible for the observed biological activities.

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*Corresponding author's email: ntordzagla@uds.edu.gh

Introduction

Plants are major sources of pharmaceuticals due to their secondary metabolites. Many of these plant samples have biological activities, which can always be used as the starting material for development of new drugs (Hamidi et al., 2014; Tordzagla et al., 2025).

Globally, traditional medicines are used by 88 % of the total number of countries and over 40 % of pharmaceutical formulations are based on natural products. Drugs such as morphine, codeine, aspirin, and artemisinin were isolated from plants (Zargaran, 2023). Some of these drugs that were isolated from plants have antimicrobial activity and antioxidant property.

There is often a positive correlation between an extract's antioxidant and antimicrobial activities. This implies that, compounds with higher antioxidant properties tend to also have stronger antimicrobial effects. This link is largely due to the presence of phytochemicals such as phenols and flavonoids, which act as antioxidants by neutralizing free radicals and can also disrupt bacterial cell membranes to kill microbial organisms. Therefore, natural extracts rich in these compounds, frequently display both biological activities (Rammali et al., 2024). *Alchornea cordifolia* is one of the plants with these qualities.

Alchornea cordifolia is a medicinal plant widely used in Africa to solve many health problems (Djimeli et al., 2017). In West Africa, the plant is used for the treatment of health conditions like headaches, colds, and control of spontaneous abortion. The fruit is used to cure asthma and cough. The treatment of urinary and gastrointestinal disorder forms part of its traditional usage. The leaves and root are used to treat fever, malaria, rheumatic pains, leprosy, snake venom, and as a mouthwash to treat ulcers of the mouth and toothache. The sap of the fruit is applied to cure eye problems and skin diseases. A cold infusion of the dried and crushed leaves act as a diuretic (Akoto et al., 2019).

Even though, *Alchornea cordifolia* leaves (Appendix Fig. A) are used extensively in Ghana, there is little scientific significant studies on it. Therefore, this study was designed to investigate antimicrobial and antioxidant activities of *Alchornea cordifolia* leaf extract and to evaluate its acute toxicity in rats.

Materials and methods

Sample collection, identification, and processing

The *Alchornea cordifolia* leaves were collected from Ejisu in the Ashanti region of Ghana and authenticated by Mr. Emmanuel Adom a Lecturer at the Department of Pharmacognosy and Herbal Medicine, University for Development Studies, Tamale, Ghana. The specimen was deposited in the herbarium of the department with a voucher number UDS/PHM/NR/24/002. The leaves were cleaned and air-dried in the shade at room temperature for two weeks. The dried leaves were then milled into fine powder by using an electronic grinding machine. The powdered sample was kept for extraction.

Extraction of plant sample

A 562 g of the powdered sample was weighed and cold macerated with 2.0 L of methanol for three days. The residue and filtrate were then separated using a Whatman filter paper after 72 h. A water bath (Buchi, B-491) and rotavapor (Buchi R-210) at 40°C, were used to concentrate the filtrate. The dried extract was screened for phytochemicals.

Phytochemical screening

The methanol extract was subjected to phytochemical screening to detect the presence of alkaloids, tannins, flavonoids, saponins, sterols, cardiac glycosides, terpenoids, and reducing sugars using standard protocol previously described (Kuete, 2013; Evans, 2002).

Determination of antimicrobial activity of plant extract

Test microorganisms

Test microbes were obtained from the Department of Pharmaceutics and Microbiology, School of Pharmacy and Pharmaceutical Sciences, University for Development Studies, Tamale, Ghana. They

include both Gram positive and Gram negative as well as a fungus.

Inoculum preparation of test microorganisms

The test strains of a fungus and bacteria were streaked on 20 mL sterile Sabouraud dextrose agar and nutrient agar plates, respectively. Their colonies were fished and suspended in 10 mL of sterile water in test tubes, after incubation. The turbidity was matched to the 0.5 McFarland standard and then assessed visually.

Agar well diffusion assay

The agar well diffusion assay method was used to determine the antimicrobial properties of the methanol extract of *Alchornea cordifolia* leaf as well as the standard drugs as described by Reeves (1989). In this assay, 100 µL of standardized inoculum of each test organism was spread onto sterile Muller–Hinton Agar and Sabouraud Dextrose Agar for bacteria and fungus respectively. A sterile cork-borer was used to make 8 mm diameter wells in the solidified agar, and 100 µL of the methanol extract of *Alchrornea cordifolia* leaf in different concentrations: 12.5, 25, 50, and 100 mg/mL placed into each well. Also, 0.1 mg/mL each of ciprofloxacin and fluconazole used as positive controls for bacteria and fungi respectively, then placed in separate wells.

After allowing the extract to diffuse properly into the agar for one hour at room temperature, the plates were incubated for twenty-four hours at 37 °C. There were duplicates made for every concentration. The concentration of the extract having antimicrobial activity inhibited the growth of the test organisms, and clear zones were formed. With the help of a ruler, the zones of inhibition of the extract and standard drugs were measured in milliliters as described by Raid *et al.* (2014).

Determination of minimum inhibitory concentration of Alchornea cordifolia extract

Minimum inhibitory concentrations of methanol extract of *Alchornea cordifolia* were determined in accordance with reference experimental protocols previously described (Andrews, 2001). Eight different concentrations of the extract and standard drugs were prepared ranging from 100-0.781 mg/mL and 0.10-0.000781 mg/mL respectively. Total volume of 200 uL made up of 100 uL of the agar, 80 uL of the sample and 20 uL of each test organism were placed into a 96-well bottom flat microtiter plate. The plates were then incubated at 37 °C for 24 h and MIC of the extract and standard drugs were determined as the lowest concentration that inhibited the growth of microbes after the addition of 20 µL of 1.25 mg/mL 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to the medium. The results were validated by performing all the tests in triplicates (Andrews, 2001).

Determination of minimum bactericidal/ fungicidal concentration of the extract

Regarding the MBC, the concentrations were prepared by subculturing 100 uL of all negative or yellow cultures from MIC into another fresh100 uL sterile broth in 96 well plates. The plates were incubated at 37 ℃ for another 24 h and observed for MBC (Andrews, 2001). The same procedures were used for minimum fungicidal concentration (MFC) assay. All the assays were performed in triplicates.

Determination of total phenolic contents in the extract

The total phenolic content of the methanol extract of *Alchornea cordifolia* was determined with the Folin–Ciocalteu reagent and disodium carbonate (Na_2CO_3) solution assay (Cheok et al., 2013) with some modifications. To about 3 mL of prepared concentrations (500, 250, 125, 62.5, 31.25, 15.625, 7.8125, and 3.90625 µg/mL) of the *Alchornea cordifolia* extract, 4.5 mL of Folin–Ciocalteu reagent was added and allowed to stand for 5 minutes and then 7.5 mL of 20% Na_2CO_3 was added. The mixture was incubated in the dark for 2 hours. After which 250uL of the mixture was pipetted into a 96-bottom flat plastic micro-well plate and the absorbance was measured at 760 nm in a Microplate Reader. The results were expressed in milligrams equivalents of gallic acid per milligram of dry weight. The calibration curve was established using 500, 250, 125, 62.5, and 0.00 µg/mL of gallic acid (Hatami et al., 2014). The experiment was repeated in triplicates.

DPPH antioxidant activity of the extract

The antioxidant capacity of the extract was assessed using a DPPH radical scavenging activity assay. Test tubes were serially made with various doses of the extract and ascorbic acids (500 µg/mL, 250 µg/mL, 125 µg/mL, 61.25 µg/mL, 31.25 µg/mL, 15.625 µg/mL, 7.8125 µg/mL, and 3.90525 µg/mL). Nine (9) mL of 0.3 mM DPPH freshly prepared in methanol were combined with 1.0 mL of the prepared concentration in newly cleaned test tubes. The resulting solutions were incubated for 30 min at room temperature in the dark, 200 µL of each was pipetted into a bottom flat 96-well plate, and the absorbance was measured using a microplate reader at 517 nm. The experiment was repeated in triplicates. The following formula was used to determine the DPPH scavenging activity (Warsi & Sholichah, 2017):

$$Radical\ Scavenging\ Percentage\ (\%\ Inhibition) = \left(\frac{Abs\ of\ control - Abs\ of\ sample}{Abs\ of\ control}\right) * 100\%$$

Abs of control denotes mean absorbance of the negative control.

Abs of sample denotes mean absorbance of the extract or ascorbic acid (standard).

Animals and ethical clearance

The animals used were Sprague-Dawley Rats (SDR) of both sexes. They were acquired from the animal house of Center for Plant Medicine Research, Mampong-Akwapim, Ghana. The animals were housed in the animal house facility of the Department of Pharmacology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi, Ghana, where acute toxicity study was done. The animals used in the study were treated humanely in accordance with the Public Health Service Policy on Humane Care and Use of Laboratory Animals and the Animal Welfare Regulations. Approval of the research work was granted by the Research and Ethics Committee, University for Development Studies Institutional Review Board (UDSIRB) with approval number UDS/ RB/ 140/24 before the commencement of the study.

Acute toxicity study of the extract

An acute toxicity study of *Alchornea cordifolia* leaf extract was conducted by first grouping the experimental animals. Six groups of pathogen-free Sprague-Dawley Rats (SDR) of both sexes were randomly selected. The experimental rats were allowed to acclimatize to the laboratory environment for seven days and fasted overnight with access to water. Before the extract was given orally to the rats at doses of 500 mg/kg (Group II), 1000 mg/kg (Group III), 2500 mg/kg (Group IV), and 5000 mg/kg (Group VI), the rats were weighed. Group I, the positive control group, were given 10 mL/kg of distilled water. The experimental animals were observed closely for weight loss, diarrhea, change in locomotion, skin fur position, lethargy, salivation, urination and death at 0 min, 30 min, 60 min, 120 min, 200 min, 24 h, 7 days, and 14 days after administration of the extract.

Statistical analysis

Data analysis was done by using SPSS Software and GraphPad Prism 6.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results and discussion

Percentage yield of Alchornea cordifolia leaf

The powdered *Alchornea cordifolia* leaf was brown in colour and its methanolic extract was dark-green with a percentage yield of 5.15 %.

Phytochemical composition

The leaves of *Alchornea cordifolia* have been shown to contain secondary metabolites such as cardiac glycosides, saponins, tannins, flavonoids, sterols, terpenoids, and reducing sugars. Reported studies show that these secondary metabolites serve as a major source of drugs (Tordzagla *et al.*, 2024; Owoseni et al., 2015; Suphiratwanich et al., 2024).

Tannins inhibit the growth of diverse microbes, such as bacteria, fungi, and yeasts (Farha et al., 2020). Also, tannins can inhibit the growth of both

Gram-positive and Gram-negative bacteria (Boakye et al., 2016).

The antimicrobial activity of saponins from various plant sources against various bacterial and fungal strains is well documented. Saponins are reported to possess antibacterial property against *Staphylococcus aureus, S. epidermidis and Bacillus cereus*, causing severe damage through bacterial cell wall degradation followed by disruptions of the cytoplasmic membrane and membrane proteins (Dong et al., 2020).

In addition, flavonoids, which are also found in the extract and they have numerous biological activities including antimicrobial and antioxidant activities (Dias et al., 2021). Flavonoids demonstrate antioxidant activity by neutralizing free radicals, donating electrons and hydrogen atoms, and binding metal cations (Hassanpour & Doroudi, 2023). The presence of these phytochemicals in the leaf extract of *Alchornea cordifolia* could attribute to its biological activity.

Antimicrobial activity of Alchornea cordifolia extract

Agar well diffusion

The extract proved to have a broad-spectrum antimicrobial activity, as both Gram-negative and Gram-positive bacteria as well as fungus were susceptible to the extract across a range of concentrations.

The extract had the ability to inhibit the growth of the test microorganisms at varying degrees. From the results obtained, the lowest concentration at which the extract recorded a zone of inhibition was 12.5 mg/mL and the most active concentration was 100 mg/mL. From Table 1, it is evident that, at the concentration of 100 mg/mL, all the test microbes were susceptible to the extract. At the highest concentration used in this study, ciprofloxacin was also active against the tested microbes. Fluconazole recorded inhibition of 16.4±1.8 against *C. albicans* at a concentration of 100 µg/mL.

In other studies, methanol extract of *Alchornea cordifolia* leaf recorded similar results for *E. faecalis*, *E. coli* and *S. aureus* (Banso et al., 2924). These results agree with the other findings, which reported that leaf extracts were able to inhibit both Gram-positive and Gram-negative bacteria (Okoye et al., 2014).

MIC, MBC and MFC of the extract of and the standard drugs

The MIC results showed that the extract exhibited a broad-spectrum antimicrobial activity against the test organisms. The MIC values ranged from 3.125 mg/mL to 12.5 mg/mL. The highest activity observed by the extract was against *S. aureus* and *C. albicans* with MIC value of 3.125 mg/mL, followed by *S. typhi* and *S. typhimurium* with MIC of 6.25 mg/mL and the remaining test organisms were inhibited at 12.5 mg/mL as shown in Table 2.

The results in this study conform with other studies where 70 % ethanolic extract of *Alchornea cordifolia* leaves showed activity against the test microorganisms; *S. aureus, S. typhi, K. pneumoniae, E. coli* and *P. aeruginosa* with MIC values ranging from 1.5625 to 12.5 mg/mL (Fadehan et al., 2015).

Chloramphenicol was the standard antibiotic used as a control in this research work on the bacterial strains. The MIC of chloramphenicol against the microorganisms ranged from 0.0125 mg/mL to 0.05 µg/mL as presented in Table 2. The chloramphenicol standard was most active against *N. gonorrhoea, S. aureus* and *E. coli* at MIC value of 0.0125 mg/mL but less active against *P. aeruginosa* and *K. pneumoniae* with MIC value of 0.05 mg/mL as shown in Table 2.

The MBC test allows determination of the minimum concentration of the plant extract necessary to achieve a bactericidal effect. The MBC values of the extract against the twelve selected test microorganisms ranged from 50 mg/mL to 100 mg/mL. As compared to the MIC values, there were significant differences in the MBC values obtained for the respective test organisms with none of the organisms recorded the same values for MIC and MBC. The summary of the MIC, MBC and MFC values of the extract and standard drugs are shown in Table 2.

Table 1: Antimicrobial activity (Zone of inhibition) of the extract and the standard drugs

Test Organism	Mean Zone of Inhibition (mm)					
	Methanol Extract				Control	
	100 mg/mL	50 mg/mL	25 mg/mL	12.5 mg/mL	Chloramphenicol 0.10 mg/mL	Fluconazole 0.10 mg/Ml
E. coli	20.0±0.1	19.0±4.2	19.0±1.4	16.5±0.7	26.5±2.1	NA
S. aureus	19.0±1.4	18.0±2.8	-	-	27.5±0.7	NA
E. coli (ESBL)	16.5±0.7	14.0±0.0	-	-	22.0±1.4	NA
B. cereus	16.8±0.3	-	-	-	22.5±0.7	NA
MRSA	21.0±1.4	19.5±2.1	18.0±2.8	16.5±2.1	25.0±4.2	NA
N. gonorrhoea	18.0±0.0	-	-	-	19.0±1.4	NA
C. albicans	24.5±2.1	22.5±2.1	21.5±2.1	19.0±1.4	NA	16.4±1.8
E. faecalis	24.0±5.6	19.5±0.7	19.0±1.4	18.0±1.4	19.0±1.4	NA
S. typhi	22.0±2.8	21.0±1.4	18.5±4.9	15.5±0.7	18.4±0.2	NA
S. typhimurium	21.5±4.9	17.5±3.5	17.0±1.4	-	26.5±3.5	NA
P. aeruginosa	27.0±2.8	20.0±0.0	17.5±0.7	-	22.5±3.5	NA
K. pneumonia	14.3±3.6	-	-	-	20.0±2.1	NA

Key: Values presented as Mean ± SD; ND =Not applicable, - = No inhibition

Table 2: Summary of MIC, MBC and MFC values of the extract and the standard drugs

Test Organisms	*Extract (mg/mL)*			*Chloramphenicol (mg/mL)*		*Fluconazole (mg/mL)*	
	MIC	*MBC*	*MFC*	*MIC*	*MBC*	*MIC*	*MFC*
S. typhi	*6.25*	*100*	*NA*	*0.025*	*0.10*	*NA*	*NA*
S. typhimurium	*6.25*	*100*	*NA*	*0.025*	*0.10*	*NA*	*NA*
N. gonorrhoea	*12.5*	*100*	*NA*	*0.0125*	*0.10*	*NA*	*NA*
K. pneumonia	*12.5*	*50*	*NA*	*0.050*	*0.10*	*NA*	*NA*
B. cereus	*12.5*	*50*	*NA*	*0.025*	*0.10*	*NA*	*NA*
S. aureus	*3.125*	*100*	*NA*	*0.0125*	*0.05*	*NA*	*NA*
E. faecalis	*6.25*	*50*	*NA*	*0.025*	*0.10*	*NA*	*NA*
E. coli	*12.5*	*100*	*NA*	*0.0125*	*0.05*	*NA*	*NA*
P. aeruginosa	*12.5*	*50*	*NA*	*0.050*	*0.10*	*NA*	*NA*
MRSA	*12.5*	*50*	*NA*	*0.025*	*0.10*	*NA*	*NA*
E. coli (ESBL)	*12.5*	*100*	*NA*	*0.025*	*0.05*	*NA*	*NA*
C. albicans	*3.125*	*NA*	*50*	*NA*	*NA*	*0.025*	*0.10*

NA= Not applicable

Total phenolic content (TPC)

Total phenolic content of the extract was calculated from the regression equation of calibration curve, (A = 0.002789*C + 0.268151; R^2 = 0.9950) and expressed as mg gallic acid equivalents (GE) per gram of sample in dry weight (mg GAE/g) and the results are shown in Table 3.

The total phenolic content of the extract was determined across a range of concentrations from 3.90625 to 500µg/mL. The data (Table 3) shows that as the concentration increases, the TPC values of the extract also increase. From the concentrations 3.90625 to 31.25 µg/mL, there was a steady increase in TPC, from 73.97±9.45 to 119.63±0.03 mg GAE/g. However, beyond the concentration of 31.25 µg/mL, there was a sharp increase in the TPC values. This sharp rise at higher concentrations could be attributed to an increase in phenolic compounds as higher weights of the extract were used to prepare those solutions; hence, the solution became saturated with phenolic compounds.

Table 3: The total phenolic content of *Alchornea cordifolia* in mg GAE/g of dry extract across a range of concentrations

Concentration (µg/mL)	TPC (mg GAE/g)
3.90625	73.97±9.45[a]
7.8125	82.25±3.44[a]
15.625	100.87±14.84[a]
31.25	119.63±0.03[b]
62.5	175.50±4.92[c]
125	314.41±18.79[d]
250	425.40±9.12[e]
500	531.20±20.45[f]

The results were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. The superscript letter 'a' indicates no significant difference. However, the superscript letters b, c, d, e, and f indicate significant difference at $p < 0.05$.

Radical scavenging activity (%) of the extract and ascorbic acid (standard drug)

The scavenging percentage of the extract increased from 34.14±0.33 % at a concentration of 3.90625 µg/mL to 93.60±0.28 % at 500 µg/mL whereas, the scavenging activity of ascorbic acid, the reference drug used in this study increased from 17.71±0.48 % at a concentration of 3.90625 µg/mL to 97.12±0.42 % at 500 µg/mL. These results indicate that both the extract and ascorbic acid exhibited a concentration-dependent increase in scavenging activity (Table 4).

At lower concentrations, ascorbic acid showed slightly lower scavenging activities in contrast to the extract. For instance, at the same concentration of 3.90625 µg/mL, the extract exhibited a scavenging activity, which was twice that of ascorbic acid. This implies that the extract might contain a mixture of compounds with different antioxidant activity capacities that work synergistically.

However, at a higher concentration, ascorbic acid showed a significantly greater increase in scavenging activity as compared to that of the extract. Scavenging percentages of 78.81±0.29 % and 59.45±0.22 % were recorded for ascorbic acid and the extract respectively, at a concentration of 62.5 µg/mL. From concentrations of 125-500 µg/mL the scavenging percentage of the methanol extract of *Alchornea cordifolia* and ascorbic acid increased only slightly and this could be as a result of near-maximal therapeutic benefit has been already be achieved at 125 µg/mL concentration, and further increases may offer limited additional advantage. At 500 µg/mL, the scavenging activity of the extract (93.59±0.27 %) was slightly lower than that of ascorbic acid (97.12±0.41 %). The IC_{50} of the extract was calculated to be 42.98±0.02 µg/mL. The extract, though very potent, might contain other compounds and some of these compounds may not contribute to the scavenging activity of the extract as compared to ascorbic acid, hence, the reason for the observed antioxidant activity.

Table 4: Radical scavenging percentage of the methanol extract of *Alchornea cordifolia* and ascorbic acid (standard drug).

Concentration (µg/mL)	Scavenging Percentage (%)	
	Extract	Ascorbic acid
3.90625	34.14±0.33[a]	17.71±0.48
7.8125	33.06±0.29[a]	19.59±0.12
15.625	37.19±0.16[a]	27.19±0.25
31.25	45.60±0.24[a]	42.67±0.28
62.5	59.45±0.22	78.81±0.29[a]
125	87.50±0.23	95.88±0.16[a]
250	93.41±0.25	96.57±0.48[a]
500	93.59±0.27	97.12±0.41[a]

The superscript letter 'a' indicates significant difference between the results of the extract and ascorbic acid at $p < 0.05$.

Acute toxicity profile

The study investigated the toxicological profile of the methanol leaf extract of *Alchornea cordifolia* in Sprague rats. The change in weight of the animals were recorded at week 0, week 1 and week 2 as mean ± standard deviation as shown in Appendix Table A.

This consistency of the weight over the two-week period suggests that the intervention did not affect weight changes during the experimental period. The absence of significant weight loss in any of the groups, even at the highest dose of 5000 mg/kg, suggests that the methanol extract of *Alchornea cordifolia* could be non-toxic within the dosage range tested.

The study also observed other factors such as behavioural changes and mortality over a 14-day period. The toxicity indicators regarding the physical and behavioural reactions to the test substance have been summarized in Table 5. These indicators include death, coma, excessive salivation, movement, changes in eye colour, diarrhea, urination patterns, sleep disturbances, skin and fur condition, sensitivity to touch, and defecation.

There was no death of the experimental rats during the entire period. This result is important, as it is an indication that the doses were well tolerated by the rats, with no signs of life-threatening toxicity and that the LD_{50} of the extract is higher than 5000 mg/kg. Also, there was no case of coma, meaning that at a dose of 5000 mg/kg, the extract did not cause significant central nervous system depression. Other parameters such as locomotion, sleeping patterns, eye colour, urination, salivation, defecation, skin and fur position and reactivity to touch remained normal across all the groups indicating that these physiological and behavioral parameters were not affected by the experimental rats.

The results obtained in this current study agree with other studies (Ejeh et al., 2023). In the previous studies, no death of experimental animals was observed after the administration of 2000 and 5000 mg/kg of the aqueous and ethanol extracts of *Alchornea cordifolia* leaves respectively. Also, in terms of general appearance and behavioural observations of the experimental rats, similar results were obtained where the animals did not show any change in behaviour or signs of intoxication immediately after administration of the doses and during the observation period (Ejeh et al., 2023).

Table 5: General appearance and behavioural observations of the experimental rats in treated groups in mg/kg and the control group

Toxicity signs	Control	500	1000	2500	5000
Mortality	0	0	0	0	0
Coma	-	-	-	-	-
Reactivity to touch	μ	μ	μ	μ	μ
Locomotion	μ	μ	μ	μ	μ
Eye colour	μ	μ	μ	μ	μ
Diarrhea	-	-	-	-	-
Urination	μ	μ	μ	μ	μ
Salivation	μ	μ	μ	μ	μ
Sleep	μ	μ	μ	μ	μ
Skin and fur position	μ	μ	μ	μ	μ
Defecation	μ	μ	μ	μ	μ

The Table highlights physical appearance and behavioural observations of the experimental rats for acute toxicity study conducted. Number of rats per group, N = 6, 0 = no death, μ= normal, - = absent.

Conclusion

The methanol extract of *Alchornea cordifolia* leaf contains cardiac glycosides, saponins, tannins, flavonoids, sterols, terpenoids, and reducing sugars. The extract also possesses broad spectrum of antimicrobial activity against tested pathogenic microorganisms. The results support the traditional use of the extract to treat various microbial infections.

The extract exhibited antioxidant activity, which was comparable to ascorbic acid, a well-established antioxidant. It has been established that the extract has very high phenolic content.

The acute toxicity assessment showed no toxic reactions, even at the maximum tested dose of 5000 mg/kg, suggesting a high safety margin. These findings validate the traditional use of *Alchornea cordifolia* leaf in treatment of various microbial infections and as a tea bag. Hence, the study has provided a strong foundation for further pharmacological and clinical investigations, which should lead to isolation, characterization and identification of exact compounds responsible for the observed biological activities.

Authors contributions

Conceptualization, Nestor Tordzagla and Ronald

Bakang; investigation, Nestor Tordzagla, Ronald Bakang and Stephen Antwi; formal analysis, Nestor Tordzagla and Ronald Bakang; writing of the manuscript, Nestor Tordzagla, Ronald Bakang and Stephen Antwi; All authors have agreed to the final version of the manuscript.

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Ethical approval statement

The study on the rats was conducted with the approval of the Research and Ethics Committee, University for Development Studies Institutional Review Board (UDSIRB) with approval number UDS/ RB/ 140/24.

Conflict of interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that no AI or AI assisted technologies were used to write the manuscript.

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Appendix

Fig. A: *Alchornea cordifolia* leaves (picture taken in 2024 at Ejisu, Ghana)

Table A: The mean weight of the rats in treated groups and control

Dose (mg/kg)	Weight (g)		
	Day 0	**Day 7**	**Day 14**
500	147.08±26.07	147.78±26.94	148.95±27.34
1000	158.30±18.55	159.63±18.88	159.98±18.35
2500	199.5±11.08	200.18±11.50	197.36±10.07
5000	207.38±16.85	208.17±16.72	205.1±15.46
Control	167.68±29.71	167.98±29.83	168.38±29.86

One Way ANOVA analysis followed by a Tukey test of multiple comparisons of mean weight differences between different dose groups and a control group.