



Phytochemistry and Toxicity of Methanol Root Extract of *Costus lucanusianus*

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Authors' contributions

This work was collaboration between both authors. Author HDK designed the study and performed the statistical analysis. Author LAG managed the literature searches and wrote the first draft of the manuscript. While author HDK proof-read the manuscript to make corrections. Both authors read and approved the final manuscript.

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ABSTRACT

Costus lucanusianus (ginger lily or monkey sugarcane) is a medicinal plant commonly used to treat various ailments in tropical Africa. The leaves, stem and sometimes the root have been exploited for this purpose. However, it is a common belief in the Niger Delta of Nigeria that the root is poisonous. This study investigated the phytochemical and acute toxicity profiles of the methanol root extract of the plant. Phytochemical screening was conducted using standard procedures to test for alkaloids, flavonoids, saponins, tannins, anthraquinones, glycosides, triterpenoids/steroids and carbohydrates. The median lethal dose (LD₅₀) was determined using the Arithmetic method of Reed and Muench. This was followed by haematologic, liver and kidney functionality assays at doses of 14, 29 and 58

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mg/kg of the methanol root extract. The result obtained showed that the extract contained flavonoids, saponins, triterpenoids and steroids among others, but anthraquinones and alkaloids were not present. The median lethal dose (LD₅₀) value obtained for the extract was 288 mg/kg. The haematologic assay showed significant, $p < 0.001$, dose-dependent decrease in red blood cell parameters and also some white blood cell parameters (such as white blood cell, neutrophil, monocytes and eosinophil counts). Furthermore, the serum levels of the liver enzymes, electrolytes (except K⁺), urea and creatinine increased significantly as the doses increased. From the foregoing, it could be concluded that the root of *Costus lucanusianus* is toxic to the kidney and liver and could be a blood poison (hemotoxic). This confirms the ethnobotanical belief that this part of the plant (root) is poisonous.

Keywords: *Costus lucanusianus*; phytochemical screening; LD₅₀ determination; arithmetic method of reed and muench.

1. INTRODUCTION

Medicinal plants are a rich source of medically important compounds and since ancient time plant and plant derived products have been used as medicine in traditional and folk medical systems [1]. *Costus lucanusianus* J.Braun (Costaceae), commonly called “ginger lily” or “monkey sugarcane” is a stout, perennial and rhizomatous herb found in the forest belt from Guinea to Ethiopia, Niger, Senegal and Nigeria [2,3].

It is commonly used as a medicinal plant in tropical Africa. The parts of the plant mainly used for medicinal purposes are the leaves and stems which are commonly used to treat diarrhea, pyrexia, pain, inflammatory conditions and dysmenorrhea [4]. The stem extract is used in Ivory Coast for the treatment of threatened abortion because it is known to exhibit uterine relaxant activity [5]. However previous studies carried out on the leaves of *C. lucanusianus* showed that the aqueous leaf extract had oxytocic effect on isolated pregnant rat uterus [6]. Furthermore, the aqueous leaf extract showed anti-inflammatory and anti-nociceptive properties [7]. The root decoction has been reported to be used to treat epilepsy and mental disorders [8].

The folklorish belief by communities living in the Niger Delta area of Nigeria who consider the root of *C. lucanusianus* to be a poison and the dearth of relevant toxicological literature on this part of the medicinal plant informed the current study.

2. MATERIALS AND METHODS

2.1 Plant Collection and Identification

Fresh roots of *Costus lucanusianus* were obtained from Oyigbo Community in the

Neighbour hood of Afam Power Station, Rivers State, Nigeria. The root samples were identified and deposited in the Department of Plant Science and Biotechnology Herbarium, University of Port Harcourt, Nigeria with a voucher number, UPH/V/12120.

2.2 Plant Extraction Procedures

The roots of *C. lucanusianus* were washed immediately after uprooting and chopped into smaller bits and kept in a well-ventilated room and allowed to dry under room temperature for three weeks. The dried roots were reduced to a fine powder with a mechanical grinder, sieved, and the fine powder stored in a polyethylene bag.

The pulverized substance was macerated with 70% methanol for 72 hours and stirred intermittently. Thereafter it was filtered with a Millipore and the filtrate evaporated to dryness using rotary evaporator. It was the dry powder that was used for further analysis.

2.3 Animal Handling

Thirty albino rats of including both male and female weighing between 200 g-300 g were used for this study. The animals were allowed to acclimatize to laboratory conditions (7 days) and maintained under standard environmental conditions. The animals were provided with standard rodent pellet diet (finisher feed) and water *ad libitum*. Food was withdrawn 18 h before the experiment.

2.4 Phytochemical Screening

Phytochemical screening was carried out on the methanolic extract of the roots of *Costus lucanusianus* according to the method described by Sofowora [9,10] for testing phytochemical compounds.

Test for tannins: About 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric-chloride was added and observed for brownish green or a blue-black colouration for a positive test.

Test for phlobatannins: Deposition of a red precipitate when an aqueous extract of the plant sample was boiled with 1% aqueous hydrochloric acid was to be taken as evidence for the presence of phlobatannins.

Test for saponin: About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

Test for flavonoids: A few pieces of magnesium metal is added to 5 ml of the extract and 2 M concentrated Hydrochloric acid. A reddish colouration would indicate a positive test for flavonoids.

Test for terpenes: 3 ml of chloroform was added to 0.5 g of extract, shaken and filtered; 10 drops of acetic anhydride followed by 2 drops of Conc. sulphuric acid. A reddish brown colouration at the interface was looked out for, to show a positive result for the presence of terpenes.

2.5 Test for Cardiac Glycosides

Lieberman's Test: 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. The mixture was underlaid with 2 ml of concentrated sulphuric acid. A reddish brown coloured ring at the zone of contact with the supernatant and the colour changing from blue to green will indicate the presence of triterpenes and sterols (steroidal rings characterize the aglycones of cardiac glycosides).

Keller-Killani Test: 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

Salkowski Test: 0.5 g of the plant extract was dissolved in 2 ml of chloroform. 2 ml of concentrated sulphuric acid was carefully added to form a lower layer. A reddish brown ring observed at the interphase of the two liquids will indicate the presence of a steroidal ring which is characteristic of cardiac glycosides.

2.6 Test for Anthraquinones

2.6.1 Free hydroxy anthraquinones

The Bontrager's test for anthraquinones was used. 5 g of the extract was shaken with 10 ml of benzene and filtered. 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the lower (ammoniacal) phase was observed. A violet coloured lower phase will indicate the presence of free hydroxyl anthraquinones.

2.6.2 Combined Anthraquinones

5 mg of plant extract was boiled with 10 ml aqueous sulphuric acid and filtered while hot. The filtrate was shaken with 5 ml of benzene, the benzene layer separated and half its own volume of 10% ammonia solution added.

2.7 LD₅₀ Determination

2.7.1 Animals and experimental design

Preliminary test, with four rats per dose level, was conducted to ascertain the range of toxicity so that the proper dose levels could be established. With the pilot tests it was possible to establish the maximum dose of the extract that will kill none of the exposed animals and the minimum dose that kill all the animals. The doses used in this acute toxicity study ranged between two extremes.

Thirty rats weighing between 200-300 g were kept in 6 cages (5 per cage) and handled according to standard guidelines for the use and care of laboratory animals [11]. However food was withdrawn 18 h before the start of the experiment according to the method of Amresh et al. [12]. In between the two extreme doses, six different doses were administered intraperitoneally (i.p.). The extract was dissolved with 20% tween 80. Group A received 100mg/kg of the extract, Group B, C, D, E, F received 200, 250, 300, 350 and 400 mg/kg of the extract respectively via intra peritoneal administration.

General symptoms of toxicity and mortality were observed over 24 hour period. The median lethal dose was determined using Arithmetic method of Reed and Muench [13].

2.7.2 Sub-acute toxicity

Twenty (20) wistar rats of both sexes were randomly divided into four groups of five animals each. Group 1, the control group, was given 20% tween 80 (the vehicle) while animals in groups 2, 3 and 4 were administered with 58, 29 and 14 mg/kg (i.p.) methanol root extract of *C. lucanusianus* respectively, daily for 14 days. Food and water were given *ad libitum* during the experiment.

Effect of extract on hematological parameters

Animals were sacrificed at the end of 14 days of treatment with the extract, and blood was obtained by cardiac puncture. The collected blood samples were used for the estimation of haematological parameters such as packed cell volume (haematocrit), haemoglobin concentration, erythrocyte and leucocyte counts and differential cell count as described by Cheesbrough [14]. The erythrocyte and leucocyte counts were determined using the improved Neubauer haemocytometer method.

The haemoglobin (Hb) concentration and packed cell volume were determined using the cyanomethaemoglobin and techniques respectively.

Effect of extract on biochemical parameters

Blood samples obtained by cardiac puncture were collected into bottles for determining plasma activity of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline.

Phosphatase (ALP) and levels of creatinine, urea, sodium, potassium and chloride. The blood was centrifuged at 4000rpm for 10 mins after which the plasma was separated from the coagulated cells and tipped into a separate vial. The vial was placed in micro centrifuge tubes, capped and stored at -20°C until analysis.

The plasma biochemistry determinations were done using commercial test kits. The activities of ALT and AST were measured according to the method described by Reitman and Frankel [15] using diagnostic kits from Randox Laboratories, Northern Ireland.

The serum ALP activity was determined by the thymolphthalein monophosphate method according to Roy [16] using Teco Diagnostics kits. Plasma urea and creatinine was determined by the Urease-Berthelot method described by Fawcett and Scott [17] and the modified Jaffe method [18] respectively, using diagnostic kits from Randox Laboratories, Northern Ireland. The plasma potassium (K⁺), sodium (Na⁺) and chloride (Cl⁻) were determined by colorimetric method [19].

2.8 Statistical Analysis

Data was analyzed by one-way ANOVA followed by Dunnett's post-test using Graph pad prism 3.0 Software. P values less than 0.05 was considered statistically significant.

3. RESULTS

3.1 Phytochemical Analysis

Result from phytochemical screening showed that alkaloids and anthraquinones were absent while saponins, Tannins, flavonoids, triterpenoids/ steroids among others, were present in the extract.

3.2 LD₅₀ Determination

The result of LD₅₀ determination according to Arithmetic Method of Reed and Muench is presented in Table 2.

From the Table it is seen that 50% mortality lies between 250 mg/kg and 300 mg/kg.

Proportionate distance of the 50% point =
(50% - Next lowest % / Next highest % - Next lowest %)

$$\frac{50 - 30}{55.5 - 30} = \frac{20}{25.5}$$

$$= 0.78$$

The logarithm of the dose increment is computed as follows;

$$250 \text{ mg/kg} = 2.398$$

$$300 \text{ mg/kg} = 2.477$$

$$= 0.079$$

Then

$$0.079 \text{ multiply } 0.78 \\ = 0.0616$$

$$\text{Log LD}_{50} = 2.3979 + 0.06162 \\ = 2.4595$$

$$\text{LD}_{50} = \text{Antilog } 2.4595 \\ = 288$$

$$\text{LD}_{50} = 288 \text{ mg/kg}$$

Effect of extract on Hematological parameters

The extract showed significant ($p=0.5 - p<0.001$), dose-dependent decrease in all the parameters measured, when compared to control except basophils.

Effect of extract on Biochemical parameters:

Serum levels of liver enzymes significantly increased with increase in dose, Table 4.

4. DISCUSSION

The result of this study showed that the root of *Costus lucanusianus* contained flavonoids, saponins, triterpenoids and steroids among others, butanthraquinones and alkaloids are absent.

Furthermore, the LD_{50} value obtained for the extract was 288 mg/kg. This value is categorized by the rating of [20] Gosselin et al.,

as "very toxic". Furthermore, the effect of the extract on the Red blood cell parameters (PCV, Hb, RBC) showed a significant ($P < 0.001$) dose dependent decrease in value relative to control, whereas there was a significant ($P < 0.001$ and $P < 0.05$), dose dependent increase in some white blood cell values (WBC, neutrophils, monocytes and eosinophils) relative to control but there was no significant difference in basophil and lymphocyte counts. Physical observation of the animals showed that they were sluggish, ataxic and had weight decrease during the period of the study. From the foregoing, the study has demonstrated that the extract dose dependently made the animals anaemic and could therefore be classed as haemotoxic.

The functionality of the liver is often evaluated by measuring the plasma levels of liver enzymes, alkaline phosphatase (ALP), aspartate transaminase (AST) and alanine transaminase (ALT) [21,22] and other biochemicals produced by the liver including albumin. Damage to the hepatocytes leads to the release of these liver enzymes into circulation thereby increasing their plasma levels [23,24]. ALP is an enzyme in the cell lining of the biliary ducts of the liver. Its plasma level increases when liver bile duct obstruction is present or there is intrahepatic cholestasis or infiltrative diseases [25,24].

Table 1. Phytochemical profile of *Costus lucanusianus* root

Phytochemical	Test	Inference
Saponins	Frothing test	+ve
	Emulsion test	+ve
Tannins	FeCl ₃ test	+ve
	Phlobatannins	+ve
	Gelatin test	-ve
	Albumin test	-ve
	With NaOH	+ve
Anthraquinone	Bontragers test:	
	Free Anthraquinone	-ve
	Combined anthraquinone	-ve
Alkaloid	Drangedorff's test	-ve
	Mayer's test	-ve
	Hager's test	-ve
Triterpenoids/ Steroids	Liebermann-Buchard test	+ve
	Salvoski test	+ve
	Fixed oils	+ve
	Keller killani test	+ve
Cardiac Glycosides	Kedde test	+ve
	Shinoda test	+ve
Flavonoids	Lead acetate test	+ve
	Molisch test	+ve
	Fehlings test	+ve
Carbohydrates	Molisch test	+ve
	Fehlings test	+ve

Note: +ve=positive; -ve = negative

Table 2. Arithmetic method of reed and Muench

Group	Dose(mg/kg)	Log dose	Death	Survival	Cumulative			Mortality	%Mortality
					Death	Survival	Total		
A	100	2.000	0	5	0	16	16	0/16	0
B	200	2.301	1	4	1	11	12	1/12	8.3
C	250	2.398	2	3	3	7	10	3/10	30
D	300	2.477	2	3	5	4	9	5/9	55.5
E	350	2.544	4	1	9	1	10	9/10	90
F	400	2.602	5	0	14	0	14	14/14	100

Table 3. Assessment extract on haematological parameters

Parameters	Control	14 mg/kg	29 mg/kg	58 mg/kg
PCV(%)	30.75 ± 2.29	21.23± 0.96 ^{***}	18.00 ± 0.53 ^{***}	14.10 ± 0.24 ^{***}
RBC($10^{12/l}$)	5.68 ± 0.53	2.06 ± 0.32 ^{***}	2.00 ± 0.20 ^{***}	1.02 ± 0.10 ^{***}
Hb(g/dl)	10.81± 0.63	6.88± 0.54 ^{***}	5.04 ± 0.20 ^{***}	4.12 ± 0.10 ^{***}
WBC($\times 10^9/l$)	5121.00±65.00	5631.00±99.50 ^{***}	5121.7±83.19 ^{***}	3403.00±75.00 [*]
Neutrophils(%)	68.75 ± 0.90	80.67±4.01 ^{***}	79.00 ± 2.06 ^{***}	78.22± 1.12 ^{***}
Basophils(%)	1.25 ± 0.48	2.67 ± 1.20 ^{ns}	0.75 ± 0.75 ^{ns}	0.50 ± 0.00 ^{ns}
Monocytes(%)	6.25 ± 1.75	14.33 ± 1.33 ^{***}	13.33 ± 1.33 ^{***}	12.75 ± 0.18 [*]
Lymphocytes(%)	12.75 ± 3.33	15.33± 3.84 ^{ns}	14.30 ± 1.45 ^{ns}	13.00±1.20 ^{ns}
Eosinophils (%)	2.25±0.63	9.60±2.53 [*]	8.70 ± 1.16 [*]	6.50± 1.00 [*]

Significance relative to control: ^{ns}= Not Significant, ^{*}P=0.05, ^{***}P<0.001

Table 4. Effect of extract on liver enzymes

Dose mg/kg	AST(IU/l)	ALT (IU/l)	ALP (IU/l)
Control	40.00±1.83	41.75±1.55	131.00±3.19
Extract	14	43.00±0.75 [*]	145.00±3.42 ^{ns}
	29	46.67±0.88 ^{***}	159.30±1.76 ^{***}
	58	47.33±1.02 ^{***}	175.70±5.78 ^{***}

Values are expressed as Mean±SEM; (n=4); Significance relative to control: ^{ns}= Not Significant, ^{*}P=0.05, ^{***}P<0.001

Table 5. Effect of extract on electrolytes and other toxicity biomarkers

Dose mg/kg	Cl ⁻ (mmol/l)	Na ⁺ (mmol/l)	K ⁺ (mmol/l)	Urea (mmol/l)	Creatinine (mmol/l)	
Control	100.00 ± 0.91	137.00± 1.29	4.33±0.13	2.48±0.17	52.00±1.83	
Extract	14	106.00±1.20 [*]	143.00± 0.36 [*]	4.63± 0.13 ^{ns}	3.93±0.30 [*]	59.10±0.03 ^{ns}
	29	107.10±1.58 [*]	146.30±1.22 ^{***}	4.87±0.18 ^{ns}	4.07±0.40 [*]	60.00±2.40 [*]
	58	108.33±2.33 ^{***}	148.30±2.60 ^{***}	5.00±0.38 ^{ns}	5.53±0.55 ^{***}	61.00±2.66 [*]

Values are expressed as Mean±SEM; (n=4); Significance relative to control: ^{ns}= Not Significant, ^{*}P=0.05, ^{***}P<0.001

The result obtained from the current study showed that the liver enzymes (AST and ALT) had significant ($P < 0.001$) increase in values relative to control, this may suggest the hepatotoxic property of the extract. *Costus afer* (which is of the same family Costaceae with *Costus lucanusianus*) have been shown increase in the levels of AST, ALT and ALP indicative of liver damage [26].

The kidneys play an important role within the body and have three main functions- excretion of

waste, maintenance of extracellular fluid and hormone synthesis. When the kidneys are not functioning efficiently, waste products and fluids begin to accumulate instead of being excreted which can cause serious health problems. Elevation of kidney electrolytes indicates poor renal function and in the case of potassium both elevated and depleted levels have a negative impact [27]. The result of this study showed that electrolytes (Na⁺, Cl⁻, urea and creatinine) showed significant increase in values relative to control, however, there was no significant

difference in K⁺ levels in all doses administered relative to control. Elevation of these parameters indicated renal impairment caused by the administered doses of the extract.

5. CONCLUSIONS

From the foregoing, it could be concluded that the root of *Costus lucanusianus* is toxic to the kidney and liver and could be a blood poison or hemotoxic. This confirms the ethnobotanical belief that this parts of the plant (root) is poisonous.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The study protocols were duly approved by the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt. The rats for the study were humanely handled in accordance with the Ethics and Regulation guiding the use of research animals as approved by the University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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