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In Vitro Evaluation of the Cytotoxicity and Elemental Composition of *Alchornea cordifolia* Leaf Extracts

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Abstract

The maximum therapeutic and minimum side effects of herbal remedies have been verified in numerous scientific investigations. Although, isolated or synthesized active compounds can become toxic in relatively small doses; it usually takes a much greater amount of a whole herb, with all of its components, to reach a toxic level. The study was aimed to determine the toxicity level and elemental analysis of *Alchornea cordifolia* leaf extracts collected in Niger State. Phytochemical screening of the *Alchornea cordifolia* leaf extracts was carried out using standard methods. Agar well diffusion and agar dilution methods were employed to determine the zone of inhibition, minimum inhibitory concentration, and minimum bactericidal concentration. The elemental analysis of the *Alchornea cordifolia* leaf as well as the toxicity level using brine shrimp were determined using standard protocol. The phytochemical screening of the leaf extracts revealed the presence of secondary metabolites such as flavonoids, tannins, alkaloids, steroids, glycosides. The LD_{50} values for ethanol and ethyl acetate extracts were 456.1µg/ml and 433.8µg/ml respectively which indicate that the extracts were medium toxic. Elemental analyses of *Alchonea cordifolia* leaf constituents show that lead, cobalt and manganese were within permissible limit given by WHO while chromium, Zinc, copper, iron, cadmium and Nickel were above World health organization's permissible limit. The findings of this study suggest that *Alchornea cordifolia* leaf could be employed as potential therapeutic candidate with medium toxicity for the treatment of uropathogenic infections.

Keywords: *Alchornea cordifolia*, Toxicity, extract, Elemental composition

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Introduction

Toxicity is the degree to which a substance can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity). Biological Assay is an experiment carried out on the test plant extracts to determine the level of potency or toxicity of their secondary metabolites. Bioactive compounds could be toxic to *Artemia salina* larvae. The eggs of the brine shrimp *Artemia salina* are readily available as fish food in pet shops. When placed in artificial seawater, the eggs hatch within 48 hours, providing large numbers of larvae. It is a typical experiment to investigate a dose-response relationship and one indicator of the toxicity of plant material is LD_{50} or LC_{50}, which refers to the amount of plant material that kills half of the test organisms. Brine shrimp lethality assay is considered a useful tool for preliminary assessment of toxicity and it has been used for detection of plant extract toxicity (Ali *et al*., 2024). Phytotoxins are considered a potential source of pharmaceutical drugs. Pharmacology is simply toxicology at a higher concentration and toxicology is simply pharmacology at a lower concentration, showing that dose adjustment differentiates a poison and a remedy (Onyeka *et al*, 2018). Although many plants have valuable properties, some of them are known to carry toxicological properties as well. Recent studies indicate that numerous plants are used as food sources, some of them may have mutagenic or genotoxic potential (Ogbonnia *et al*., 2016). Numerous research studies have recently focused on both pharmacology and toxicity of medicinal plants used by humans. This is of high importance in order to achieve a safe treatment with plant products (Chebaibi *et al*., 2019). The toxicity of the plants may originate from different contaminants or from plant chemical compounds that are part of the plant. Various assays are used for the research of potential toxicity of herbal extracts based on different biological models, such as *in vivo* assays on laboratory animals. *Alchornea cordifolia* (Schumach. and

Thonn.) Müll. Arg (Euphorbiaceae) (referred to as Christmas bush) is native to Senegal, East Kenya, South Tanzania, and throughout Central Africa to Angola. This plant usually grows very close to water bank, moist or marshy places to a significant height while remaining in a shrubby or scrambling habit (Nnamdi *et al*., 2017). It belongs to the subfamily Acalypholdeae and family Euphorbiaceae or Spurge family (Nnamdi *et al*., 2017). *Alchornea cordifolia* is the most studied species in the Alchornea genus (Adeshina *et al*., 2012). It is found in countries like Congo, Nigeria, Ivory Coast, and Ghana (Ishaq *et al*., 2024). *A. cordifolia* is known as buissondenoel in French and Christmas bush in English (Ngene et *al*., 2022). In Nigeria, it is known as "Bambami" in Hausa, "Ububo" in Igbo, and "Ewe Ipa", "Esinyin" in Yoruba and "mbom" in Efik (Noundou *et al*., 2016; Boniface *et al*., 2016. The aim of this study is to determine the toxicity level and elemental analysis of *Alchornea cordifolia* leaf extracts collected in Niger State.

Materials and Methods

Collection and authentication of plant material

Healthy, disease-free leaf of *Alchornea cordifolia* were collected from Madalla- Suleja road in Suleja Local Government of Niger State. Authentication of the plant was carried out at the Herbarium, Department of Plant Biology, Faculty of Life Sciences, Bayero University, Kano, by a taxonomist and accession number*; Alchornea cordifolia* – BUKHAN 0404 was given.

Pretreatment of plant parts

The leaf was washed under running tap water and then spread out in the laboratory to air-dry away from sunlight (Lalisan *et al*., 2014, Ayéna *et al*., 2022). After proper drying has been ensured (Clement *et al*., 2020), the leaf was pulverized by mechanical grinding using mortar and pestle (Lalisan *et al*., 2014). These were then weighed, packed in nylon bags and labelled.

Preparation of Plant Extracts

The powdered plant materials (500 grams each) were subjected to soxhlet extraction method using two (2) different solvents, ethanol and ethyl acetate as solvents (Dianursanti *et al*., 2020).

Extraction using the Soxhlet method

This was carried out using Soxhlet extraction apparatus described by Dianursanti *et al*. (2020) using ethanol and ethyl acetate as solvents of extraction**.**

Qualitative Phytochemical screening of the extracts

The leaf extracts was subjected to phytochemical screening in order to identify the phytochemical constituents of the leaves using the method of Ali *et al*. (2017).

Procedure for elemental analysis

The leaf of *Alchornea cordifolia* extract was used for elemental analysis using Atomic Absorption Spectrometry (AAS) (Buck Scientific Model VGP 210 Model) at the appropriate wave length, temperature and lamp current flow for each element under study, for the determination of iron, Zinc, Copper, Cromium, Cadmium, Nickel, Manganese, Cobalt and Lead (William *et al.*, 2015).

Toxicity studies

The acute toxicity (LC_{50}) value of the ethanol and ethyl acetate extract of *Alchonea cordifolia* was determined using standard conventional procedures as described by (Hamrun *et al*., 2020). A stock solution of sample was prepared by dissolving 20 mg of the sample in 2 ml ethanol to obtain the desired concentrations of 1000µg/ml, 100µg/ml and 10µg/ml, The solvent was then evaporated by leaving the vials in a vacuum desiccator for 24 hours, mixture of DMSO and sea water was used as negative control. Each extract in three concentrations (1000, 100 and 10 ppm) was taken into small sterile vials in triplicate (9-vials/extract). Ten shrimps were added to each vial using Pasteur's pipette. Volumes of each were adjusted to 5 ml sea water immediately after adding the shrimps. The vials were maintained under illumination at room temperature and survivors were counted after 24 hours. The mean percentage mortality was plotted against the logarithms of the concentrations. The concentration LC_{50} at which 50% of the larvae is killed was determined from the graph.

Results

Phytochemical Constituents of *Alchornea cordifolia* leaves Extracts

The qualitative phytochemical screening of the ethanol and ethylacetate extracts of *Alchornea cordifolia* leaf revealed the presence of the following phytochemicals constituents; carbohydrate, cardiac glycosides, resins, flavonoids, tannins and alkaloids, steroids and triterpenes in all the extracts. However, saponins were present in ethanol extract but absence in ethyl acetate extract. Anthraquinones derivatives were absence in both extracts (Table 1).

Determination of Elemental constituent

Elemental analyses of *Alchonea cordifolia* leaf constituents show that lead, cobalt and manganese were within permissible limit given by WHO while chromium, Zinc, copper, iron, cadmium and Nickel were above World health organization's permissible limit (Table 2).

Determination of toxicity of the extracts

The toxicity of ethanol and ethyl acetate extracts expressed as LC_{50} were 452.81µg/ml and 421.42 µg/ml respectively at the concentration of 10 µg/ml, 100µg/ml and 1000µug/ml as shown in table 3. Brine shrimps' toxicity test was carried out on the extracts which shows that 100% of the nauplii were dead at 10µg/ml concentration, 73% death at 100µg/ml and 0 % death at 1000 µg/ml in ethanol extract while in ethyl acetyl acetate it was 93% of the nauplii were dead at 10µg/ml concentration, 70 % death at 100µg/ml and 0% death at 1000 µg/ml. (% Death = [(Test – Control) / Survivors of control] X 100).

Table 1: Phytochemical Constituents of the Extracts of *Alchornea cordifolia* Leaf

S/N	Phytochemicals	Test Name	Ethyl Acetate	Ethanol
1.	Carbohydrates	Molisch's test	+	+
	Reducing sugar	Fehling's test	+	+
2.	Glycosides	Kella-Killiani test	+	+
	Cardiac glycosides	Kadde test	+	+
3.	Anthraquinone derivatives	Borntrager's test (modified)	–	–
	Combined anthracene	Borntrager's test	–	–
4.	Resin	Acetic anhydride test	+	+
5.	Saponin	Frothing test	–	+
6.	Flavonoids	Sodium hydroxide test	+	+
7.	Tannins	Ferric chloride test	+	+
8.	Alkaloids	Mayer's test	+	+
		Wagner's test	+	+
9.	Steroids and Triterpenes	Salkowski test	+	+
		Liberman-Burchard's test	+	+

key: += Present; - = Absent

Table 2: Elemental Constituents of *Alchornea cordifolia* Leaf Extracts

S/N	Element	Concentration (mg/kg)	WHO Permissible Limit (mg/kg)
1.	Lead (Pb)	0.177	2.0
2.	Chromium (Cr)	2.156	1.3
3.	Zinc (Zn)	58.788	50
4.	Copper (Cu)	9.175	10
5.	Cobalt (Co)	3.040	42
6.	Iron (Fe)	56.933	20
7.	Cadmium (Cd)	3.452	0.02
8.	Nickel (Ni)	54.709	67.9
9.	Manganese (Mn)	88.212	200

Table 3: In-Vitro Cytotoxicity of *Alchonea cordifolia* extracts on Brine Shrimp Nauplii

Extracts	Concentration (mg/ml)	Dead nauplii after 24 hours			Total Survivors	Mortality (%)	LC_{50} (µg/ml)
		T_1	T_2	T_3			
Ethanol	10	0	0	0	30	100	
	100	6	6	0	18	73	452.81
	1000	10	10	10	0	100	
Ethyl acetate	10	1	1	0	28	93	
	100	4	3	2	21	70	421.42
	1000	10	10	10	0	100	
Control	–	0	0	0	30	0	

Key: T_1-T_3 = Tubes

Discussion

Qualitative phytochemical screening of the extracts extracted with ethanol and ethyl acetate revealed the presence of all the following phytochemicals tested, namely alkaloids, flavonoids, reducing sugar, Tannins, resin and carbohydrates which collaborated with the findings of Djimeli *et al*. (2017) and Jia *et al.* (2017). Anthraquinones and saponins were present in the ethanolic leaves extract but were absence in ethyl acetate extract of *Alchornea cordifolia*. Cardiac glycoside and steroids were present in ethyl acetate extract but absent in ethanol extract. Moreover, the active compounds can sometimes be present in very small concentrations, which could be a hinderance when trying to elucidate antimicrobial compound structures (Ekundayo *et al.*, 2020). The presence of these phytochemicals has conferred to the leaf's extracts of *Alchornea cordifolia* their medicinal value (Arbonnier, 2004). Akbari *et al*. (2019) indicated that the recoveries of bioactive phytochemical compounds from plants are potentially affected by the conditions of extraction methods and different solvent formulations. Phenols are generally protoplasmic poisons toxic to all types of cells. Precipitation of proteins occurs with high concentration of phenol, while at low concentrations it denatures proteins without coagulating them. Phenol freely penetrated the tissue because of its denaturing activity (Adeshina *et al*., 2012). Flavonoids on the other hand have been reported to be synthesized by plants in response to microbial infection; hence they exhibit antibacterial activities (Anyanwu and Okoye 2017). The presence of flavonoids suggested that it can be used as antispasmodic and antioxidant, and confirms the reason for the use of the plant in the treatment of spasmodic bronchitis and other microbial infections.

The elemental analysis results show the presence of iron, chromium, cadmium, copper, lead, zinc, cobalt, nickel and manganese at different concentration in the leaf of *Alchornea cordifolia.* The lower concentration of some of these elements is an indication of little or no toxicity of the plant as heavy metals are known to cause cancer, liver and kidney problems (Ogugbuaja *et al.*, 2000).

The toxicity of *Alchornea cordifolia* leaf extracts expressed as LC_{50} was evaluated by comparison to Clarkson's toxicity index. According to these indexes, extracts with LC_{50} more than 1000 µg/ml are categorized as non-toxic, LC_{50} of 500-1000 µg/ml have low toxicity, extracts with LC_{50} of 100-500 µg/mL show medium toxicity, while extracts with LC_{50} of 0-100 µg/ml are highly toxic (Clarkson *et al.*, 2004, Simorangkir *et al*., 2021).

Out of the two extracts analyzed, the cytotoxicity was determined from ethanol and ethyl acetate which shows (LC_{50} = < 500µg/ml). This result agrees with the findings of Shima *et al.*, (2016) that in high concentrations of (1000 µg/ml) approximately all the shrimps were dead. Variation in the concentration of *Alchornea cordifolia* leaf ethanolic extract and *Alchornea cordifolia* leaf ethyl acetate extract in this experiment results in different mortality rates for the brine shrimp larvae. This indicates that cytotoxicity of the ethanolic extract and ethyl acetate extract were concentration dependent (Pertiwi *et al*., 2020).

The significant lethality and non-lethality of the extracts of *Alchornea cordifolia* leaf indicates that there is presence of cytotoxic compounds and also less harmful compounds in the extracts which can be ascribed to the solvents used in extraction. The death mechanism of brine shrimp larvae is related to the function of alkaloid compounds in *Alchornea cordifolia* leaf extracts which can diffuse through the cell membrane of brine shrimp larvae, causing damage or modification of the membrane's permeability and disrupting the substance transfer system which can interfere with biochemical processes and physiological processes (Sharififar *et al*., 2017).

Conclusion

The results obtained in this investigation provide a scientific rationale for the use of *Alchornea cordifolia* to treat urinary tract ailments in traditional medicine. This investigation has revealed that the leaves of *Alchornea cordifolia* studied have high phytochemical content. Thus, they could be exploited to be used in the formation of alternative antimicrobial drugs which will be used to cure and control human diseases. Brine shrimp lethality assay (BSLA) provides initial screening data that can be followed up by further precise bioassays and the extracts of *Alchornea cordifolia* leaf in this study possessed medium toxicity. It is also recommended that the search and research towards the discovery of lead compounds from the leaf of *Alchornea cordifolia* extract against the human pathogens and multi drug resistant pathogens should be intensified.

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Conflict of Interest

The authors declare no conflict of interest.

References

Adeshina, G. O. Kunle, O. F. Onaolapo, J. A. Ehinmidu, J. O. and Odama, L. E. (2012). Antimicrobial activity of the aqueous and ethyl acetate subfractions of *Alchornea cordifolia* leaf. *European Journal of Medicinal Plants*. **2**(1): 31-41.

Akbari, S. Abdurahman, N. H. and Yunus, R. M. (2019). Optimization of saponins, phenolics, and antioxidants extracted from fenugreek seeds using microwave-assisted extraction and response surface methodology as an optimizing tool. *Comptes Rendus Chim.* **22**: 714–727.

Ali, M. Yahaya, A. Zage, A. U. and Yusuf, Z. M. (2017). *In-vitro* Antibacterial Activity and Phytochemical Screening of *Psidium guajava* on Some Enteric Bacterial Isolates of Public Health Importance. *Journal of Advances in Medical and Pharmaceutical Sciences*. **12**(3): 1-7.

Anyanwu, M. U. and Okoye, R. C. (2017). Antimicrobial activity of Nigerian medicinal plants. *J int Ethnopharmacol*. **6**:240-59.

Arbonnier, M. (2004). Trees, shrubs and lianas of West African dry zones. CIRAD MARGRAF publishers, GMBH. AJ. Wageningen, *The Netherland*. 573 pp

Ayéna, A. C. T. Dosseh, K. Idoh, K. Agbonon, A. and Gbeassor, M. (2022). Comparative physicochemical screening and toxicology of hydroethanol extracts of the parts of *Pterocarpus santalinoides* l'H´er. Ex DC. (Fabaceae) in Wistar Rats. *Sci World J,* Article ID 5953094, 7.

Boniface, P. K. Ferreira, S. B. and Kaiser, C. R. (2016). Recent trends in phytochemistry, ethnobotany and pharmacological significance of *Alchornea cordifolia* (Schumach. and Thonn.) Muell. Arg. *Journal of Ethnopharmacology*. **191**: 216-244.

Chebaibi, M. Bousta, D. Chbani, L. Iken, I. and S. Achour. (2019). "Evaluation of acute toxicity of plants' mixture used in traditional treatment of kidney diseases in Morocco," Pharmacognosy Research, **11**(2):155–161.

Clarkson, C. Maharaj, V. J. Crouch, N. R. Grace, O.M. Pillay, P. Matsabisa, M. G. Bhagwandin, N., Smith, P.J., and Folb, P.I. (2004). *In vitro* antiplasmodial activity of medicinal plants native to or naturalized in South Africa. *J Ethnopharm*. **92**: 177-191.

Clement, OA. Akwasi, A. Osei, K. and Bartholomew, AB. (2020). An Assessment of the Anti-Inflammatory, Antimicrobial, and Antioxidant Activities of *Ficus sur* Stem-Bark. *Organic and Medicinal Chem IJ***. 9**(4): 555-566.

Dianursanti, D. Nugroho, P. and Prakasa, M. B. (2020). Comparison of maceration and soxhletation method for flavonoid production from *Spirulina platensis* as a sunscreen's raw material. AIP Conf Proc. 2230:020006.

Djimeli, M. N. Fodouop, S. P. C. Njateng, G. S. S. Fokunang, C. Tala, D. S. Kengni, F. and Gatsing, D. (2017). Antibacterial activities and toxicological study of the aqueous extract from leaves of *Alchornea cordifolia* (Euphorbiaceae). BMC Complement. Altern. Med. **17:**349.

Ekundayo, E. Kalu, U. and Enya, E. (2020). Detection of Beta Lactamase in Ampicillin Resistance Enterobacteriaceae isolated from Human stool In Ekiti state *Nigerian Journal of Microbiology*, **34**(1):5044 – 5052

Hamrun, N. Nabilah, T. Hasyim, R. Ruslin, M. Dammar, I. and Arianto, M. A. (2020). Toxicity test of bioactive red alga extract *Eucheuma spinosum* on shrimp *Artemia salina* leach. *Sys Rev Pharm.***11** (5):672-6.

Jia, S. Shen, M. Zhang, F. Xie, J. (2017). Recent Advances in *Momordica charantia*: Functional Components and Biological Activities. *Int. J. Mol. Sci*. **18**: 2555.

Lalisan, J. A. Nuñeza, O. M. and Uyi, M. M. (2014). Brine Shrimp (*Artemia salina*) Bioassay of the medicinal plant *Pseudelephantopus spicatus* from Iligan City, Philippines. *Int Res J Biol Sci*. **3:**47-50.

Ngene, J. P. Ngoule, C. C. Yinyang, J. K. Pouka, M. C. Eyetemou, M. Etame, L. G. and Marie, M. (2022). Antiseptic Activity of *Alchornea cordifolia* (Schumach and Thonn.) Mull. -Arg. (Euphorbiaceae) Aqueous Leaves Extract on Oral and Dental Bacteria. *Saudi J Oral Dent Res*, **7**(7): 175-181.

Nnamdi, A. Ettebong, E. and Davis, K. (2017). Antiplasmodial and antioxidant activities of methanolic leaf extract and fractions of *Alchornea cordifolia*. *J Herb Med Pharmacol*. **6**:171-177.

Noundou, X. S. Krause, R.W. M. van Vuuren, S.F., Tantoh, N. D. and Olivier, D.K. (2016). Antibacterial effects of *Alchornea cordifolia* (Schumach. and Thonn.) Müll. Arg extracts and compounds on gastrointestinal, skin, respiratory and urinary tract pathogens. *Journal of Ethnopharmacology*. **179**: 76–82

Ogbonnia, S. O. Mbaka, G. O. Akinsande, O. E. Otah, D. A. and Ayeni TA. (2016). Evaluation of acute toxicity, hypoglycaemic and hypolipidaemic effects of *Cyathula prostate* (Linn.) Blume weeds on adult rats. *Br J Pharm Res***. 9**(5):1-11

Onyeka, I. P. Suleiman, M. M. and Bako, S. P. (2018). Toxicity Effects of Methanolic Extract of *Euphorbia hirta*-Honey Mixture in Albino Rats. *J Pharmacogn Nat Prod*. **4**:147.

Pertiwi, D. Hafiz, I. and Leny L. (2020). Potential bioactivities of ethanol, ethyl acetate and N-hexane extracts from pagoda leaves (*Clerodendrum paniculatum* L.). *Rasayan J Chem*. **13**(4): 2313-6.

Sharififar, F. Assadipour, A. Moshafi, M. H. Alishahi, F. and Mahmoudvand, H. (2017). Bioassay screening of the essential oil and various extracts of *Nigella sativa* L. Seeds using brine shrimp toxicity assay. *Herbal Med J*. **2** (1):26-31.

Shima, J. Nazanin, M. Younes, G. Samira, M. Sajjad, B. and Mohammad, AM. (2016). Evaluation and Comparison of the Toxicity of Some Iranian Native Plants and Microalgae, Using Brine Shrimp Test (BST). *Trends in Pharmaceutical Sciences*. **2(**4): 285-290.

Simorangkir, M. Nainggolan, B. Juwitaningsih, T. and Silaban, S. (2021). The toxicity of N-hexane, ethyl acetate and ethanol extracts of sarang banua (*Clerodendrum fragrans* Vent Willd) leaves by brine shrimp lethality test (BSLT) method. *J Phys Conf Ser*. **11**: 53.

William, A. Shamaki, B. U. Sadiq, A. A. and Abdullahi, A. (2015). Phytochemical and Elemental Constituents, Acute Toxicity (LD_{50}) Studies of Aqueous Leaf Extract of *Calotropis procera*. *World J Pharm*, **3**(4): 696-701.

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