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Oral Toxicity Assessment and *In vitro* Antimicrobial Profile of Methanolic Leaf Extract of *Alchornea Cordifolia* on Albino Rats

Komolafe Kafilat Adenike[1, 3, *], Adeoti Olatunde Micheal[1, 2, 4], Olaoye Opeyemi Joy[1], Olufemi Olutope Samson[1], Adedokun Elizabeth Olajumoke[1], Adedoja Sulaiman Aderogba[1], Adesina David Ademola[1], Abiola Adebiyi Oladepo[1]

[1]Department of Science Laboratory Technology, Microbiology Unit, the Oke-Ogun Polytechnic, Saki, Shaki, Nigeria

[2]Department of Pure and Applied Biology, Ladoke Akintola University of Technology, Ogbomosho, Nigeria

[3]Department of Zoology, Parasitology Unit, University of Ibadan, Ibadan, Nigeria

[4]Department of Zoology, Cellular Parasitology Unit, University of Ibadan, Ibadan, Nigeria

Email address:

txy23m@yahoo.com (K. K. Adenike)

[*]Corresponding author

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Abstract: The ameliorative tendency of the leaves of Alchornea cordifolia has been reported against ailments ranging from conjunctivitis to yaws and certain parasitic infections. This necessitated investigating the in vitro antibacterial efficacy of methanol-extracted leaves of Alchornea cordifolia on hematological and histopathological of organ of toxicity on albino rats. The rats were randomly segregated into four groups of five animals in each cage. The groups were orally administered with 250, 500 and 750 mg/kg body weight and 10% Tween 80 control for 28 days. Blood samples were collected for hematological analysis and organs (liver and spleen) for histopathological analysis. The data obtained were analyzed by ANOVA and Dunnett's test at P>0.05 levels of significance. Methanol leaf extract had a significantly higher inhibitory zone in E. coli and K. pneumonia ranging 35.00± 1.73 and 35.67± 3.48 at all the concentrations tested. There was no significant effect on hematological parameters. Liver necrosis was noticed in the harvested organs of the experimental rats. The liver sections of rat treated with 750mg/kg of the leaf extract showed cloudy swelling of hepatocytes and mild Kupffer cell hyperplasia. These results suggest that Alchornea cordifolia is non-toxic but has the propensity to induce hepatic injury at high doses. Conclusively, successful antibacterial activity at all concentrations and the slight pathological effects could be indicative low toxicity and high efficacy of this plant if taken at lower doses.

Keywords: *Alchornea Cordifolia*, Hyperplasia, Conjunctivitis, Antibacterial, Histopathological

1. Introduction

Alchornea cordifolia (Schumach and Thonn) (Euphorbiaceae) is an erect and bushy perennial shrub or small tree, up to 4 m high, reproducing from seeds. The stem is woody, greyish, with lightly granulated bark [1] with many branches and bushy when young. It is geographically distributed in secondary forest usually near water, moist or marshy places. The common names are Christmas bush and dovewood. *Alchornea cordifolia* occurs widely in Africa from Senegal to Kenya, Tanzania, and throughout Central Africa to Angola. It is cultivated in Democratic Republic of Congo for its medicinal use [2].

There are many convergences in its traditional use

throughout tropical Africa including medicinal and ethnobotanical values. The leaves, stem bark, stem pith, leafy stems, root bark, roots and fruits have been reported to be commonly used in local medicine [3]. The crude aqueous methanolic ethe leaves of *A. cordifolia* has been found to show anti-inflammatory activity [4]. It is also used for the treatment of wounds ulcers, gum inflammation and conjunctivitis [3]. Despite the progress in human medicine, infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health and particularly large in developing countries due to relative unavailability of medicines and emergence of widespread drug resistance [5]. From the point of its abundance and reported efficacy the present investigation was made to evaluate the antibacterial activities and toxicity examination of *Alchornea cordifolia* against three human bacteria.

2. Materials and Method

2.1. Plant Materials and Authentication

The Plant *Alchornea cordifolia* used for this research was collected from the Polytechnic Ibadan quarters, Ibadan, Nigeria. The plant was identified and authenticated by a plant taxonomist at Forest Research Institute of Nigeria (FRIN). Sample deposited in the herbarium was assigned voucher number 111248.

2.2. Extraction of Plant Materials

The healthy leaves of the plant were harvested and air-dried for 4 weeks to a constant weight in the laboratory. The dried samples was ground into powdered form using an electric grinder and stored in air tight bottle. Using maceration method, 350g of powdered sample was soaked in 1750 ml of methanol of analytical grade, for 72 hours. Sample solution was filtered using muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated using rotary evaporator at 45°C. The extract was weighed and stored in well stopper container and kept in a refrigerator at 4°C until used.

2.3. Phytochemical Screening

The phytochemical screening was performed qualitatively using standard methods [6]. The plant sample was screened for the following classes of compounds: phenols, tannins, saponins, flavonoids, alkaloids, steroids, terpenoids, phlobatannins, anthraquinones, coumarins, Glycoside, Emodin.

2.4. Test Samples

The bacterial isolates selected for this investigation were *Escherichia coli and Staphylococcus aureus*. These isolates were obtained from the Microbiology Laboratory in the Department of Microbiology, University College Hospital (UCH), Ibadan where they were kept as stock cultures. Each isolate was sub cultured in nutrient agar for 24 hours and subjected to simple but specific tests for confirmation before use for this study.

2.5. Experimental Animals

Forty albino rats (20 males: 20 females) weighing (120-130g) required for the experiment were procured from the Animal facility of the Anatomy Department, University of Ibadan. The animals were acclimatized for two weeks under 12 hours light/dark cycle at 28.0±1.0°C room temperature with available standard feed and water prior to commencement of experiment. Animals were handled according to standard protocols for the use of laboratory animals and approved protocol by Animal Care and Use in Research Committee (ACUREC), University of Ibadan.

2.6. Antibacterial Susceptibility Screening

Antibacterial activity of the leaf extract was determined using the Kirby-Bauer disc diffusion method [7]. Bacterial cell suspensions were prepared in fresh normal saline and the turbidity of the resulting suspensions was adjusted to 0.5 McFarland turbidity standards. 1 ml inoculums of each selected organism were spread by glass spread on nutrient agar media. The sterile discs (6 mm diameter) of Whatman's No. 1 filter paper were impregnated with 20 µl of the extract solutions to achieve desired concentrations of 50, 100 and 200mg/ml on disc and placed separately in the inoculated agar plates. Ciprofloxacin and Ampiclox (30µg/ml/disc) was used as positive control and disc impregnated with DMSO was used as negative control. Each experiment was carried out in 3 replicates. The antibacterial assay plates were incubated at 37°C f or 24hr and mean diameters of the inhibition zones were recorded.

2.7. Oral Toxicity Study

Animals were used for the oral sub-acute toxicity study carried out according to OECD guideline 407 [8]. Forty albino rats of both sexes with mean body weight (120 -130) g were randomly divided into four groups of ten rats (5 males: 5 females) each comprising three experimental (250, 500 and 750mg/kg) and one control group. Extracts were orally administered daily for 28 days. Body weight was weekly recorded, and the animals were daily observed for clinical signs of toxicity. Animals were euthanized with ketamine (0.02ml) on 29th day. Blood samples were collected by cardiac puncture for hematological and selected organs were harvested, weighed and stored in 10% formalin for pathological examination.

Collection of Blood Samples and Organ Harvesting

Blood samples were collected from the rats via the cardiac puncture into (EDTA) bottles for blood parameter analysis. The uncoagulated blood was analyzed immediately for the following packed cell volume (PCV), red blood cell count (RBC), white blood cell count (WBC), Haemoglobin level (Hb) as described by [9]. Neutrophils, eosinophils, platelets, lymphocytes, monocytes were determined using Automated Haematologic Coulter Analyser in accordance with the standard procedures [10], MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean

corpuscular hemoglobin concentration). Analyses were carried out in the Department of Veterinary Clinical Pathology, University of Ibadan. Organs (liver, kidney, spleen, ovary/ testis) were harvested post euthasia, blotted dry with clean filter paper, weighed and preserved in 10% formalin solution in properly labeled sample bottles for 24 hours. Sectioning and staining with hematoxylin and eosin for analysis followed methods of [11] and [12] with slight modification and later observed under a light microscope with objective lens 400x; scale: 0.500µm/pixel, and obj 100x; scale: 0.049µm/pixel.

2.8. Statistical Analysis

Statistical analyses were carried out using statistical package for social science (SPSS). The data was presented as Mean ± SEM (standard error of mean). The statistical differences between groups were analyzed using (ANOVA) followed by Dunnett Multiple range test, (GraphPad Prism software, USA). Value of ($P<0.05$) indicates statistically significant difference between compared data.

3. Results and Discussion

3.1. Phytochemical Screening

Table 1. *Qualitative Screening of the Methanol Leaf Extract of Alchornea cordifolia.*

PARAMETER	INFERENCE	REMARK
Phenol	+++	Present in high concentration
Tannin	++	Present in moderate concentration
Saponin	+	present
Flavonoid	+	present
Alkaloid	+	Present
Steroid	+	Present
Terpenoids	+	Present
Phlobatannins	-	Absent
Anthraquinone	+	Present
Coumarins	+	Present
Glycoside	-	Absent
Emodin	+	Present

3.2. Antibacterial Screening

The methanolic leaf extract from *Alchornea cordifolia* were tested against the bacteria strain of *E. coli* and *S. aureus.* The extract appeared more efficacious at all the concentrations tested with *E. coli* and *S. aureus* been more sensitive recording inhibition zone of 35.00 ±1.73 and 33.60± 2.73 at 50mg/ml respectively (Table 2).

Table 2. *Antibacterial Activity of the Methanol Leaf Extract of Alchornea cordifolia.*

Plant extract	Concentration (mg/ml)	*E. coli*	*S. aureus*
	50mg/ml	35.00±1.73	33.60±2.73
	100mg/ml	27.60±6.09	20.60±3.84
	200mg/ml	29.00±2.08	26.30±4.98
	Positive control Ciprofloxacin (30µg/ml)	25.00±1.00	19.00±1.79
	Ampiclox (30µg/ml)	25.80±2.80	6.30±1.79
	Negative control DMSO 5%	-	-

SEM: Standard Error Mean

3.3. Oral Toxicity

3.3.1. Body Weight of Animals

There was increase in the body weight of rats in all the treated and control group throughout the experimental period without any significant difference when compared to control groups (Figure 1).

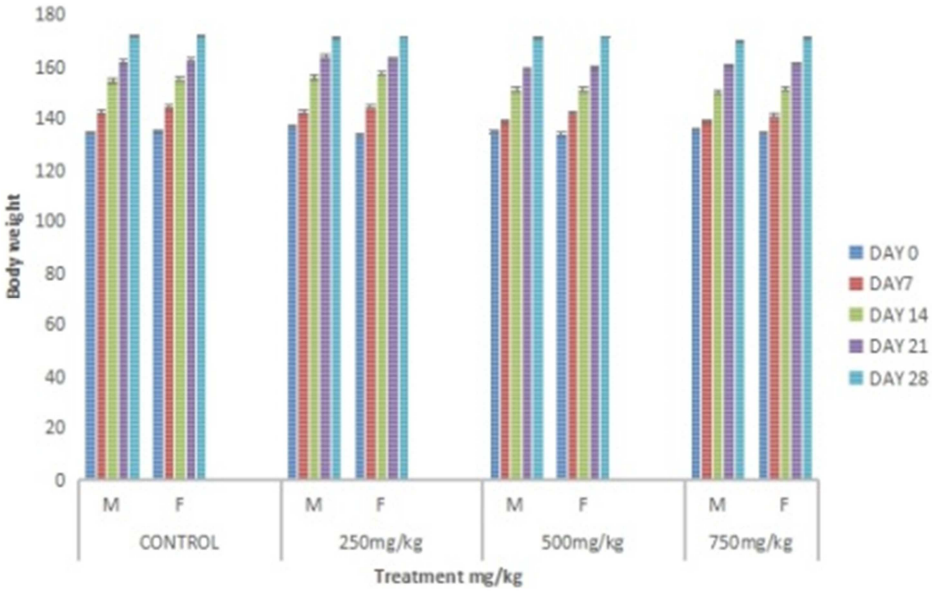

Figure 1. *Comparison effect of methanol leaf extract of Alchornea cordifolia on the body weights (g) of male and female albino rats treated for 28 days.*

3.3.2. Haematological Analyses on Animals Exposed to the Leaf Extract of Alchornea cordifolia

There is no significant effects (P>0.05) on the PCV, Hb, RBC, WBC of both the male and female rats treated with the methanol leaf extract of *Alchornea cordifolia* (AC). Slight decrease in was recorded in the PCV level of female rats administered AC500mg/kg (38.00±1.00) and 750mg/kg (38.00±0.00) compared to the control (41.50±2.50). There was no significant differences (P<0.05) in MCV, MCH and MCHC indices of both the male and female rats in the treated groups compared to the control. No significant differences (P>0.05) were recorded in the WBC count and platelets, Lymphocytes, Neutrophils, Eosinophils and Monocytes of both the male and female rats in all treated groups compared to control groups (Table 3).

The sections of the liver from female and male control groups, showed a few foci of thinning of hepatic cords with dilation of hepatic sinusoids (Figure 2a). The liver section of rats given 250 and 500mg/kg of the extract showed closely packed hepatic plates and mild Kupffer cell hyperplasia (KCH) in the male (Figure 2b). In rats given 750mg/kg of the extract revealed closely packed hepatic plates, random foci of single-cell hepatocellular necrosis and moderate KCH (Figure 2c).

There were no histomorphological changes in spleen of female and male rats in the control groups (Figure 2d). There were moderate large peri-arteriolar lymphoid sheaths with a few germinal centres and a few aggregates of dark brown pigments (haemosiderosis) were observed in both male and female rats treated with 250mg/kg, 500mg/kg and 750mg/kg of *Alchornea cordifolia* (Figure 2e).

3.3.3. Histopathological Examination on Exposed Rats

Table 3. *Effect of methanol leaf extracts of Alchornea cordifolia on the haematological parameters on whole blood of rats.*

Parameters	Control		Doses (mg/kg)					
			250mg/kg		500mg/kg		750mg/kg	
	Male	Female	Male	Female	Male	Female	Male	Female
PCV (%)	38.00±0.00	41.50±2.50	44.50±1.50	40.00±2.00	43.00±1.00	38.00±1.00	40.50±2.50	38.00±0.00
Hb (g/dl)	12.35±0.05	13.70±1.00	14.95±0.45	13.40±0.40	14.00±0.00	12.75±0.45	12.10±0.70	12.55±0.05
RBC (10^6/µl)	6.30±0.07	6.84±0.43	7.33±0.08	6.69±0.47	7.30±0.19	6.31±0.11	6.81±0.51	6.34±0.03
MCV (f l)	60.47±0.53	60.72±0.12	60.69±1.38	59.88±1.22	58.95±0.12	60.21±0.54	59.53±0.79	59.99±0.24
MCH (pg)	19.40±0.14	20.13±0.22	20.39±0.39	20.09±0.81	19.24±0.49	20.20±0.36	17.95±2.37	19.81±0.16
MCHC (g/dl)	32.50±0.13	32.99±0.42	33.60±0.12	33.53±0.68	32.58±0.76	33.54±0.30	30.10±3.59	33.03±0.13
WBC (10^3/µl)	2875±975	24400±1200	1850±450	2375±1125	3850±1450	4050±750	5200±1200	3575±275
Platelets (10^3/µl)	123000±33000	81000±21000	12450±26500	120500±8500	72000±26000	107000±77000	85500±19500	63500±19500
Lymphocytes (%)	67.00±1.00	70.50±2.50	72.00±0.00	69.00±2.00	70.50±0.50	67.50±1.50	66.00±4.00	65.00±0.00
Neutrophils (%)	30.00±1.00	26.50±3.50	25.00±1.00	28.00±1.00	25.50±0.50	28.00±2.00	30.50±4.50	32.50±0.50
Monocytes (%)	1.50±0.50	1.00±0.00	2.50±0.50	2.50±0.50	1.50±0.50	1.50±0.50	1.50±0.50	1.50±0.50
Eosinophils (%)	1.50±0.50	2.00±1.00	0.50±0.50	0.50±0.50	2.00±1.00	3.00±0.00	2.00±1.00	1.50±0.50

Data are mean ± SEM (n= 5). One-way ANOVA followed by Bonferroni's multiple comparison test: Compare all pairs of column at (p>0.05). (p>0.05) are not significantly different from the control. RBC: red blood cell; WBC: white blood cell, MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration.

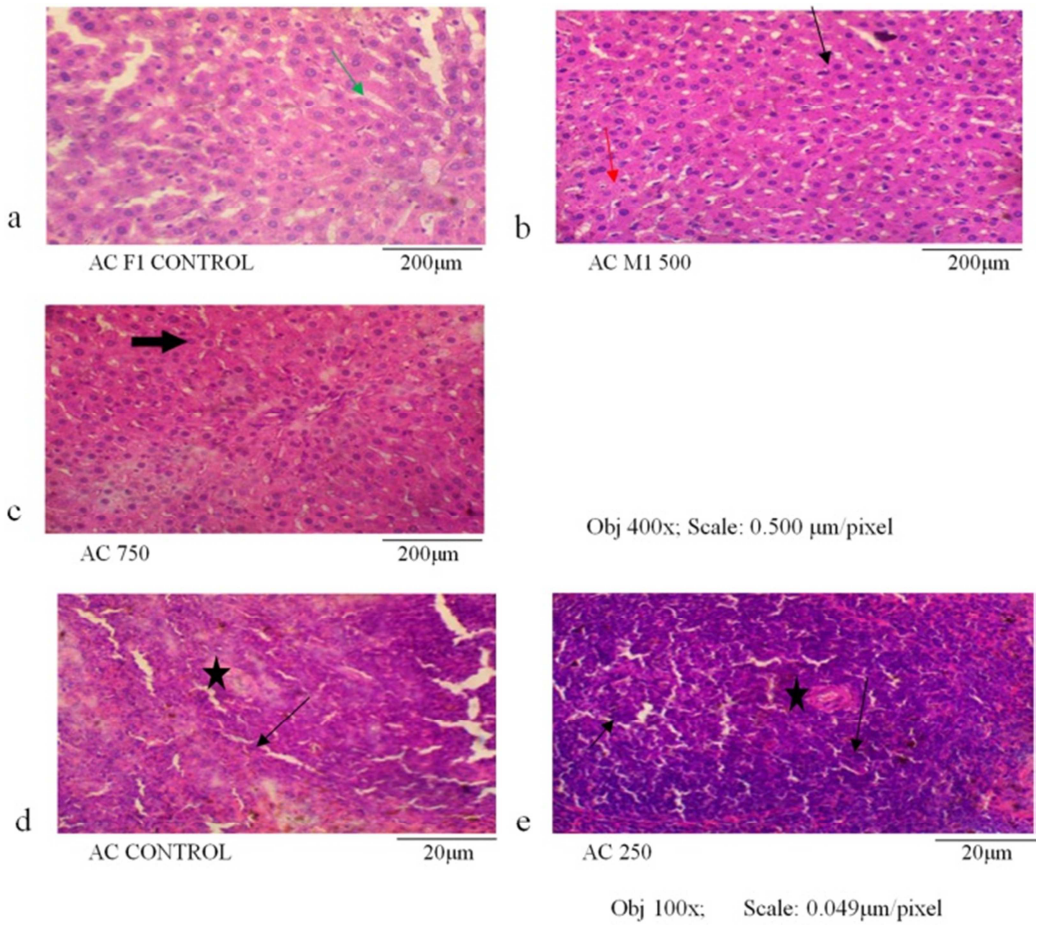

Figure 2. *Photomicrograph showing Histopathological Examination on exposed rats.*

a. Photomicrograph of a female and male rat liver showing a few foci of thinning of hepatic cords with dilation of hepatic sinusoids (green arrows)
b. Photomicrograph of a rat liver showing closely- packed hepatic plates. There is mild Kupffer cell hyperplasia (red arrows).
c. Photomicrograph of a rat liver showing a few megalocytes (hepatocytes with large nuclei) (thick arrow).
d. Photomicrograph of a rat spleen showing no histomorphological changes
e. Photomicrograph of a rat spleen showing large peri-arteriolar lymphoid sheaths (arrow) with a few germinal centres (star) with a few aggregates of dark brown pigments (black arrows).

4. Discussion

4.1. Phytochemical Screening and Antibacterial Activities

The leaf extract of *Alchornea cordifolia* at tested doses showed antibacterial efficacy against all bacteria tested. *E. coli* showed the largest zones of inhibition at lowest concentration of 50mg/ml. The sensitivity of *E. coli* to methanol leaf extract of *Alchornea cordifolia* is of great interest as it could be used to curb incidence of food poisoning [13]. The antibacterial activities of *Alchornea cordifolia* appeared to be broad spectrum since it was effective against both gram-positive and gram-negative as these could be ascribed to the bioactive constituents present in the leaf extract which exhibit antibacterial activity. The phytochemical components found in the leaves included terpenoids, steroid, glycosides, flavonoids, tannins, saponins, alkaloid, and several constituents was similar to the work of [14, 15]; which could be responsible for its efficacy as antimicrobial [15].

4.2. Oral Toxicity Studies

The observed increase in body weight after the 28-days treatment was normal especially when the animals were well-fed ad libitum [16]. The animals were daily observed for clinical signs of toxicity and non of the animal showed toxicity signs and no mortality rate was recorded. On 29th day, animals were humanely anaesthesised and blood and some organ of toxicity were collected for blood parameters, biochemical and organ pathology analyses.

4.3. Haematology

The haematopoetic system is an important index of physiological and pathological status in man and animals [17] and a sensitive target for toxic compounds [18]. This has a predictive value for toxicity in humans and animals and therefore analysis of blood is relevant to risk evaluation [19].

The hematological analysis showed that *Alchornea cordifolia* has little or no effect on White Blood Cell, Lymphocytes, Neutrophils, Red Blood Cells, Haemoglobin Concentration, Pack

Cell Volume, and platelets. However, the slight decrease in WBC of female rats administered 250mg/kg may be caused by viral infections that temporarily disrupt the work of bone marrow. A dose dependent decrease in neutrophils (NEU) was observed. Neutrophils interact with foreign compounds and microorganisms and destroy them by the process of phagocytosis. Other parameters were not affected in treated animals. *Alchornea cordifolia* has been shown to contain flavonoids [20]. Some of these flavonoids have been demonstrated to inhibit nephrotoxicity because of its strong antioxidant activity [21]. *Alchornea cordifolia* has also been reported to contain tannins and tannins are known to offer protection against nephrotoxicity [22]. It is possible that these constituents offered protection to the treated animals in the present study.

4.4. Histopathological Analysis

Alchornea cordifolia could have evoked mild liver damage rather at high dose of 750 mg/kg in the present work. This may be intriguing, because of the reported several components in the extract with different pharmacological actions. The proportion of specific toxicants could also be increased at high doses. There were no histomorphological changes noticed in spleen based on the widely recommended dosage of dried *Alchornea cordifolia* leaves (maximum 50 g per litre of water; 3 cups daily) in traditional medicine. Similar recommendation was made in the study of [23] that 50 g of the plant product per litre of water; and those 4 cups could be taken daily. Therefore, a daily intake of 200 mg/kg of the extract could be recommended as the maximum threshold in humans. Though this appeared to be lower than the doses tested in this present study, and the high doses could be resulted in the sign of liver damage observed in the rat. Therefore, further studies are call for on the standard doses that can be recommended.

5. Conclusion

Methanolic extract of the leaves of *Alchornea cordifolia* contain bioactive ingredients and demonstrated high potency against the tested organisms. It could be exploited for use as an antibacterial drug for the treatment of infections caused by enteric bacteria. Although, methanolic leaf extract of *Alchornea cordifolia* was non-toxic to hematological indices; these findings could not be directly extrapolated to man in view of possible species differences and differences in metabolic activation. However, its usage should be taken with caution especially at high doses and over a prolong usage of plant.

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