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Chemical and toxicological study of leaf extracts from *Deinbollia boinensis* Capuron (Sapindaceae), a malagasy medicinal plant

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Abstract

The aim of this study was to investigate the toxicity of *Deinbollia boinensis*, a Sapindaceae endemic to Madagascar. A purification process involving precipitation with 50% ethanol, dialysis and fractionation with n-butanol was used to obtain a partially purified toxic extract (PPE) from the cold crude aqueous leaf extract (LCE). The active compounds were thermostable and soluble in water, ethanol and n-butanol. They could be precipitated by neutral lead acetate and were adsorbed on activated charcoal. Phytochemical screening of LCE and PPE revealed the presence of tannins, polyphenols, saponins, deoxyoses, triterpenes and sterols. PPE caused symptoms in mice that suggested damage to the nervous system, as well as tissue lesions in some organs characterised mainly by capillary dilatation and hemorrhagic areas in the brain, lungs and kidneys. In the liver, architectural destruction was observed. The PPE LD_{50} was in the order of 60.25 to 66.55 mg/kg of mice. PPE was also toxic to other warm-blooded animals such as guinea pigs, rats, chicks and cold-blooded animals including mosquito larvae, carp alvins and frog tadpoles. It caused lysis of sheep red blood cells. LCE inhibited the germination of seeds of some plants and the growth of young maize and cowpea bean seedlings. LCE had a stimulating effect on the development of axillary buds, while PPE had an inhibitory effect. LCE and PPE had no effect on the microorganisms tested.

Keywords: *Deinbollia boinensis*; Sapindaceae; Leaf toxicity; Warm-blooded animals; Cold-blooded animals; Effects on plants

1. Introduction

Medicinal plants are widely used as a source of medicines, especially in developing countries. The World Health Organization (WHO) estimates that up to 80% of these countries rely on locally available plant resources for their primary health care because they are easily accessible and less expensive [1]. Despite their therapeutic properties, medicinal plants must be used with extreme caution as they can be toxic [2]. The WHO recommends strengthening research and evaluating the safety and efficacy of herbal products [1].

Among the many plant families with medicinal uses is the Sapindaceae family, which contains several genera, including *Deinbollia*. This genus has 41 recognised species, including one shared between Africa, La Réunion and Madagascar, 5 endemic to Madagascar and 35 restricted to sub-Saharan continental Africa [3].

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Certain species of *Deinbollia* are used in a number of empirical applications. The leaves of *D. pinnata* are used to treat sickle cell disorder [4]. The roots and leaves of this species are used in folk medicine as a febrifuge and analgesic, and to treat intercostal bronchitis, intestinal pain, jaundice, cough, asthma and infections [5, 6, 7].

The roots of *D. oblongifolia* macerated in water are drunk to treat stomachache [8], and boiled roots heal diarrhea [9].

A decoction of *D. borbonica* roots is used to treat diabetes. Dried leaves and roots are used externally to accelerate wound healing [10].

A few species of *Deinbollia* were the subject of biological studies. *D. pinnata,* the most studied species was widely reported for its antibacterial [11], anti-inflammatory [12] and antioxidant [13] activities. *D. oblongifolia* has antibacterial activity [14].

Deinbollia boinensis, a Malagasy medicinal species, has been used by aged traditionalist for the treatment of various ailments such as fever, headaches and stomachaches and it is also cultivated as an ornamental plant in gardens and as a hedge plant [15].-However, rumours about its side-effects and toxicity persist, limiting its use as a medicine.

D. boinensis was chosen as study material in the present work. This choice was made for a number of reasons. Firstly, it is a plant that has never been the subject of any research other than botanical research. Secondly, it is used in traditional medicine, but rumours circulate about its toxicity, which were confirmed during preliminary toxicity tests carried out on mice in our laboratory. The aim of our research was to determine the chemical composition and to study the toxicity of *D. boinensis* leaf extracts on various organisms.

2. Materials and methods

2.1. Plant Material

2.1.1. Deinbollia boinensis

D. boinensis (Figure 1), a shrub of 3 to 6 m, grows on calcareous soils in the west of Madagascar from Antonibe peninsula to the Onilahy river basin. It is known under the vernacular names such as Ampelamainty, Ampoly fotsy, Dovy, Fandriatotoroka, Kamoty, Tsiramiramy.

(Source: the authors)

Figure 1 *Deinbollia boinensis*: the whole plant and leaves

Fresh leaves of *D. boinensis* were collected in Marofandiliha forest, located in the west of Madagascar, in the Morondava region, in September during its vegetative stage.

Shade-dried leaves were ground into a fine powder and then stored at -20 °C.

2.1.2. Plant Seeds

Seeds came from the collection of National Research Center for Farming (FOFIFA, Antananarivo) (Table 1).

Table 1 Plants whose seeds were used for germination assays

Plant families	Monocotyledons	Dicotyledons	Common name
Poaceae	*Zea mays*		Maize (corn)
	Oryza sativa		Rice
Apiaceae		*Daucus carota*	Carrot
		Petroselinum crispum	Chinese parsley
Asteraceae		*Lactuca sativa*	Lettuce
Brassicaceae		*Brassica sp*	Tissam white
Cucurbitaceae		*Cucurbita pepo medullosa*	Pumpkin, Zucchini
Fabaceae		*Phaseolus vulgaris*	Bean
		Pisum sativum	Pea
		Vigna unguiculata	Cowpea bean
		Vigna subterranea	Bambara pea
Lamiaceae		*Ocimum basilicum*	Basil
Liliaceae		*Allium cepa*	Onion
Solanaceae		*Solanum nigrum*	Black nightshade
		Solanum tuberosum	Potato

2.2. Animals

2.2.1. Mice

OF-1 strain Albino mice (*Mus musculus*), weighing 25 ± 2 g, came from the Pasteur Institute of Madagascar (IPM) breeding farm.

2.2.2. Tadpoles

Apode frog tadpoles (*Ptychadena mascareniensis*) were harvested from the ponds in the vicinity of the Antananarivo University site.

2.2.3. Alvins

The 2-month-old *Cyprinus carpio* alvins were provided by a fish farmer in Manjakandriana, 45 km east of Antananarivo. They were allowed to acclimatize to the aquarium conditions for a few days before testing.

2.2.4. Mosquito Larvae

Stage 3 mosquito larvae, *Culex quinquefasciatus*, came from stagnant water around the Antananarivo University Campus.

2.2.5. Chiks

One day-old chicks (*Gallus gallus domesticus*), Hubbard classic strain, were provided by poultry farmer.

2.2.6. Other Species

Tricoloured guinea pigs (*Rattus norvegicus*) came from an approved private supplier and white rats (*Rattus rattus*) of the WISTAR strain from the breeding farm of the Department of Animal Physiology of Antananarivo University.

2.3. Microorganisms Strains

Microorganisms used in this study were supplied by the Environmental Microbiology Laboratory (LME) of the National Environmental Research Centre (CNRE). They consisted of 7 strains of bacteria including 1 Gram (+) and 6 Gram (-) and 1 yeast (Table 2).

Table 2 List of germs used

Microorganisms	Strains	GRAM
Bacteria	*Staphylococcus aureus*	+
	Yersinia pestis	-
	Alcalescens dispar	-
	Escherichia coli	-
	Shigella sonnei	-
	Klebsiella oxytoca	-
	Klebsiella rhinoscleromatis	-
Yeast	*Candida albicans*	

2.4. Preparation of Extracts

2.4.1. Cold Aqueous Extraction

Leaf powder was suspended in distilled water in a ratio of 1/10 (w/v). The mixture, subjected to magnetic stirring for 3 h at room temperature, was then left to macerate overnight at 4°C. The macerate was filtered through four layers of gauze to remove insoluble residues. The filtrate obtained was centrifuged at 3,000 rpm for 30 min. The supernatant was recovered and the pellet discarded.

2.4.2. Purification

Crude extract was purified using Razanatseheno *et al.* [16] purification methods, based on solubility, molecular weight or electric charge of active principles. Partially purified extract obtained of this purification was named PPE.

2.5. Phytochemical Screening

The detection reactions of chemical groups on leaf powder and extracts were carried out according to the methods of Fong *et al*. [17] and Marini-Bettolo *et al*. [18].

2.6. Acute Toxicity Test in Animals

2.6.1. Effect on Mice

Toxic effect on mice was evaluated by intraperitoneal route (i.p). The extract was injected at a volume of 0.3 mL per 25 g of body weight.

All changes in intoxicated mice behavior were observed during 24 h after extract administration and other physiological activities or death was noted.

The LD_{50} (24 h) of extract was determined on mice by calculation and graphical methods [19]. Seven different doses of PPE were injected by i.p route on seven groups of five male mice. Another group receiving physiological serum served as control.

2.6.2. Anatomopathological Study

Histopathological examination was carried out as described by Rasoatahina *et al*. [20]. Brain, lungs, heart, stomach, liver, kidneys and intestine of mice were harvested.

The preparation of the organ sections for histopathological examinations was carried out using a classical method including the following steps: body fixation, inclusion, organ sections, preparation by microtomy, glass slide mounting, staining and microscope examination.

2.6.3. Hemolytic Activity Test

The hemolytic activity of PPE was evaluated as described previously by Razanatseheno *et al.* [16] with a slight modification.

The red blood cells (RBC) were washed three times with physiological saline. The solution was centrifuged at 3,000 rpm for 5 min. The pellet was recovered and the supernatant removed by aspiration. A 100% red cell suspension was obtained at the end of the third wash. The suspension was diluted with 2% phosphate buffered saline (PBS).

The 2% red cell suspension was distributed in the wells of a V-bottom microplate. The extract to be tested, diluted in cascade with PBS was poured into the wells. Two controls were performed: a positive control (C+) and a negative control (C-). After a gentle stirring, plate was incubated in an oven at first at 37°C for 3 h and then in a fridge at 4°C for 24 h.

The composition of the medium in each well is shown in Table 3.

Table 3 Composition of the medium for the hemolytic test

Wells n°	C+	C-	3	4	5	6	7	8	9	10	11	12
Final Concentration of PPE (µg/ml)	0	0	1000	500	250	125	62.5	31.25	15.62	7.81	3.90	1.95
PPE 1 mg/ml (µl)	0	0	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.10
PBS (µl)	0	50	0	25	37.5	43.75	46.87	48.44	49.22	49.61	49.81	49.90
2% red cell suspension (µl)	50	50	50	50	50	50	50	50	50	50	50	50
Distilled water (µl)	50	0	0	0	0	0	0	0	0	0	0	0
Final volume of mixture (µl)	100	100	100	100	100	100	100	100	100	100	100	100

2.6.4. Acute Toxicity Test in Cold Blooded Animals

Different concentrations of extract were tested in alvins and frog tadpoles according to Razanatseheno *et al*. [16] techniques. The graphical method of linear regression by Boyd [21] was used to determine the LC_{50} (24 h) or lethal concentration that killed 50% of the animals tested in 24 h.

For the mosquitoes, larvae were placed in crystallizers each containing a final volume of 200 ml of fresh water. Different concentrations of extract were added in the medium. After 24 h, dead and moribund larvae were counted.

2.7. Assays in Plants

2.7.1. Assays on Seed Germination

Batches of 10 seeds of each species were used. These seeds were first soaked in water at 30°C in darkness for 48 h. The soaked seeds were transferred on cotton wool, soaked in extract or in water in a Petri dish. Seed germination was observed 72 h later. The lifting or not of seed dormancy was assessed.

2.7.2. Effects on Seedling Growth

The effects of extract were studied on epicotyl and hypocotyl growth of rice and bean according to the method developped by Razanatseheno *et al.* [16].

2.7.3. Assays on Axillary Bud Growth

Assays were realized on 15-day-old pea seedlings previously sectioned above the second axillary bud as described by Rakoto *et al.* [22]. Effects of extracts were compared with those of the plant growth regulators gibberellins and auxin.

2.8. Antimicrobial Assays

Antimicrobial activity was assessed using the methods detailed in our previous articles [23, 24]. They consisted in measuring the inhibition zone diameter (IZD) using the disc diffusion method [23].

The results were interpreted according to the IZD scale [25]: bacteria were considered non-susceptible with an IZD ≤ 8 mm; susceptible with an 9 ≤ IZD ≤ 14 mm; highly susceptible with an 15 ≤ IZD ≤ 19 mm and extremely susceptible with an IZD ≥ 20 mm. Neomycin was used as the reference antibiotic and miconazole as the reference antifungal agent.

3. Results

3.1. Extraction Yields

Extraction of the dried leaves (50 g) of *D. boinensis* gave a dark brown colored leaf crude extract (LCE) with a pH of 4.

The extraction and purification processes are summarized in the following diagram (Figure 2).

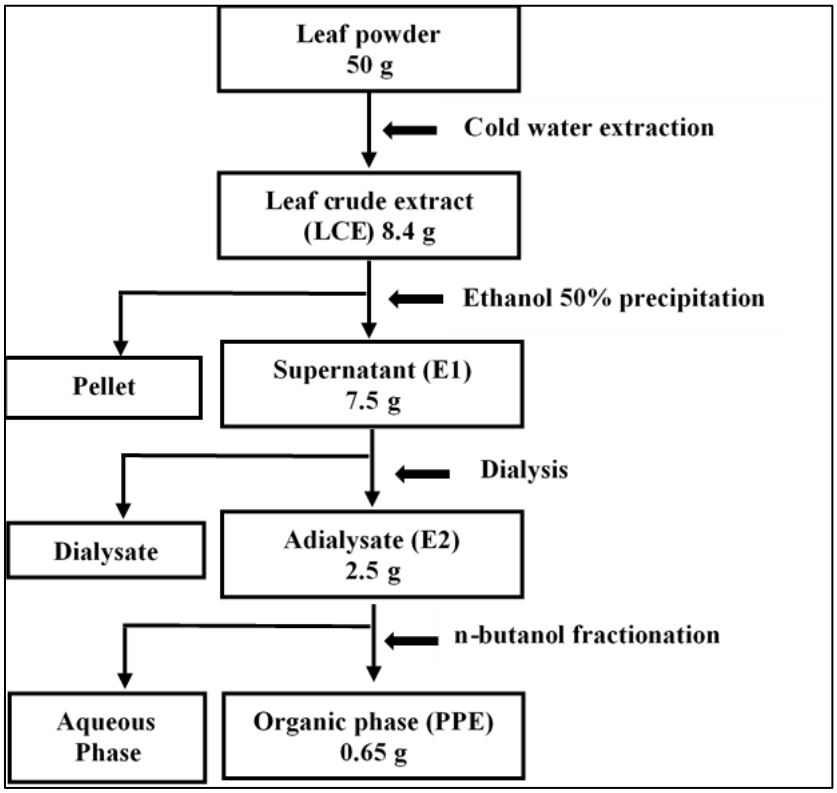

Figure 2 Diagram summarizing the extraction and the purification stages of active principles

From 50 g of *D. boinensis* leaf powder, 8.4 g of LCE and 0.65 g of partially purified extract (PPE) were obtained. The extraction yield was estimated at 16% and the purification yield 1.3%.

3.2. Phytochemical Screening

The phytochemical screening of LCE and PPE is presented in Table 4.

Table 4 Phytochemical screening results of LCE and PPE

Chemical families	Tests	Results	
		LCE	PPE
Saponins	Foam test	+	+
Tannins and polyphenols	Gelatin test	+	+
	Salted gelatin test	+	+
	Ferric chloride test	+	+
Deoxyoses	Keller – Kiliani	+	+
Iridoids	Hot HCL	-	-
Alkaloids	Wagner	-	-
	Mayer	-	-
	Dragendorff	-	-
Flavonoids and leucoanthocyanins	Wilstater	-	-
	Bate-Smith	-	-
Steroids and triterpenes	Lieberman-Burchard	+	+
	Salkowski	+	+
Anthraquinones	Bornträger	-	-

The results of phytochemical screening of LCE and PPE revealed the presence of tannins and polyphenols, deoxyoses, saponins, unsaturated sterols and triterpenes.

3.3. Effects on Animals

3.3.1. On Mice

Behavior Studies

After injection of a lethal dose of PPE (115 mg/kg), the signs of the nervous system disorders were developed by contortion of the abdomen, followed by hyperexcitation. After 20 min, its motor activity suddenly decreased. Incoordination of the limbs was observed. The respiratory systems attacks were manifested after 2 h by reduction of respiration frequency, and cyanosis. Exophthalmos and piloerection were noted. Death occurred after 3 h by ataxia and clonic convulsions. Symptoms were developed after injection of a sublethal dose of PPE (38.5 mg/kg), such as a passive state, incoordination of the limbs during rare movements accompanied by hyperemia and piloerection. A progressive remission was observed after the 10^{th} hour.

LD_{50} Values

The LD_{50} of PPE by i.p route was assessed at 60.25 mg/kg to 66.55 mg/kg body weight.

Histopathological Lesions

The study of tissue damage due to the effects of PPE was carried out on mice injected by i.p route with a lethal dose of 115 mg/kg body weight. The mice were sacrificed 3 h after the symptoms of intoxication appeared. The organs (brain, heart, stomach, liver, intestine, lungs and kidneys) were removed.

The main lesions caused by PPE on each organ are summarized in Table 5 and shown in Figure 3.

Table 5 Main lesions caused by PPE at 115 mg/kg on mice organs

Organs	Observed lesions
Brain	Dilated cerebral parenchymal capillaries
Liver	Dilated sinusoidal capillaries, altered hepatocytes
Kidneys	Retracted glomerular flocculi, hemorrhagic areas
Lungs	Dilated interalveolar capillaries
Heart	Normal myocardial tissue
Stomach	No histological lesion
Intestine	No histological lesion

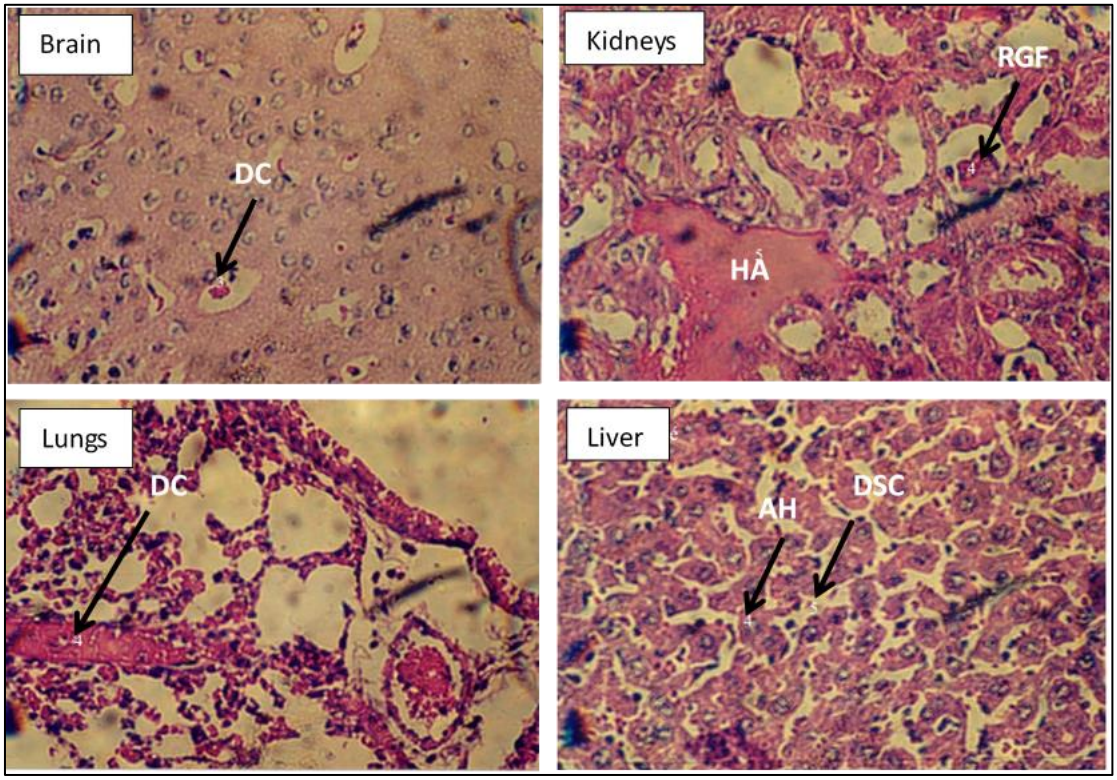

Figure 3 Main histological lesions in brain, kidney, lung and liver due to i.p. administration of the PPE at a dose of 115 mg/kg weight (magnification X 400)

- DC : dilated capillary; HA : hemolytic area; RGF : retracted glomerular flocculi; AH : altered hepatocyte; DSC : dilated sinusoidal capillary

Histopathological lesions were observed in the brain, liver, lungs and kidneys. The main detoxifying organs (liver and kidneys) were the most affected. No histological lesion was observed in intestine, heart and stomach.

3.3.2. Effects of PPE on Sheep Red Blood Cells

The hemolytic activity of PPE on sheep red blood cells is shown in Table 6 and Figure 4.

Table 6 Effects of different concentrations of PPE on sheep red blood cells

Concentration (µg/ml)	1.95	3.90	7.81	15.62	31.25	62.5	125	250	500	1000
Effect	-	-	-	-	-	+	++	++	++	++

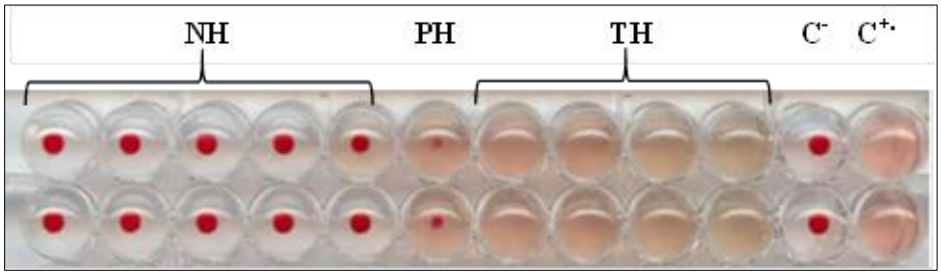

Figure 4 Hemolytic activity of PPE on sheep red blood cells

C+: Positive control; C-: Negative control; TH: Total hemolysis; PH: Partial hemolysis; NH: No hemolysis.

The hemolytic activity of PPE varied with concentration. No hemolysis was observed at a concentration ≤ 31.25 µg/ml. Partial hemolysis with sedimentation of intact red blood cells was observed at concentrations between 31.25 µg/ml to 62.5 µg/ml. Hemolysis was total at concentrations ≥ 125 µg/ml.

3.3.3. Effects on Cold-Blooded Animals

Effects on Frog Tadpoles

Seven concentrations of PPE ranging from 45.63 µg/ml to 61.15 µg/ml (reason 1.8) were tested on seven batches of frog tadpoles. The results of these tests are presented in Table 7.

Table 7 Effects of different concentrations of PPE on frog tadpoles

Concentration (C) in µg/ml	log C	Number of dead	% of death
61.15	1.786	10	100
58.23	1.765	10	100
55.46	1.743	10	100
52.82	1.722	10	100
50.30	1.701	10	100
47.91	1.680	10	100
45.63	1.659	0	0

According to these results, the PPE effect obeyed the « all-or-nothing law »: at 45.63 µg/ml the mortality rate was 0%, whereas at 47.91% it was already 100%.

Effects on Carp Alvins

Eight PPE concentrations ranging from 40.65 µg/ml to 45.12 µg/ml, with a geometric reason of 1.015, were tested. The results are shown in Table 8.

Table 8 Effects of different concentrations of PPE on carp alvins

Concentration (C) en µg/ml	log C	Number of dead	% of death
45.12	1.654	10	100
44.45	1.647	10	100
43.79	1.641	10	100
43.14	1.634	10	100
42.51	1.628	10	100
41.88	1.622	0	0
41.26	1.615	0	0
40.65	1.609	0	0

According to these results, the « all-or-nothing law » was also observed: at 41.88 µg/ml the mortality rate was 0%, whereas at 42.51% it was already 100%.

Effect on Mosquito Larvae

A batch of twenty larvae was tested at a concentration of 2 mg/ml of PPE. After 24 h, ten of the twenty larvae tested died. The LC_{50} (24 h) for mosquito larvae was therefore estimated at 2 mg/ml.

3.3.4. Effects on Chicks

At 115 mg/kg, a lethal dose on mice, chicks died of ataxia and a series of convulsions in about 40 min after administration of PPE by i.p route.

3.3.5. Effects on Rats and Guinea Pigs

In rats and guinea pigs, symptoms developed after injection of PPE at 115 mg/kg body weight, which was lethal to mice, did not lead to death. Symptoms were similar to those observed on mice, with the exception of hyperaemia. Progressive remission occurred after 20 h.

3.3.6. Effects on Plants

Effects of PPE on Seed Germination Capacity

The effect of PPE (1 mg/ml) was evaluated on seed germination (Table 9). On one hand, PPE did not affect the germination of peas (*Pisum sativa*), cowpeas bean (*Vigna inguiculata*), and potatoes (*Solanum tuberosum*) (0% inhibition). On the other hand, for lettuce (*Lactuca sativa*), bambara pea (*Vigna subterranea*), basislic (*Ocimum basilicum*) and onion (*Allium cepa*), germination was 100% inhibited. For other seeds, inhibition varied from 10% (*Phaseolus vulgaris*) to 60% (*Cucurbita pepo medullosa*).

Table 9 Effects of PPE on seed germination

Plant families	Species	Common name	Germination rate (%)	Inhibition rate (%)
Poaceae	*Zea mays*	Maize (corn)	100	0
	Oryza sativa	Rice	80	20
Apiaceae	*Daucus carota*	Carrot	50	50
	Petroselinum crispum	Chinese parsley	60	40
Asteraceae	*Lactuca sativa*	Lettuce	0	100
Brassicaceae	*Brassica sp*	Tissam white	80	20
Cucurbitaceae	*Cucurbita pepo medullosa*	Pumpkin, Zucchini	40	60

Fabaceae	*Phaseolus vulgaris*	Bean	90	10
	Pisum sativum	Pea	100	0
	Vigna unguiculata	Cowpea bean	100	0
	Vigna subterranea	Bambara pea	0	100
Lamiaceae	*Ocimum basilicum*	Basil	0	100
Liliaceae	*Allium cepa*	Onion	0	100
Solanaceae	*Solanum nigrum*	Black nightshade	60	40
	Solanum tuberosum	Potato	100	0

LCE Effects on the Growth of Young Seedlings

Experiments were carried out on maize and cowpea beans at LCE doses ranging from 0.038 mg/ml to 9.81 mg/ml (Table 10).

Table 10 Growth inhibition rate of maize and cowpea hypocotyls and epicotyls

		Control (water)	**LCE (mg/ml)**				
			0.038	**0.153**	**0.613**	**2.453**	**9.81**
Zea mays	Epicotyl (cm)	22	20	18	15	14	4
	Inhibition (%)	0	9.09	18.18	31.81	36.36	81.81
	Hypocotyl (cm)	14	14	12	10	3.5	0.5
	Inhibition (%)	0	0	14.28	28.57	75	96.43
Phaseolus vulgaris	Epicotyl (cm)	14	12	10	4.5	0	0
	Inhibition (%)	0	14.28	28.57	67.85	100	100
	Hypocotyl cm)	9	8	5	0	0	0
	Inhibition (%)	0	11.11	44.44	100	100	100

At 0.038 mg/ml, LCE had no significant effects on hypocotyl and epicotyl development. At concentrations ≥ 0.153 mg/ml, growth inhibition was observed. This inhibition increased with dose. At 9.81 mg/ml, inhibition was almost total.

Effects of LCE and PPE on the Development of Apical Dominance

The experiments were carried out on pea (*Pisum sativum*) seedlings. The effects of LCE and PPE were estimated by measuring the growth of axillary buds on treated seedlings (Table 11).

Table 11 Effects of extracts on the growth rate of seedling axillary buds

Solution tested	**% growth**	**Effect**
Water (control)	100	Normal
Gibberellin	191.66	Stimulator
Auxin	12.5	Inhibitor
LCE	137.5	Stimulator
PPE	75	Inhibitor

LCE stimulated axillary bud development. However, the stimulation was less than that of gibberellin, a plant hormone known for its stimulating effects.

On the other hand, PPE had an inhibitory effect but this was less than that of auxin, a hormone known to inhibit axillary bud growth.

3.3.7. Effects on Microorganisms

At high concentrations of LCE and PPE (153 mg/ml and 11.5 mg/ml respectively), no antimicrobial activity was observed.

4. Discussion

Several extraction techniques were tried but the cold aqueous extraction method was chosen for the preparation of the leaf crude extract (LCE). It was less expensive and LCE was less heterogeneous than the other extracts obtained by the other extraction methods tested but much more toxic. The extraction yield was 16% at this leaf harvesting time.

The purification techniques selected or not, provided information on the physico-chemical properties of the active principles. They were thermostable compounds, soluble in polar solvents such as water and ethanol, and in organic solvents such as n-butanol. They can be precipitated by heavy metal salts such as PNA. The molecular weight of the active principles could be greater than 15,000 Da, as they were unable to cross the dialysis membrane. Their strong adsorption to activated charcoal suggested that they probably had aromatic or cyclic rings in their structure. The same chemical groups were found in LCE and PPE of *D. boinensis* such as tannins and polyphenols, saponins, sterols and triterpenes.

The active principles in *D. boinensis* leaf extract were toxic to various animal and plant organisms. Intraperitoneal administration of the extract induced on mice symptoms suggestive of damage to the central nervous system.

The LD_{50} (24 h) of PPE ranged from 60.25mg/kg to 66.55mg/kg in mice. According to the Hodge and Sterner scale [26], the extract belonged to the category of moderately toxic substances (50 mg/kg ≤ LD_{50} ≤ 500 mg/kg). To the best of our knowledge, there are no yet reports of any form of intoxication or side effects from the medicinal use of other *Deinbollia* species. In comparison with the toxicity of other species of the Sapindaceae family in mice, PPE was more toxic than the aqueous extract of *Paullinia pinnata* leaves (LD_{50} of 1131 mg/kg) [27] but less toxic than the methanolic extract of *Dodonea madagascariensis* seeds (LD_{50} of 36.12 mg/kg) [28].

Histopathological examinations revealed that PPE caused varying degrees of damage to certain organs. The kidneys and liver were the most affected. In the kidneys, retraction of the glomerular flocculi and hemorrhagic areas were observed. In the liver, severe dilatation of the sinusoidal capillaries and alteration of the hepatocytes led to architectural disorganisation of the organ. The hemorrhagic zones were certainly due to the surface-active and haemolytic activities of the active principles. In the brain and lungs, the lesions were characterised mainly by dilatation of the capillaries. Intestine, heart and stomach appeared normal.

PPE has strong hemolytic activity, which is certainly due to saponins. These compounds interact with sterols (such as cholesterol) present in the cell membranes of red blood cells. This interaction creates pores in the membrane, increasing its permeability and facilitating the movement of ion: Na^+ and H_2O enter, K^+ leaves, the membrane bursts, allowing hemoglobin to leave [29]. Compared with the hemolytic potency of the partially purified extract of *Gambeya boiviniana*, which induced total hemolysis *in vitro* at 250 mg/ml [20], PPE was much more active (125 µg/ml). Its hemolytic effect was similar to that of Albodorin, a pure saponoside isolated from *Albizia odorata* (125 µg/ml) [30].

These results on the toxicity of *D. boinensis* on mice justified the rumours about the side effects and toxicity of this plant.

PPE was also highly toxic to cold-blooded animals. It acted at very low doses, in the order of 40 µg/ml. Alvins were more sensitive than tadpoles. In both species, the « all-or-nothing law » was observed. The high toxicity of *D. boinensis* to cold blooded animals could be exploited as a potential natural herbal plant to combat harmful animals as insects.

In plants, PPE inhibited the germination of various vegetable seeds. Tegumental inhibition of germination was linked to the impermeability of the seeds, which was the result of the presence of specific compounds [31]. This inhibition could be due either to a direct effect of the toxic principles on the embryos, leading to their destruction, or to an indirect effect by preventing the embryos from using the reserves contained in the cotyledons, or by inactivating the enzymes involved in their germination. The growth of young maize and cowpea bean seedlings was also inhibited by LCE at different concentrations. This inhibition was dose-dependent. At 0.613 mg/ml, the cowpea bean seeds rotted after a few days. LCE had a stimulatory effect on the development of axillary buds, whereas PPE had an inhibitory effect.

LCE and PPE at the doses used had no effect on the microorganisms studied including *Candida albicans*, *Staphylococcus aureus*, *Yersinia pestis*, *Alcalescens dispar*, *Escherichia coli*, *Shigella sonnei, Klebsiella oxytoca* and *Klebsiella rhinoscleromatis*.

5. Conclusion

The results obtained, although preliminary, confirmed the presence of toxic principles in *D. boinensis*. In view of the results, further toxicological studies will be required on the different organs of the plant. Harvesting at different seasons could be beneficial, as the toxic compounds generally vary according to the plant's stage of growth. They constituted the first scientific results on the physico-chemical, the chemical nature and the toxicological properties of the toxic principles contained in the leaves

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interests.

Statement of ethical approval

All the tests on animals were approved and in line with the standard established by Ethics Committee of the IPM.

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