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Anticancer Potential of the Leaf Extracts from the Selected *Croton* Species

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Abstract

The reported investigations were aimed at establishing anticancer potential of the leaf extracts from *Croton dictyophlebodes* (CDE), *C. kilwae* (CKE) and *C. longipedicellatus* ssp *austrotanzanicus* (CLE). *In vitro* cytotoxicity assays of the extracts were performed against human laryngeal cancer cell line (HEp-2) and human breast cancer cell line (MCF-7), while in vivo toxicological studies were done using Swiss albino mice (*Mus musculus*) following the Organization for Economic Cooperation and Development guideline number 423 of 2001. Results for *in vitro* assays revealed that CDE and CLE induced cytotoxicity to MCF-7 and HEp-2 cell lines at EC_{50} values of 86 and 63 µg/mL, respectively, while CKE was inactive to HEp-2 cells at 100 µg/mL. For *in vivo* studies, mice were given calculated doses based on globally harmonized standards and monitored for 14 days to observe signs of toxicity. Haematological analysis was also carried out to obtain information on changes in blood conditions. The findings showed that LD_{50} for CKE and CDE were > 2000 mg/kg, while for CLE was 175 mg/kg body weight (b.w.). The oral administration of CDE and CKE did not reveal significant changes in haematological parameters of treated mice, while CLE provoked some. Thus, the studied extracts may be considered potential cancer remedies with safety precautions that warrant further investigations.

Keywords: *Croton* species, Phytochemicals, Toxicity, Cancer remedy.

Introduction

Cancer is a genomic disease characterized by uncontrolled growth of abnormal cells due to dysregulated gene structure and/or functions (Cullen and Breen 2017). It is the second leading global health burden for more than two decades after cardiovascular diseases causing high morbidity and mortality to people (Desai et al. 2008). Statistics show that cancer cases by both incidences and deaths in Tanzania since 1970s to date are on the increase (MoHCDEC 2017). Current data indicate new cancer cases have reached 40,464 and 26,946 and new deaths were reported for Tanzania in the late 2020 (IARC 2020). Despite utilization of chemotherapy, radiotherapy, brachytherapy, surgery, immunotherapy and gene therapy as treatment options for different types of cancer, the disease has remained the second major global health threat for more than two decades after cardiovascular diseases due to a number of challenges (WHO 2022, Chhikara and Parang 2023). These challenges include the difficulty in early identification of neoplasms through diagnosis and screening

whereby, most cancers are identified at late or advanced stages (WHO 2022). Other challenges are the lack of community readiness in participating in the national volunteering programs aimed at carrying out public cancer diagnoses (Chhikara and Parang 2023), the adverse side effects posed to patients by almost all conventional cancer therapies (Greenwell and Rahman 2015) and the exposure to potent environmental carcinogens from either laboratory or industrial settings (WHO 2022).

Apart from conventional therapeutic approaches used in cancer management, countless number of plants offer phytochemicals that are known to possess safe pharmacodynamics and efficient in treating various human diseases including cancer (Salatino et al. 2007, Asare et al. 2011, Greenwell and Rahman 2015, Jain et al. 2016). It is known that nearly 60% of cancer chemotherapeutic drugs are derived from medicinal plants (Solowey et al. 2014, Cragg and Pezuto 2016). Thus, many species of the genus *Croton* (Euphorbiaceae) including *C. urucurana, C. macrobotrys, C. caracasana, C. sphaerogynus* and *C. tiglium* are ethnomedicinally used against cancer due to possession of pharmacologically active phytochemicals including terpenoids, volatile oils, alkaloids and polyphenols (Salatino et al. 2007, Suarez et al. 2009, Santos et al. 2015, Jain et al. 2016, Njoya et al. 2018). Tanzania is also endowed with a number of plants exhibiting medicinal potential including *Croton* species reported in this study namely; *C. dictyophlebodes, C. kilwae* and *C. longipedicellatus* ssp *austrotanzanicus*. Thus, *C. dictyophlebodes* is used in ethnomedicine to treat intestinal worms, colds and coughs (Munissi et al. 2020). The diterpenoids isolated therefrom were inactive against NCI 60 tumor cell line at a single dose of 10^{-5} M (Munissi et al. 2020). There is no information available on ethnomedicinal uses of *C. kilwae* however; constituents therefrom have shown anticancer activities against human breast cancer (MCF-7), human laryngeal cancer (HEp-2) and human cervical cancer (HeLa) cells (Nyamhagata 2021, Mahambo et al. 2023). *C. longipedicellatus* ssp *austrotanzanicus* is similarly not reported to be used in ethnomedince, however, antiviral diterpenoids have recently been characterized from the root and stem barks of this species (Mahambo 2023). There is no any documented *in vivo* toxicological study for the three *Croton* species reported in this paper. Therefore, due to the alarming statistics on cancer and potential of plants in cancer treatment, the present study aimed at evaluating the leaf extracts of selected *Croton* species for anticancer properties along with *in vivo* toxicological profiles.

Materials and Methods

Chemicals and reagents

Chloroform (Thomas Baker Pvt Ltd, Mumbai, India) was used for anesthetization of mice, dichloromethane (Loba Chemie Pvt Mumbai, India) and methanol (Loba Chemie Pvt Mumbai, India) for extraction. Dulbecco's Modified Eagles Medium (DMEM) (Fisher Scientific, UK) was used as a culture medium for cancer cells, alamarBlue and CellTiter 96 AQeous Solution (Promega, Madison, WI) were used as cell viability reagents, Heparin (Aculife Healthcare PVT Ltd, India) as an anticoagulant for blood. Dimethyl sulfoxide (DMSO, Fisher Scientific, UK) was used as a drug vehicle in control panels during cytotoxicity assays and Tween 80 (Inqaba Biotec East Africa, Tanzania Branch) as a drug vehicle for mice in control group and ethanol (Scharlab SL, Spain) as a solvent for plant materials extraction.

Plant materials collection

The leaves of *Croton dictyophlebodes* were collected on 14th September, 2021 from Mazumbai forest reserve in Lushoto District, Tanga Region in Tanzania at an altitude of 1522 meters and GPS Location of S 04°49.39 and E 038°30.21.70. The leaves of *C. kilwae* were collected on 28th November, 2018 from areas along the road from the junction of B2 main road to Rushungi village in Kilwa District, Lindi Region in Tanzania at an altitude of 103 meters and GPS Location of S 09°27.55.1 and E 039°36.17.3. The leaves of

C. longipedicellatus ssp *austrotanzanicus* were collected on 29th November, 2018 from areas around Lake Lutamba and Litipo River in Lindi District, Lindi Region in Tanzania at an elevation of 190 meters and GPS Location of S 10°02.26.9 and E 039°28.12.8. The plant species were identified by a taxonomist at the Botany Department, University of Dar es Salaam where the voucher specimens FMM 4136, FMM 3904 and FMM 3906 for *C. dictyophlebodes*, *C. kilwae* and *C. longipedicellatus* ssp *austrotanzanicus*, respectively are preserved.

Experimental animals

Swiss albino mice, *Mus musculus* were selected in this study to carry out *in vivo* toxicity screening for the leaf extracts of the selected *Croton* species. About 48 male mice used in the study were purchased from Tanzania Veterinary Laboratory Agency (TVLA). The animals were then maintained in animal house at the Department of Zoology and Wildlife Conservation and were given two (2) weeks to acclimatize before subjected to the experiments. They were supplied with unlimited food and water and temperature and humidity were at 28 ± 1.5 ℃ and 51%, respectively at 12-hours dark/light cycle.

Extraction of plant materials

At the Natural Products Research Laboratory, Chemistry Department, University of Dar es Salaam, the leaves of *C. dictyophlebodes*, *C. kilwae* and *C. longipedicellatus* ssp *austrotanzanicus* were air-dried and pulverised. Leaves (50 g) of each plant were soaked twice in 1:1 v/v dichloromethane (DCM):methanol (MeOH) for 72 h. The extracts were concentrated using rotary evaporator at 40 °C yielding 3.1, 3.4 and 3.8 g of crude extracts from *C. dictyophlebodes*, *C. kilwae* and *C. longipedicellatus* ssp *austrotanzanicus*, respectively. The extracts obtained were stored in a refrigerator at 4 °C until required for bioassays.

Cytotoxicity assays and determination of the effective concentration (EC_{50})

Cytotoxicity screening of the crude extracts from the leaves of the selected plants was carried out at the University of Gothenburg (Sweden). Two cancer cell lines namely, human breast cancer cell line (MCF-7) and human laryngeal cancer cell line (HEp-2) were used to investigate cytotoxic effects of the leaf extracts. Thus, cytotoxicity assays of the leaf extracts from *C. kilwae* and *C. longipedicellatus* ssp *austrotanzanicus* against HEp-2 cells were carried out following recently reported procedure (Mollel et al. 2022, Mahambo et al. 2023). A volume of 50 µL of 10% DMEM was added to each well of 96-well plates for both control and treatment panels. Then, using micropipette 2 x 10^4 HEp-2 cells were measured and added to each well of 96-well plates containing 50 µL of 10% DMEM, seeded and incubated in a humidified incubator at 5% CO_2 and 37 °C for 72 h. After 72 h incubation, the cells were washed and 50 µL of fresh 10% DMEM were added. Then, the cells in control panel were treated with the corresponding volume of DMSO used for preparation of stock solution of the test samples while those in treatment panels were treated with 0.016–100 µg/mL of crude extracts. Then, the cells were re-incubated in an incubator at 5% CO_2 and 37 °C for 72 h. After 72 h of re-incubation period, 15 µL of CellTiter 96 AQeous Solution were added to each well of all the assay plates. The plates were then shaken and placed into an incubator for further 4 h, and absorbance measured using microplate reader at 490 nm. Moreover, cytotoxicity assay of the leaf extract from *C. dictyophlebodes* was performed following the above reported procedure except MCF-7 cells (cell line) and alamarBlue (cell viability reagent) were used (Maeda et al. 2020), in which the absorbance was measured using microplate reader at 570 to 600 nm. EC_{50} values (the concentration required to obtain a 50% cytotoxic effect) for each extract were calculated, from three independent replicate experiments, using two-fold dilution intervals (Maeda et al. 2020).

Acute toxicity assays and determination of lethal dose (LD_{50})

The assays included evaluation of clinical signs of toxicity and haematological parameters conditions following OECD (2001) guideline number 423 and previously reported procedure (Malekela et al. 2020). In brief, forty-eight (48) Swiss male albino mice of age ranging 8–10 weeks were used. The animals were acclimatized for five (5) days prior to dose administration and managed for fourteen (14) days after dose administration at the ZWCD with temperature ranges of 26 °C in March 2021 and 33 °C in April 2022 and humidity of 80 and 85%, respectively. The mice were fed with animal mash and pellets, and provided with drinking water twice each day for 5 days of acclimatization and 14 days of experiments. The laboratory used for experiments was provided with 12 h of light and darkness each as well as sufficient ventilation. The general cleanliness of the animal cages was maintained once daily for all 19 days of acclimatization and experiments. After acclimatization, each mouse was weighed and its dose was calculated based on globally harmonized standard doses (GHSDs) of 50, 300 and 2000 mg/kg b.w.; and globally harmonized vehicle dosing volume (GVDV) of 10 mL/1000 g b.w. Then each mouse was fasted for 2 h, given its dose using oral gavage and monitored for 14 days to determine any clinical signs of toxicity. On the 15th day after experiments, the survived mice were humanely sacrificed and the blood samples were collected for haematology. On the other hand, all mice found in moribund state (state of delayed death) in the course of experimental period were humanely killed and their bloods collected for haematology analysis. Determination of LD_{50}, the concentration of the studied extract that was sufficient to kill 50% of a population of the experimental mice within 14 days of experiment was done as previously reported by Malekela et al. (2020).

Analysis of haematological parameters

All mice found in moribund state, and those that survived in the course of experiments including those in control groups were anesthetized with chloroform and dissected at the ZWCD laboratory. Blood samples were directly collected from the pulsating hearts of dissected mice using standard gauge needles and syringes, transferred into EDTA tubes and stored in a standard refrigerator at 2 °C for not more than 7 days before analysis. Complete blood counts/full blood pictures (CBCs/FBPs) analyses were carried out at the University of Dar es Salaam, Muhimbili University of Health and Allied Sciences Haematology Research Laboratory, Nairo Link Tz Ltd and Lancet Labs using haematology analysers.

Statistical analysis

Data were analysed using InStat™ software, v. 2.01/93 (GraphPad). Statistical significance was evaluated using One-Way Analysis of Variance (ANOVA) followed by Turkey-Kramer's Multiple Comparisons tests for gross body weights and haematological parameters**.** Data are presented as means plus standard error of the mean (SEM) with significance level set at $p < 0.05$.

Results and Discussion

Results

Cytotoxicity against cancer cells lines

Cytotoxic effects of the studied plant extracts against the chosen cancer cell lines are presented in Table 1. *Croton dictyophlebodes* leaf extract (CDE) was cytostatic to human breast cancer cells (MCF-7) at EC_{50} of 86 µg/mL, while *C. longipedicellatus* ssp *austrotanzanicus* leaf extract (CLE) exhibited cytotoxic effects against human laryngeal cancer cells (HEp-2) at EC_{50} of 63 µg/mL. *C. kilwae* leaf extract (CKE) did not induce cytotoxic effects against HEp-2 at concentrations ≤ 100 µg/mL.

Table 1: Cytotoxicity of the leaf extracts from the selected *Croton* species

Extract	Cancer cell lines	Cytotoxicity (EC_{50}, µg/mL)
CDE	MCF-7	86
CKE	HEp-2	>100
CLE	HEp-2	63

Toxicological tests of selected croton species in mice

Mean percentage mortalities of 16.7% and 33.3% were observed in mice administered *oral suspension (*o.s.) 300 and o.s. 2000 mg/kg b.w. of CDE, respectively. Despite the mortalities observed, the survived mice that received the same doses of CDE displayed no other signs of toxicity (Table 2) However, using Tukey's multiple comparison test a significant decrease in final body weights was observed between group of mice that received 2000 mg/kg b.w. and 300 mg/kg b.w. of CDE (Table 3). All clinical signs of toxicity were normal for mice given o.s. 300 and o.s. 2000 mg/kg b.w. of CKE (Table 2).

Similarly, mean percentage mortalities of about 22.2% and 77.8% were observed in mice administered o.s. 50 and o.s. 300 mg/kg b.w. of CLE, respectively. While the mice that received o.s. 50 mg/kg b.w. of CLE displayed normal signs, those administered with o.s. 300 mg/kg b.w. of CLE experienced other clinical signs of toxicity apart from death (Table 2). However, there was no significant difference between the means of the final body weights of mice in treatment and control groups (Table 3) The LD_{50} for CKE and CDE were > 2000 mg/kg, while that of CLE was 175 mg/kg b.w. as shown in Table 4.

Table 2: Signs of toxicity of tested *Croton* leaf extracts to mice

	Test extract and groups								
	CDE (N = 15)			CKE (N = 15)			CLE (N = 21)		
Toxic signs	Control (n = 3)	300 mg/kg b.w. (n = 6)	2000 mg/kg b.w. (n = 6)	Control (n = 3)	300 mg/kg b.w. (n = 6)	2000 mg/kg b.w. (n = 6)	Control (n = 3)	50 mg/kg b.w. (n = 6)	300 mg/kg b.w. (n = 9)
Tremors	NS	NS	NS	NS	NS	NS	NS	NS	NS
Convulsions	NS	NS	NS	NS	NS	NS	NS	NS	NS
Diarrhoea	NS	NS	NS	NS	NS	NS	NS	NS	NS
Vomiting	NS	NS	NS	NS	NS	NS	NS	NS	NS
Hyperactivity	NS	NS	NS	NS	NS	NS	NS	NS	NS
Sleeping	N	N	N	N	N	N	N	N	A
Grooming	N	N	N	N	N	N	N	N	A
Lacrimation	NS	NS	NS	NS	NS	NS	NS	NS	NS
Salivation	NS	NS	NS	NS	NS	NS	NS	NS	NS
Pinna reflex	N	N	N	N	N	N	N	N	R
Breathing	N	N	N	N	N	N	N	N	R
Alertness	N	N	N	N	N	N	N	N	R
Touch response	N	N	N	N	N	N	N	N	R
Urination	NS	NS	NS	NS	NS	NS	NS	NS	NS
Lateral Position	NS	NS	NS	NS	NS	NS	NS	NS	S
Recumbence	NS	NS	NS	NS	NS	NS	NS	NS	S
Vocalization	N	N	N	N	N	N	N	N	A
ARFW	N	N	N	N	N	N	N	N	R
AED	N	N	N	N	N	N	N	N	U
Mean % Mortality	0	16.7	33.3	0	0	0	0	22.2	77.8

A = Abnormal; AED = Able to Eat and Drink; ARFW = Able to Reach Food and Water; N = Normal; Nn = None; NS = Not Seen; R = Reduced; U = Unable.

Table 3: Comparison between mean values (mg ± SEM) for the final body weights of mice

Extract	Groups			One-Way ANOVA
CDE	Control	300 mg/kg	2000 mg/kg	P-value
(N = 15)	(n = 3)	(n = 6)	(n = 6)	
(g ± SEM)	26.2 ± 0.6	29.1 ± 0.6	19.8 ± 1.7	0.0201,*
CKE	Control	300 mg/kg	2000 mg/kg	P-value
(N = 15)	(n = 3)	(n = 6)	(n = 6)	
(g ± SEM)	25.3 ±1.9	32.7 ± 5.8	23.0 ± 1.5	0.2942, ns
CLE	Control	50 mg/kg	300 mg/kg	P-value
(N = 21)	(n = 3)	(n = 6)	(n = 9)	
(g ± SEM)	26.0 ± 0.6	30.2 ± 0.4	27.6 ± 5.4	0.6670, ns

*Means there was significance difference between groups; ns indicates that there was no significance difference.

Table 4: Lethal dose (LD_{50}) of the tested *Croton* leaf extracts to mice (mg/kg b.w.)

Extract	LD_{50} (mg/kg)
CDE	> 2000
CKE	> 2000
CLE	175

Haematological profile of mice given selected *Croton* leaf extracts

In analysis of quantitated complete blood counts/full blood pictures (CBCs/FBPs) of mice, the previously established mouse blood reference ranges (Silva-Santana et al. 2020) were used in representation and interpretation of the results obtained (Tables 5–7). In haematological evaluation, the mice given o.s. 300 and 2000 mg/kg b.w. of CDE indicated non-significant change in white blood cell counts (WBC), total red blood cells (RBC) and haemoglobin (HGB). However, a significant decrease of haematocrit (HCT) and platelets (PLT) counts was recorded in animals that received o.s. of 300 mg/kg b.w. in comparison with the control and 2000 mg/kg groups (Table 5). The mice that were given o.s. 300 and 2000 mg/kg b.w. of CKE showed non-significant change in comparison to control groups in all measured haematological parameters (Table 6). Similarly, the same trend was observed in that mice that were given o.s. 50 and 300 mg/kg b.w. of CLE in comparison to control group (Table 7).

Table 5: Effects of *Croton dictyophlebodes* leaf extract (CDE) on mice blood

Parameter	SI Unit	Control (Tween 80) (n = 3)	CDE (300 mg/kg) (n = 6)	CDE (2000 mg/kg) (n = 6)	Kruskal-Wallis Test
		Mean ± SEM	Mean ± SEM	Mean ± SEM	P – value
WBC	C x 10^9/L	11.4 ± 0.60	5.2 ± 2.55	3.9 ± 0.22	0.0743, ns
Neu#	C x 10^9/L	2.2 ± 1.35	0.7 ± 0.65	0.2 ± 0.22	0.3807, ns
Lym#	C x 10^9/L	7.9 ± 2.60	0.5 ± 0.5	3.0 ± 0.03	0.0888, ns
RBC	C x 10^{12}/L	8.8 ± 0.31	5.6 ± 0.54	8.9 ± 0.86	0.1717, ns
HGB	g/dL	13.8 ± 1.00	8.1 ± 2.10	13.1± 0.80	0.1164, ns
HCT	%	41.6 ± 2.30	26.6 ± 5.75	40.4± 2.20	0.0024, s
PLT	C x 10^9/L	824 ± 87	400 ± 179.67	54 ± 17.33	0.0359, s

C = number of cells in a given volume of blood

Table 6: Effects of *Croton kilwae* leaf extract (CKE) on mice blood

Parameter	SI Unit	Control (Tween 80) (n = 3)	CKE (300 mg/kg) (n = 6)	CKE (2000 mg/kg) (n = 6)	Kruskal-Wallis Test
		Mean ± SEM	Mean ± SEM	Mean ± SEM	P-value
WBC	C x 10^9/L	5.2 ± 1.92	6.8 ± 0.45	3.5 ± 1.62	0.3982, ns
Neu#	C x 10^9/L	0.3 ± 0.14	0.0 ± 0.00	0 ± 0.01	0.1398, ns
Lym#	C x 10^9/L	2.5 ± 0.49	2.4 ± 2.42	3.1 ± 1.39	0.9584, ns
RBC	C x 10^{12}/L	7.3 ± 1.25	8.1 ± 0.21	9.1 ± 0.22	0.3669, ns
HGB	g/dL	13.1 ± 2.75	11.9 ± 1.00	13.1 ± 0.95	0.8749, ns
HCT	%	38.3 ± 6.10	35.5 ± 2.40	43.0 ± 2.65	0.5100, ns
PLT	C x 10^9/L	1062.5 ± 325.50	983.0 ± 226.00	507.0 ± 184.00	0.4606, ns

Table 7: Effects of *Croton longipedicellatus* ssp *austrotanzanicus* leaf extract (CLE) on mice blood

Parameter	SI Unit	Control (Tween 80) (n = 3)	CLE (50 mg/kg) (n = 6)	CLE (300 mg/kg) (n = 9)	Kruskal-Wallis Test
		Mean ± SEM	Mean ± SEM	Mean ± SEM	P-value
WBC	C x 10^9/L	5.2 ± 1.92	4.0 ± 2.85	3.5 ± 1.62	0.3982, ns
Neu#	C x 10^9/L	0.3 ± 0.14	7.5 ± 2.75	0 ± 0.01	0.1398, ns
Lym#	C x 10^9/L	2.5 ± 0.49	4.8 ± 3.90	3.1 ± 1.39	0.9584, ns
RBC	C x 10^{12}/L	7.3 ± 1.25	8.5 ± 0.04	9.1 ± 0.22	0.3669, ns
HGB	g/dL	13.1 ± 2.75	14.1 ± 0.05	7.8 ± 4.20	0.3051, ns
HCT	%	38.3 ± 6.10	41.9 ± 0.60	23.5 ± 10.45	0.1740, ns
PLT	C x 10^9/L	667 ± 217.67	1053.33 ± 150.65	445.66 ±122.67	0.1408 ns

Discussion

Before examining the pharmacological characteristics and potential applications of any plant extract or compounds, it is essential to carry out toxicity screening. The current study examined the cytotoxicity of *Croton dictyophlebodes* leaf (CDE), *C. kilwae* leaf (CKE) and *C. longipedicellatus* ssp *austrotanzanicus* leaf (CLE) extracts against human breast cancer cells (MCF-7) and human laryngeal cancer cell lines (HEp-2). CDE was cytostatic to MCF-7 cell lines, while CLE was found to induce cytotoxic activity to HEp-2 cell lines in which CKE was inactive. In addition, CDE and CLE caused mortality to mice at different percentages which was not the case with CKE.

As aforementioned, the cytotoxicity assay carried out in the present study revealed the ability of the CDE to affect viability of MCF-7 cell lines. This could be attributed by the synergetic effects of the phytochemical constituents of the plant. Previous study by Munissi et al. (2020) stated the presence of the diterpenoids as major phytoconstituents of the CDE. In addition, several studies have reported the presence of terpenoids, alkaloids and flavonoids in the genus croton (Salantion et al. 2007) where some have been demonstrated to have anti-cancer activity among others. Contrary to our findings, pure diterpenoids from the root and stem extracts of the CDE were inactive to NCI 60 tumour cell lines (Munissi et al. 2020).

Similar to CDE, CLE was found to induce cytotoxic activity to HEp-2 cell lines. Currently, the constituents of the leaf extract (CLE) from this plant have not yet been established. However, the presence of clerodane and *seco*-labdane diterpenoids with cytotoxic activity against HEp-2 cell lines have been reported from the root and stem barks of the same plant (Mahambo 2023). The observed cytostatic potent of CLE against HEp-2 cell lines postulates presence of similar phytoconstituents as in the case of stem and root bark extracts of this plant.

Cytotoxicity studies on other *Croton* species, such as those on *C. bonplandianus* extract showed cytotoxicity against chronic myelogenous leukemia (K562) and Bakkitt lymphoma (Raji) cell lines (Suresh et al. 2021), while that of *C. caudatus* extract caused cytotoxic effects to HeLa cell lines (Shantabi et al. 2020) suggesting that the compounds present in the two plant species might be effective against the above mentioned cancer cell lines.

On the other hand, CKE was unable to induce anticancer activity to HEp-2 cell lines. This suggests that the collective effects of phytoconstituents present in CKE do not have cytotoxic activity against the tested cell lines. However, findings from Mahambo et al. (2023) found that crotofolanes and other diterpenoids isolated from CKE have cytotoxic activities against HEp-2 and human cervical cancer cell (HeLa) lines, respectively indicating that the diterpenoids present in CKE may be responsible for induction of necrosis to the cell lines.

Generally, the cytostatic behaviour of the extracts from the plants reported in this paper namely *C. dictyophlebodes* and *C. longipedicellatus* ssp *austrotanzanicus* may be considered potential sources of anticancer agents. With respect to *in vivo* studies, insignificant mortality was observed following administration of o.s. 300 and 2000 mg/kg b.w. of leaf extract of CDE and o.s. 50 mg/kg b.w. of leaf extract of CLE to mice. However, death was observed in the mice that received the higher dose (o.s. 300 mg/kg b.w.) of CLE, indicating the dose dependent mortality. Other species of croton showed that a dose of 2000 mg/kg b.w. of *C. grewioides* extract caused mortality to mice (Silva et al. 2016), while *Croton polyandrus* essential oil induced mortality at equivalent to 447.18 mg/kg b.w. (Meireles et al. 2016). On the other hand, in the current study there were no mortalities observed following administration of o.s. 300 and 2000 mg/kg b.w. of CKE to mice indicating that the constituents therein were less or non-toxic to mice.

A positive gain in mean gross body weight (GBW) was maintained by mice administered with o.s. 300 and 2000 mg/kg b.w. of CDE and o.s. 50 and 300 mg/kg b.w. of CLE, while insignificant decrease in mean GBW was seen following administration of o.s. 2000 mg/kg b.w. of CKE to mice. The findings indicate that extracts may contain nutraceuticals that support the growth of animals as modelled by mice.

The deviation in any haematological parameter following administration of a chemical substance predicts human intoxication. The findings on haematological parameters including WBC, neutrophils, lymphocytes, total RBC and haemoglobin were not significantly altered upon exposure to studied *Croton* species indicating their safety. However, a significant decrease in number of haematocrit and platelets was recorded only in animals that received o.s. of 300 and 2000 mg/kg b.w. of CDE, respectively in comparison with the control groups. The observed significant decreases in haematocrit and PLT# of mice following administration of CDE might be the reason for the observed mice mortalities at the corresponding doses. It is known that, thrombocytes play a major role in homeostasis and immunity in both innate and adaptive immune responses (Koutchiko et al. 2022, Silva-Santana et al. 2020) as well as in preventing bleeding due to injuries. Thus, these significant decreases in PLT# might have led to the observed mortalities possibly due to impairment of blood coagulation molecules by the compounds contained in CDE. In contrast, others studies on *Croton* species such as *C. gratissimus* extract was reported to cause significant increase in platelets (PLT) in the treated rats (Koutchiko et al. 2022), while the PLT# of rats administered *Maerua classifolia* was not affected (Christian et al. 2017).

Therefore, based on obtained toxicological data on behavioural signs, body weights, percentage mortalities, and the haematological parameters, the studied plant extracts can be considered safe to mice when administered below their LD_{50} values. Therefore, further dose dependent toxicological studies prior to clinical trials are recommended in order to justify medicinal

use of these investigated plant extracts and their possible utilization as sources of anticancer agents.

Conclusion

The leaf extracts of *Croton dictyophlebodes* and *C. kilwae* are non-toxic to mice whereas that of *C. longipedicellatus* ssp *austrotanzanicus* is toxic to mice at doses > 50 mg/kg b.w. In cytotoxicity assay, *C. dictyophlebodes* and *C. longipedicellatus* ssp *austrotanzanicus* were effective against tested cancer cell lines, while *C. kilwae* was ineffective at EC_{50} of 100 µg/mL. Collectively, the studied plant extracts may be considered potential sources of anticancer agents. However, further dose dependent toxicological studies prior to clinical trials are recommended to validate their medicinal use.

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