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Growth inhibitory and cytotoxic study of *Ceiba pentandra* (L.) Gaertn. Leaf (Malvaceae) and *Cascabela thevetia* (L.) Lippold root bark (Apocynaceae)

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Abstract
Ceiba pentandra (L.) Gaertn. leaf (Malvaceae) and *Cascabela thevetia* (L.) Lippold (Apocynaceae) root bark were investigated for growth inhibitory effect on guinea corn (*Sorghum bicolor*) seeds and cytotoxicity on tadpoles (*Raniceps ranninus*) *in vitro.* Methanol extract and solvent fractions of both plants at 0.25 – 5.0 mg/ml gave significant decreases in mean radicle length of guinea corn seeds (anti-proliferative action) which was concentration-dependent. Growth inhibition of root radicle at the most effective concentration, 5 mg/ml, was not dependent on incubation period. *Ceiba pentandra* aqueous fraction gave remarkable growth inhibition (86%) at 24h while other tested agents were similar in activity (78-76%). At 48h, the performance was methanol extract > aqueous fraction = chloroform fraction > ethyl acetate fraction (89-69%). *Cascabela pentandra* gave reasonable maximum growth inhibition only at 24h: chloroform fraction = ethyl acetate (69%) and aqueous fraction = methanol extract (59%). *Ceiba pentandra* (LC_{50} 4.8-6.0 mg/ml) proved to be more cytotoxic on tadpoles than *Cascabela thevetia* (LC_{50} 5.3-6.4 mg/ml) within 24h. Cytotoxicity ranking with *Ceiba pentandra* was ethyl acetate fraction > methanol extract > chloroform fraction > aqueous fraction, while *Cascabela thevetia* gave ethyl acetate fraction > aqueous fraction > methanol extract. Data reported herein showed that *Ceiba pentandra* was the more potent cytotoxic and growth inhibitory plant. These findings hereby justify the folkloric purpose of *Ceiba pentandra* and *Cascabela thevetia* in the treatment of oxidative stress-induced diseases.

Keywords: *Ceiba pentandra, Cascabela thevetia,* growth inhibitory activity, guinea corn seeds, cytotoxicity, tadpoles

1. Introduction
The plant sources constitute an important source of anticancer drugs like vinblastine and vincristine alkaloids isolated from *Catharanthus roseus* [1]. An upsurge in cancer research to discover plant-derived anticancer compounds, many of which have been used in traditional herbal treatments for centuries is ongoing. Cytotoxic agents are substances used in the treatment of malignant growth and other diseases, and are designed to destroy rapidly growing cancer cells.

Cancer as a leading cause of death worldwide has witnessed great advancements in its treatment and control. Significant setbacks in cancer therapy and prospects for progress have been discussed [1]. However, such chemotherapy is sometimes characterised by various undesired side effects which can be overcome through the use of plant-derived products. To date, there are a few plant products being used to treat cancer aside from the many similar products possessing very promising *in-vitro* anti-cancer activities awaiting clinical evaluation [1, 2]. *Ceiba pentandra* (L.) Gaertn (Malvaceae) is a tropical tree growing up to 10-30m tall [3]. The tree is specially valued for its fibre, food and medicinal purposes. The bark decoction is a folkloric remedy for infertility, diuresis and type 2 diabetes [3]. Leaves are implicated in skin infections, fevers, bleeding in pregnant women, conjunctivitis. Externally, pounded leaves are used for dressing sores, tumours and whitlows. Macerations of root bark are drunk against dysmenorrhoea and hypertension. Apart from the report of Tundis *et al.* [4] on cancer cell growth inhibition of *Bombax ceiba* flower extracts, and that of Kumar *et al.* [5] on antitumor activity of the stem bark, no other information is available in the literature on anticancer activity of *Ceiba pentandra, Cascabela thevetia* (L,) Lippold (Apocynaceae) is a poisonous plant up to 6m high, native to Mexico and Central America, but is now cultivated widely as an ornamental [6]. Traditionally, the root infusion is used to treat snake bite and tumours while leaf decoction is applicable for treating jaundice, fever and as a purgative for intestinal worms [3, 6]. Anticancer potential of the fruit methanolic extract has been reported [7].

The extract was found to have critical effects in the proliferation of human breast and colorectal cancer cells, and apoptosis induction in human prostate and lung cancer cell lines.

The objective of this research was to compare the growth inhibitory and cytotoxic activities of *Ceiba pentandra* leaf and *Cascabela thevetia* root bark reputed for various uses in African traditional medicine.

2. Materials and Methods

2.1 Collection and preparation of plant materials: Fresh leaves of *Ceiba pentandra* were harvested from trees growing in Okada village, Ovia north east LGA, Edo state in August 2016. *Cascabela thevetia* roots were obtained from the southern part of Edo state in Nigeria in September 2016**.** They were authenticated (voucher nos. *C. pentandra*: IUO/16/131 and *C. thevetia:* IUO/16/031) (Table 1) at the Department of Pharmacognosy herbarium, IUO, Edo State, Nigeria.

2.2 Plant extraction and fractionation: *Ceiba pentandra* leaves were dried at room temperature (25-28 ºC) for one week, while peeled root bark of *Cascabela thevetia* were cut into pieces dried for 3 weeks and ground into a coarse state using the locally fabricated laboratory milling machine. 500g of each sample were extracted to exhaustion with methanol in a Soxhlet apparatus. The crude extract was concentrated *in vacuo*, weighed (*Ceiba pentandra* 6.12%; *Cascabela thevetia* 3.91%) and refrigerated until needed. An appropriate amount of the crude methanol extract of each plant was fractionated with chloroform, ethyl acetate in a separatory funnel to yield chloroform, ethyl acetate and aqueous fractions. All fractions were reduced *in vacuo.*

2.3 Phytochemical screening: Basic phytochemical screening was carried out on the crude methanol extract of each plant according to Evans [8] and suspected secondary metabolites recorded.

2.4 Determination of growth inhibitory effect of crude extract and solvent fractions on guinea corn (*Sorghum bicolor*): Guinea corn (*Sorghum bicolor*) seeds were obtained from a local market in Okada Town, Ovia North East Local Government Area, Edo state. Viability test was performed according to Ikpefan *et al.* [9]. The seeds were put in a beaker of distilled water, stirred and viable seeds which sank were decanted and used. They were surface-sterilized with 95% ethanol for 1 minute, rinsed again with distilled water to make ready for use. According to Ikpefan *et al.* [9], twenty sterilized seeds were introduced into each petri-dish (10cm diameter) lined with absorbent cotton wool and overlaid with Whatman no.1 filter paper. Concentrations of 5mg/ml, 2.5mg/ml, 1mg/ml, 0.5mg/ml and 0.25mg/ml of the methanol crude extract and each of the aqueous, chloroform, and ethyl acetate fractions were separately applied. A negative control (water) and positive control (chloramphenicol, 0.5mg/ml) were similarly set up. All experiments were replicated thrice, and petri-dishes incubated in dark cupboard at room temperature for 72h. The length (cm) of the radicles emerging from the seeds were measured at 24h, 48h and 72h, average determined and recorded (±SEM). Results were subjected to standard statistical analyses.

Percentage inhibition of growth of seeds at 72h with 5mg/ml of all test agents was determined using the formula:

$$\% \text{ inhibition} = \frac{(\text{MRL of negative control} - \text{MRL of test agent})}{(\text{MRL of negative control})} \times 100$$

MRL= mean radicle length

2.5 Determination of cytotoxic effects of extract and partitioned fractions on tadpole: Tadpoles (5-7 day old) were scooped from stagnant water in Crown estate, IUO. Using the method of Obuotor and Onajobi [10], ten viable tadpoles were selected with the aid of pipette into different beakers containing 30ml natural water from tadpole habitat. The volume was made up to 49ml with distilled water and 1ml of stock solution containing 1mg/ml, 2mg/ml, 5mg/ml, 10mg/ml and 20mg/ml of extract and various fractions. The experiment was replicated thrice and assay beakers incubated at room temperature for 24h. A negative control (water) and positive control (chloramphenicol, 0.5mg/ml) were similarly set up. Mortality was determined and mean ±SEM recorded.

Statistical analysis: Results of triplicate determinations were expressed as mean±SEM were subjected to statistical analysis using one-way analysis of variance (ANOVA) using graph pad prism 6 version computer software packages. Differences at $P < 0.05$ and $P < 0.01$ were considered significant.

3. Results and Discussion

The phytochemical screening yielded tannins, flavonoids, alkaloids, anthraquinones and terpenoids as the secondary metabolites in both plants. The profile of the two plants investigated in this study is presented in Table 1.

Table 1: Profile of *Ceiba pentandra* and *Cascabela thevetia*

Parameter	*Ceiba pentandra* (L.) Gaertn	*Cascabela thevetia* (L.) Lippold
Family	Malvaceae	Apocynaceae
Common name	Kapok tree	Yellow oleander
Voucher no	IUO/16/131	IUO/16/031
Location	Okada village, Ovia North east LGA, Edo state	Southern part of Edo state
Morphological part	Leaf	Root bark

Ceiba pentandra and *Cascabela thevetia* investigated in this study, demonstrated concentration-dependent growth inhibitory activity (as indicated by decreases in mean radicle length of guinea corn seeds over the entire incubation period) when treated with 0.25-5.0 mg/ml of their methanol extract, aqueous, ethyl acetate and chloroform fractions (Table 2). Peak activity was achieved with 5mg/ml. Methanol extract of *Ceiba pentandra* gave mean radicle length range of 0.69-0.23 cm (at 0.25- 5.0mg/ml) to 0.91-0.47 cm between 24- 72h. while *Cascabela thevetia* gave 0.28- 0.15cm to 4.63- 3.45cm. In addition, their aqueous, ethyl acetate and chloroform fractions yielded positive growth inhibitory results: 0.58- 0.13 cm to 0.94-0.30 cm for *Ceiba pentandra* and 0.29- 0.14cm to 4.98- 2.26cm for *Cascabela thevetia* with aqueous fractions. Data for the chloroform fraction were: 0.46- 0.24cm to 0.73-0.28cm for *Ceiba pentandra* and 0.27- 0.11cm to 5.07-2.11cm for *Cascabela thevetia* within 72h, while mean radicle lengths of 0.73-0.24cm to 0.96-0.48cm (*Ceiba pentandra*) and 0.43-0.11cm to 5.28-3.07cm (*Cascabela thevetia)* for ethyl acetate fractions were recorded. These effects were significantly less than those of the standard growth inhibitory (anti-proliferative) agent, chloramphenicol (tested at 0.5mg/ml) at the respective incubation period. However, growth inhibitory activity of *Ceiba pentandra* extract and fractions approached that of the positive control at 72h. The choice of guinea corn root model of growth inhibitory assay

was based on its meristematic tissues which have the tendency to proliferate under favourable conditions culminating in the increase in the length of the radicles [9, 11]. This indicates that guinea corn root radicle is reputed for active cell division similar to cancer cells. Mechanism of growth inhibitory action of plant extracts has been attributed to interference with biochemical system or other growth-related systems [4, 11] have published anticancer potential of *Bombax ceiba* (syn. *Ceiba pentandra*) flower extract on seven human cancer cell lines.

Table 2: Mean length (cm) of guinea corn root radicle treated with *Ceiba pentandra* and *Cascabela thevetia*

Concentration (mg/ml)	24h		48h		72h	
	Crude methanol extract					
	Ceiba pentandra	***Cascabela thevetia***	***Ceiba pentandra***	***Cascabela thevetia***	***Ceiba pentandra***	***Cascabela. thevetia***
0.25	0.69±0.01	0.28±0.01	0.84±0.13	2.27±0.11	0.91±0.21	4.63±0.20
0.5	0.58±0.13	0.25±0.25	0.69±0.21	2.33±0.04	0.54±0.11	5.10±0.04
1.0	0.40±0.11	0.20±0.01	0.55±0.19	2.01±0.03	0.64±0.39	4.40±0.11
2.5	0.31±0.11	0.15±0.01*	0.45±0.22	1.47±0.02	0.56±0.17	3.88±0.08
5.0	0.23±0.01*	0.15±0.01*	0.38±0.18*	1.10±0.01*	0.47±0.12*	3.45±0.11
PC	0.24±0.10*		0.63±0.06*		1.07±0.12*	
NC	0.36±0.01		2.25±0.01		4.49±0.11	
Concentration (mg/ml)	**Aqueous fraction**					
	Ceiba pentandra	***Cascabela thevetia***	***Ceiba pentandra***	***Cascabela thevetia***	***Ceiba pentandra***	***Cascabela. thevetia***
0.25	0.58±0.21	0.29±0.01	0.75±0.22	2.24±0.01	0.94±0.	4.88±0.11
0.5	0.50±0.11	0.25±0.01	0.69±0.18	2.02±0.03	0.79±0.29	4.32±0.04
1.0	0.39±0.11	0.25±0.02	0.45±0.12	1.65±0.12	0.63±0.31	3.85±0.02
2.5	0.33±0.12	0.22±0.03	0.39±0.10	1.30±1.43	0.44±0.18	2.88±0.06
5.0	0.13±0.01*	0.14±0.01*	0.24±0.11*	1.09±0.04	0.30±0.02*	2.26±0.07
PC	0.24±0.10		0.63±0.06*		1.07±0.12	
NC	0.36±0.01		2.25±0.01		4.49±0.11	
Concentration (mg/ml)	**Chloroform fraction**					
	Ceiba pentandra	***Cascabela thevetia***	***Ceiba pentandra***	***Cascabela thevetia***	***Ceiba pentandra***	***Cascabela. thevetia***
0.25	0.46±0.21	0.27±0.01	0.59±0.21	0.25±0.01*	0.73±0.17	5.07±0.02
0.5	0.44±0.08	0.22±0.01	0.53±0.11	1.93±0.04	0.56±0.08	4.52±0.14
1.0	0.37±0.19	0.19±0.01	0.42±0.12	1.80±0.03	0.47±0.08	3.61±0.21
2.5	0.33±0.11	0.16±0.01	0.37±0.02	1.33±0.03	0.39±0.11*	2.67±0.06
5.0	0.21±0.02	0.11±0.01*	0.25±0.09*	1.06±0.02	0.28±0.04*	2.11±0.04
PC	0.24±0.10		0.63±0.06*		1.07±0.12	
NC	0.36±0.01		2.25±0.01		4.49±0.11	
Concentration (mg/ml)	**Ethyl acetate fraction**					
	Ceiba pentandra	***Cascabela thevetia***	***Ceiba pentandra***	***Cascabela thevetia***	***Ceiba pentandra***	***Cascabela. thevetia***
0.25	0.73±0.01	0.41±0.01	0.80±0.01	3.38±0.18	0.96±0.06	5.28±0.09
0.5	0.67±0.01	0.34±0.01	0.71±0.01	2.94±0.03	0.90±0.01	4.97±0.03
1.0	0.49±0.01	0.29±0.02	0.63±0.01	2.41±0.03	0.74±0.01	4.64±0.03
2.5	0.30±0.11	0.20±0.01	0.49±0.01	1.95±0.05	0.60±0.01	3.95±0.09
5.0	0.22±0.08	0.11±0.01*	0.37±0.02*	1.47±0.02	0.48±0.02*	3.07±0.02
PC	0.24±0.10		0.63±0.06		1.07±0.12	
NC	0.36±0.01		2.25±0.01		4.49±0.11	

PC, positive control, (chloramphenicol, 0.1mg/ml); NC, negative control (distilled water);
The values above are mean of three replicates. n=3. Mean ± SEM. Values with * superscript indicate significant difference $P<0.05$ relative to negative control (vehicle)

The growth inhibitory assay employed in this study based on reduction in mean length of guinea corn root radicle suggested probable anti-tumour, allelopathic and herbicidal effects of the plants [11]. Growth inhibitory activity has been reported for some Nigerian [9, 11-13], Vietnamese [14] and other plants grown elsewhere [15]. Recently, Zaid *et al.* [16] reported isolation of growth inhibitory (anti-proliferative) triterpenes from the genus, *Monothec*.

Furthermore, percentage growth inhibition of root radicles at 5mg/ml generally decreased with time, 86-64% for *Ceiba pentandra* (Fig. 1) and 69-32% for *Cascabela thevetia* from 24-72h (Fig. 2). Based on maximum inhibitions at 24h for *Cascabela thevetia*: chloroform fraction = ethyl acetate (69%) and aqueous fraction = methanol extract (59%). With *Ceiba pentandra*, apart from the aqueous fraction that was most effective (86%) at 24h, other tested agents gave similar growth inhibitions (76-78%). Therefore, the more active aqueous and chloroform fractions of *Ceiba pentandra*, and aqueous and ethyl acetate fractions of *Cascabela thevetia* appear to be promising growth inhibitory agents in the development of cytotoxic drugs. The work reported herein on the growth inhibitory effect of *Cascabela thevetia* root bark serves to expand the horizon of its anticancer potentials.

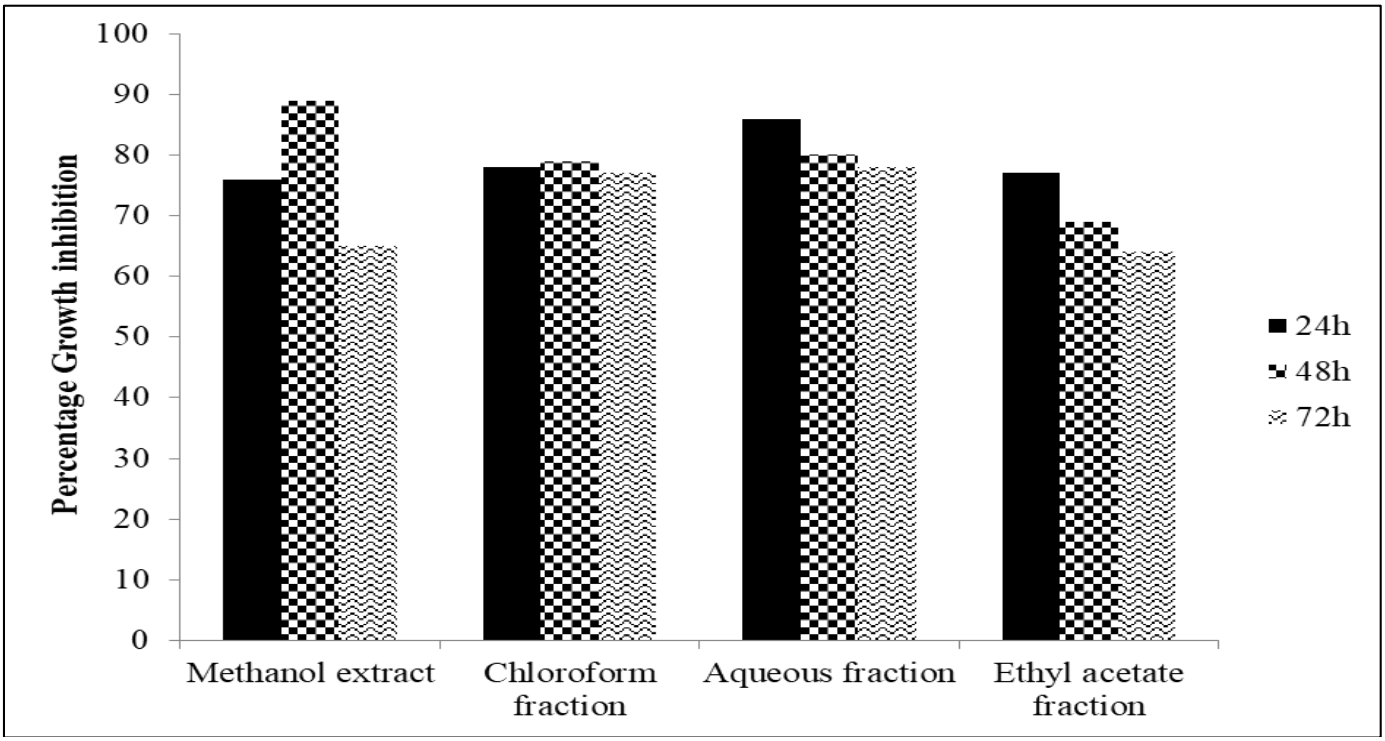

Fig 1: Percentage growth inhibition (%) of *Ceiba pentandra* test agents (5 mg/ml) on guinea corn root radicle

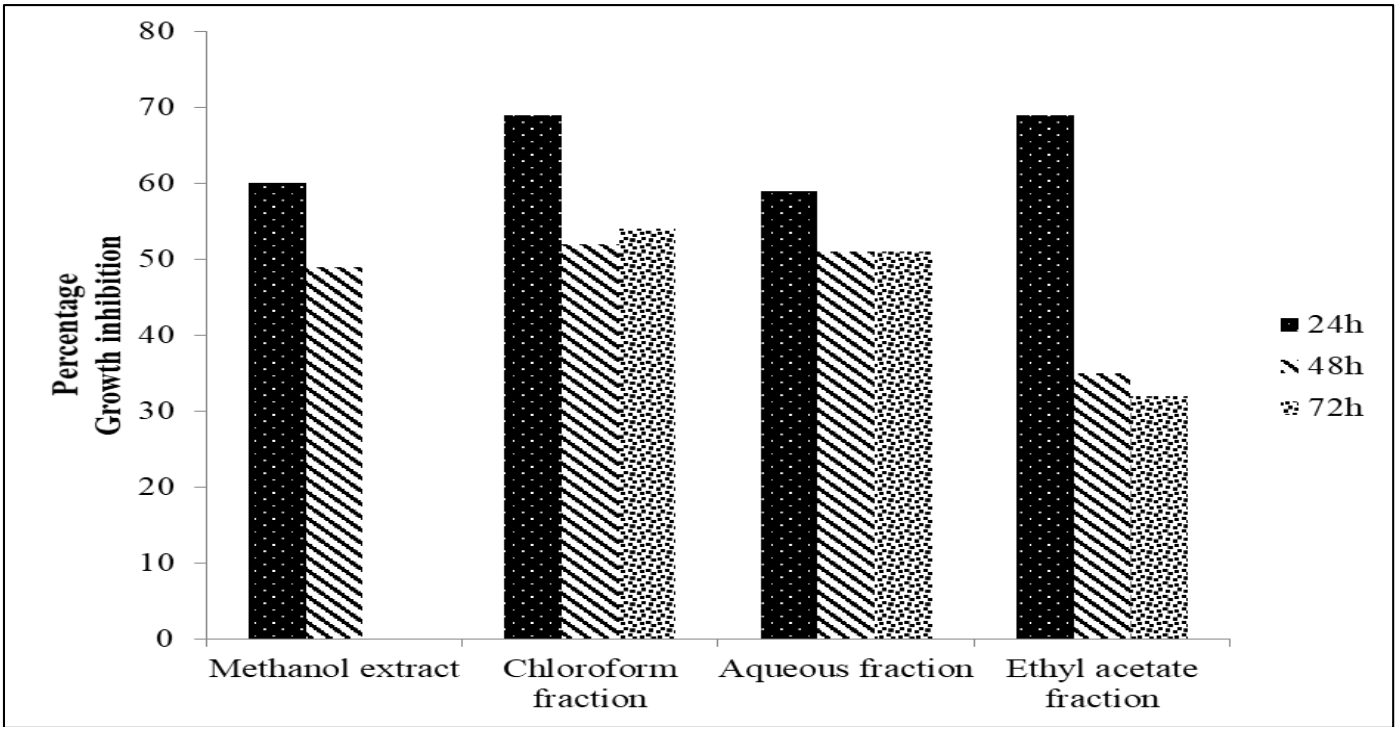

Fig 2: Percentage growth inhibition (%) of Cascbela thevetia test agents (5 mg/ml) on guinea corn root radicle

In the cytotoxicity study using tadpole model, complete mortality equivalent to that of the positive control, chloramphenicol tested at 0.5 mg/ml was recorded for all tested agents of *Ceiba pentandra* except the aqueous fraction at the maximum concentration of 20mg/ml, and the aqueous and ethyl acetate fractions of *Cascabela thevetia.* Order of cytotoxic potency was ethyl acetate fraction > methanol extract > chloroform fraction > aqueous fraction for *Ceiba pentandra* (LC_{50} 4.8 -6.0 mg/ml) in 24h (Table 2). *Cascabela thevetia* ranking was ethyl acetate fraction > aqueous fraction > methanol extract ((LC_{50} 5.3- 6.4 mg/ml). In this regard, aqueous and ethyl acetate fractions of and ethyl acetate fraction of *Ceiba pentandra* could be adjudged as promising and qualify for further isolation studies. According to this investigation, *Ceiba pentandra* leaf was the more active cytotoxic agent. Selective cytotoxicity of *Ceiba pentandra* stem bark extract on human breast and mouse melanoma cancer cell lines have been reported [5], as well as on *in vivo* model. An isoflavone glycoside [17] from the stem bark, two sesquiterpene lactones [18] from the root bark, and an isoflavone glucoside, vavain glucoside and its aglycone,[19] from the stem bark have been isolated. Four human cancer cell lines were reported to be susceptible to fruit methanol extract of *Cascabela thevetia* [7]. Moreover, seed kernel cold methanol extract of *Thevetia peruviana* (syn. *Cascabela thevetia*) exhibited cytotoxicity *in vitro* on two different cell

lines, and the cardiac glycosides, neriifolin and its acetyl derivative [20] and other cardenolides [21] have been confirmed to be responsible for this potency. This present investigation on a different morphological part of the plant is a further contribution to the cytotoxic properties of *Cascabela thevetia.*

Table 2: Mean mortality (%) of crude extract and fractions of *Ceiba pentandra* and *Cascabela thevetia* on tadpoles.

Test agent/ concentration	1mg/ml	3mg/ml	5mg/ml	10mg/ml	20mg/ml	LC_{50} (mg/ml)
	Ceiba pentandra					
Methanol extract	0	26.6	53.3	80	100*	4.9
Chloroform fraction	20	33.3	46.6	86.6	100*	5.6
Aqueous fraction	6.6	26.6	40	80	93.3	6.0
Ethyl acetate fraction	13.3	33.3	53.3	80	100*	4.8
NC	0					
PC	100*					
Cascabela thevetia						
Methanol extract	6.7	20	33.3	66.6	93.3	6.4
Chloroform fraction	0	13.3	33.3	46.6	60	13.0
Aqueous fraction	26.6	33.3	46.6	66.6	100*	5.5
Ethyl acetate fraction	13.3	26.6	46.6	86.6	100*	5.3
NC	0					
PC	100*					

PC, positive control, (chloramphenicol, 0.5mg/ml); NC, negative control (distilled water); The values above are mean of three replicates. n=3. Mean ± SEM. Values above are mean of three replicates. n=3. Mean ± SEM. Values with * superscript indicate significant difference $P<0.05$ relative to negative control (vehicle)

The positive cytotoxic control, chloramphenicol, is an old antibiotic. It is known to exhibit cytotoxic property by inhibiting multiple myeloma [22] leukaemia [23], and hence appeared suitable for this purpose. Tadpole qualifies as suitable model for cytotoxicity studies because the cells are eukaryotic in nature like human cancer cells, and any plant extract or fraction that causes mortality of tadpoles will most likely inhibit growth of tumour producing cells. Sesquiterpene lactones [18] and isoflavones [19] present in *Ceiba pentandra* have been suggested to be responsible for its cytotoxicity. The literature has abundant information on cytotoxic medicinal plants [2, 11, 24-26]. Interestingly, fourteen Nigerian medicinal plants were reported to be cytotoxic against human rhabdomyosarcoma cell line in an earlier survey by Ogbole *et al*. [27]. Also, a recent study by Gbolade *et al.* [13] reported cytotoxic action of *Piliostigma thonningii* and *Delonix regia* stem bark using tadpole model and linked it to the rich flavonoid and phenol contents. All these publications are a pointer to the increasing discovery of cytotoxic potential of African biodiversity in combating cancer. Coumarins [24, 28], flavonoids [24] and other phytochemicals [2, 25] have been linked to cytotoxic property.

Notable published works have appeared on anticancer activity of *Cascabela thevetia* particularly those of Ramos-Silva *et al.* [7] on the fruit methanolic extract on human cancer cell lines, and Managit *et al.* [29] on flower ethanolic extract on human cervical cancer cells. Therefore, our present investigation on *Cascabela thevetia* root bark is a further contribution to the anticancer potential of this poisonous plant. Cheng *et al.* [21] and Wen *et al.* [30] have attributed the anticancer properties of *Cascabela thevetia* to its cardenolides. The two plants investigated in this study will serve to update compendium of medicinal plants that can be recommended for the treatment of oxidative stress-induced ailments such as cancer and heart diseases.

4. Conclusion

This study has established growth inhibitory and cytotoxic activities for *Ceiba pentandra* leaf and *Cascabela thevetia* root bark which have been used for various purposes in African traditional medicine. The more active aqueous and ethyl acetate fractions of both plants will require chromatographic studies to isolate the bioactive compounds. Furthermore, *Cascabela thevetia* was established as the more promising cytotoxic plant, and *Ceiba pentandra* as the more potent growth inhibitory agent.

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