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Chemical and toxicological study of extracts from the stem bark of *Uapaca thouarsii* Baill. (Phyllanthaceae), a medicinal plant endemic to Madagascar

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Abstract

The aim of this study was to assess on different organisms the toxicity of extracts from the stem bark of *Uapaca thouarsii,* a medicinal plant endemic to Madagascar. An aqueous crude extract (CE) was prepared from bark powder. CE was successively dialysed and fractionated with n-butanol. A partially purified toxic extract (E2) was obtained with a yield of 0.13%. The bitter-tasting toxic principles were thermostable, precipitable by neutral lead acetate and adsorbed by activated charcoal. Phytochemical screening of the bark powder revealed the presence of triterpenes, leucoanthocyanins, unsaturated sterols and a small amount of saponins. By intraperitoneal (i.p.) route, the LD_{50} (24 h) was between 396.12 mg/kg and 398.56 mg/kg of body weight (b.w.). No symptoms were observed when E2 was administered by gavage. CE and E2 caused sheep erythrocyte lysis. They were toxic to frog tadpoles with LC_{50} of 35 µg/ml and 25 µg/ml respectively and to carp alvins by an action following the all-or-nothing law. CE significantly inhibited seed germination of lettuce, tomato, zucchini, basil and onion, while no inhibition of germination was observed for pea, cowpea, rice and maize. In the antibiogram test, of the 14 strains tested, *Shigella sonnei, Escherichia coli and Listeria ivanovii* were very sensitive to CE and sensitive to E2, while *Listeria seeligeri* was very sensitive to CE but resistant to E2.

Keywords: *Uapaca thouarsii*; Toxicity; Hemolytic property; Antibacterial property; Germination inhibition

1. Introduction

Worldwide, traditional medicine accounts for 40% of healthcare, with phytotherapeutic treatments making up 85% of practices. Plants have long been the world's main source of medicines, with more than 50,000 species of medicinal plants recorded [1]. Traditional medicine is also attracting growing interest because of its accessibility and low cost [2].

According to the World Health Organization (WHO) [3], 80% of the rural population in developing countries still depended on phytotherapy or alternative medicine. In modern medicine, plants are also recognised for their therapeutic properties. Although regulations on traditional medicine are still limited, the WHO encourages the promotion of the rational and safe use of these plants, as well as further research and evaluation of the safety and efficacy of phytotherapeutic products. It is essential to use these plants with caution because of their potential toxicity [1].

The genus *Uapaca*, which belongs to the Phyllanthaceae family (formerly Euphorbiaceae), comprises 60 species found in Tropical Africa and Madagascar [4] including 8 endemic to Madagascar [5]. Species belonging to this genus are known

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for their antioxidant [6], anti-inflammatory, antihyperglycemic [7], antimicrobial [8], anti-ulcer, analgesic, [9, 10], and antiplasmodial properties [11].

Uapaca thouarsii is a common plant in forests near the coast at altitudes of up to 500 m, in temperate and humid climates, especially on the eastern slopes of Madagascar [12]. This plant is traditionally used to treat abdominal colic [13] and may have antimicrobial properties. According to villagers, the bark of this plant is used in decoction as a tonic. The plant is also used as an aphrodisiac [14] and the roots are used to treat tiredness, neuralgia, and insomnia [15]. However, according to Boiteau [16], *Uapaca thouarsii* is classified as a toxic Malagasy plant or one with powerful active principles in the traditional pharmacopoeia.

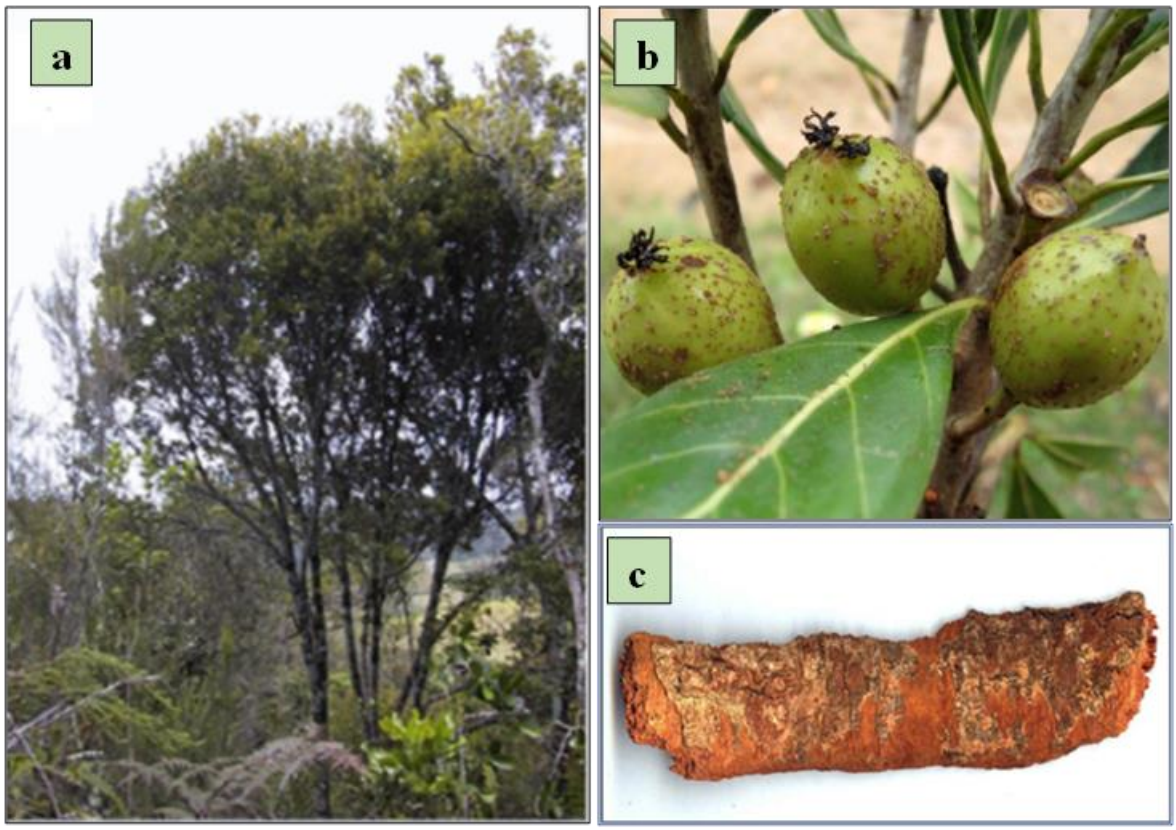
As part of our research programme on the potential toxicity of Malagasy medicinal plants, we analysed the chemical composition and toxicity of *Uapaca thouarsii* stem bark extracts on various organisms in order to ensure the safe use and to investigate its other biological properties.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

Uapaca thouarsii is a 10-20 m tall tree (Figure 1) with adventitious roots at the base of the trunk. The plant material was collected in the Mandraka (Manjakandriana district, Analamanga region) in March. Its vernacular name is "Voapaka". The fresh stem bark from this plant was air-dried for a week. It was then ground into powder using a microgrinder. The powder obtained, stored at room temperature, was used as study material.



(source: the authors)

Figure 1 *Uapaca thouarsii* a) whole plant b) fruits c) stem bark

2.1.2. Plant seeds

Seeds of 9 plants from different families were used for toxicity study on plants. They were provided by the National Centre for Applied Research for Rural Development (FOFIFA/ CENRADERU).

2.1.3. Mice

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

2.1.4. Frog tadpoles

Legless frog tadpoles (*Ptychadena mascareniensis)* were collected from rice fields on the campus of the University of Antananarivo. They were placed in a rainwater aquarium a few days before the test.

2.1.5. Fishes

Four-week-old *Cyprinus carpio* alvins and weighing an average of 1.2 g, came from an approved breeding centre in Maharidaza (Analamanga region, Manjakandriana district). They were acclimatised in the laboratory aquarium for a week before use.

2.1.6. Mosquito larvae

Larvae of the mosquito *Culex quinquefasciatus*, in the third stage of development, were collected near the campus of the University of Antananarivo.

2.1.7. Microorganisms

The microbial strains used included Gram (-) and Gram (+) bacteria, as well as a unicellular yeast, which were pathogenic germs from the collection of the Environmental Microbiology Laboratory (L.M.E.) of the National Environmental Research Centre (C.N.R.E.) in Tsimbazaza.

2.2. Methods

2.2.1. Extraction methods

The stem bark powder was suspended in cold distilled water at a ratio of 1:30 (w/v). The mixture was stirred for 3 h at room temperature under magnetic stirring. It was then macerated at +4°C for 1 night. The mixture was then stirred again for 30 min before being filtered through four layers of gauze. Then, the filtrate was centrifuged at 16,000 x g for 10 min. The supernatant obtained was evaporated under reduced pressure. The residue obtained, dissolved in distilled water, constituted the aqueous crude extract.

2.2.2. Phytochemical screening

The reactions used to detect the chemical groups were those developed by Fong *et al*. [17] and Hemingway and Karchesy [18].

2.2.3. Treatment with neutral lead acetate (NLA)

A specific volume of 20% NLA was added dropwise to CE under magnetic stirring. This procedure was repeated until no precipitate was formed. The resulting precipitate was then removed by centrifugation at 3,000 x g for 5 min. Excess lead in the supernatant was precipitated with a 10% disodium phosphate solution and then removed by centrifugation. The supernatant was concentrated by evaporation under reduced pressure.

2.2.4. Activated charcoal treatment

Treatment with activated charcoal was carried out using the Jeannoda method [19].

2.2.5. Precipitation with ethanol

Absolute ethanol was poured dropwise in the extract until no precipitate appeared. The mixture was magnetically stirred and left at +4°C for 15 min. The precipitate was removed by centrifugation at 12,000 x g for 15 min. The alcohol was evaporated and the extract concentrated.

2.2.6. Dialysis

A volume of extract, introduced into a 15,000 Da dialysis membrane, was dialysed for 24 h at room temperature against frequently renewed distilled water. At the end of dialysis, the solutions inside (adialysate) and outside (dialysate) the membrane were concentrated by evaporation and then centrifuged at 3,000 x g for 10 min to remove any precipitates.

2.2.7. Fractionation by n-butanol

The aqueous crude extract was diluted by half with distilled water and fractionated with an equal volume of n-butanol (v/v). The mixture was stirred manually, then left to settle until the phases separated. The organic and aqueous phases were collected separately, and the interphase was discarded. The aqueous phase was subjected to two further successive fractionations with n-butanol. The fractions obtained were then concentrated and returned to the starting volume by evaporation under reduced pressure.

2.2.8. Qualitative analysis by thin layer chromatography (TLC)

The extracts were deposited on 60 F_{254} silica gel plates using capillary tubes and developed in a chromatography vessel with a mobile phase consisting of n-butanol/acetic acid/water in a 60/20/20 (w/w/w) ratio. The developed TLC plates were dried and observed under ultraviolet light at 254 nm and 366 nm. They were then sprayed with vanillin/sulphuric acid reagent (development) and dried with a hair dryer.

2.2.9. Methods for studying effects on animals

Assessment of toxicity in warm-blooded animals

- Acute toxicity test on mice

Two routes (intraperitoneal or i.p. and oral routes) were used to assess acute toxicity in mice where 0.3 ml of extract was administered per 25 ± 2 g of body weight (b.w.) and the animals were then observed for a period of 24 h. A mouse given physiological solution (NaCl 9‰) served as a negative control.

- Estimation of the median lethal dose (LD_{50}) [20]

Six doses of E2, in geometric progression r = 1.2, from 252.6 mg/kg (0% mortality) to 628.8 mg/kg (100% mortality), were injected intraperitoneally into six batches of 5 mice each. A batch of 5 mice given 0.3 ml physiological water (NaCl 9‰) was used as a control. The results were interpreted using the Néné-Bi *et al.* scale [21].

- Determination of haemolytic activity

The haemolytic activity of CE and E2 was assessed on sheep erythrocytes, as described by Rasoatahina *et al.* [22]. Two controls were performed: a positive control (C+) containing 50 µl of distilled water mixed with 50 µl of 2% red cell suspension (appearance of haemolysis) and a negative control (C-) containing 50 µl of PBS mixed with 50 µl 2% red cell suspension (absence of haemolysis). The microplate was incubated at 37°C for 3 h and then at +4°C overnight.

Acute toxicity test in cold-blooded animals

Tests on cold-blooded aquatic animals (frog tadpoles, carp alvins, and mosquito larvae) were conducted based on the methods described by Razanatseheno *et al.* [23]. The graphical linear regression method [24] was used to determine the LC_{50} (24 h).

2.2.10. Assays on seed germination

Before any handling, the seeds to be tested were disinfected by immersion in a 1% bleach solution for 10 min and then rinsed with distilled water. For each species, two batches of 10 seeds each were soaked in the test extract at a concentration of 1 mg/ml. The first batch of 10 seeds was germinated on cotton wool soaked in the test extract. The seeds were sprayed with the extract every other day to prevent them from drying out. The control batch (second batch) was placed on cotton wool soaked in tap water and also sprayed with this water. The seeds were left in the dark for 48 h at 30°C. The results obtained were expressed as germination percentage.

2.2.11. Antimicrobial assays

The method used to determine antimicrobial activity was the agar diffusion method (antibiogram method) detailed in a previous study [25]. The results were interpreted according to Ponce *et al.* criteria [26]**.** Bacteria were classified according to the diameters of their inhibition zone diameter (IZD): insensitive for IZD ≤ 8 mm, sensitive for 9 mm ≤ IZD ≤ 14 mm, very sensitive for 15 mm ≤ IZD ≤ 19 mm and extremely sensitive for IZD ≥ 20 mm.

3. Results

3.1. Extraction and purification process

The diagram summarizing the extraction and purification steps is shown in Figure 2.

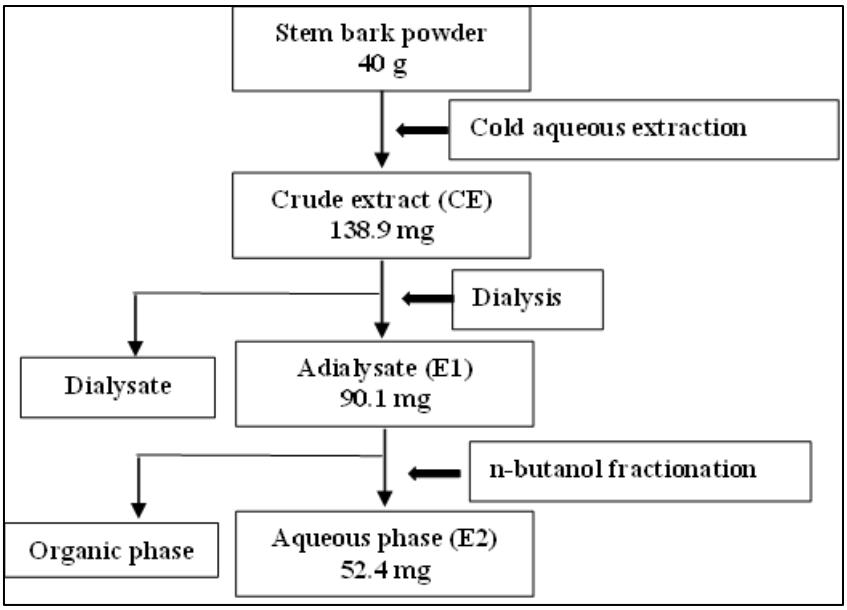


Figure 2 Diagram summarizing the extraction and the purification steps of active principles

3.2. Chemical families present in stem bark powder

The main chemical groups present in stem bark powder are shown in Table 1.

Table 1 Results of screening for *U. thouarsii* stem bark powder

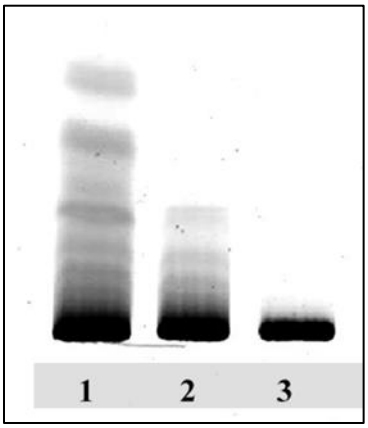
Chemical groups	Test	Result
Alkaloids	Mayer	-
	Wagner	-
	Dragendorff	-
Flavonoids	Willstatter	-
Leucoanthocyanins	Bate- Smith	+
Tannins and polyphenols	Gelatin test	-
	Salted gelatin test	-
	Ferric chloride test	-
Unsaturated sterols	Salkowski	+
Triterpenes	Lieberman- Burchard	+
Steroids		-
Deoxyoses	Keller- Kiliani	-
Iridoids	Hot HCl	-
Saponins	Foam test (foam height 1 cm)	±

- : negative; ±: weakly positive ; +: positive;

Stem bark powder contained mainly leucoanthocyanins, unsaturated sterols, triterpenes and a small amount of saponins. Alkaloids, flavonoids, tannins, polyphenols, deoxyoses, steroids and iridoids were absent.

3.3. Extract homogeneity

The homogeneity of the extracts obtained after the different steps was assessed by thin layer chromatography (TLC) (Figure 3). CE showed 10 major bands visible under UV light at 366 nm. Contaminants were progressively removed, resulting in a much more purified E2 extract. Vanillin sputtering revealed the same bands.



1: Crude extract (CE) ; **2** : Adialysate (E1) ; **3** : Aqueous phase (E2)

Figure 3 Summary chromatograms of the purification of toxic principles from *U. thouarsii* stem bark

3.4. Effects of extracts on mice

3.4.1. Influence of the route of administration on E2 toxicity

After injection at a dose of 628.8 mg/kg b.w., E2 was toxic to mice by the i.p. route. One to two min after injection, mice showed intermittent abdominal torsion. After 2 h, their ears were pulled forward and dilation of the auricular capillaries was observed. Around the sixth hour, enophthalmos was noted: the hair bristled, the mice huddled in a corner of the cage and showed motor hypoactivity. Intermittent diarrhoea was also observed. The mice died after 15 to 18 h due to respiratory difficulties. However, to the same dose of E2, but administered orally, the mice showed no symptoms of intoxication.

Following injection of the sublethal dose of 252.6 mg/kg b.w., the mice showed the same symptoms, except for respiratory difficulty. They recovered 48 h after injection.

3.4.2. Determination of the LD_{50}

The LD_{50} (24 h) of E2 extract by the i.p. route was assessed at 398.56 mg/kg and 396.12 mg/kg b.w., by calculation and graphical methods respectively.

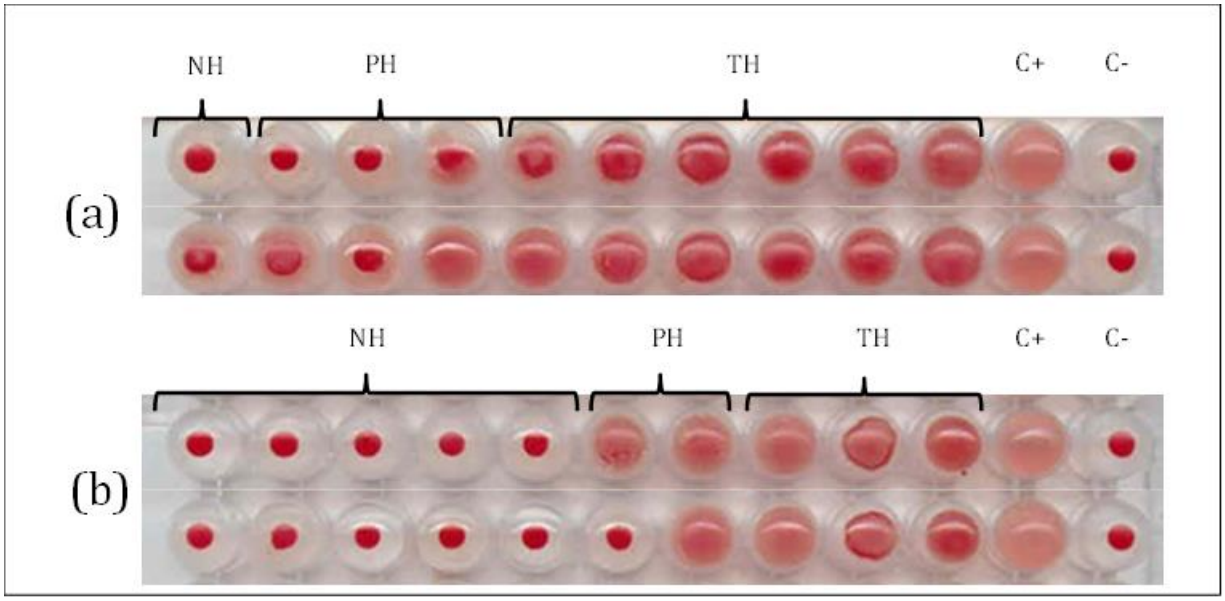
3.4.3. Assessment of haemolytic activity

The effects of CE and E2 on sheep erythrocytes were studied using increasing concentrations ranging from 0.9 µg/ml to 500 µg/ml. Total haemolysis was observed from a concentration of 15.6 µg/ml for CE and 125 µg/ml for E2. Partial haemolysis was noted between 1.9 µg/ml and 7.8 µg/ml for CE, and between 31.25 µg/ml and 62.5 µg/ml for E2. Table 2 and Figure 4 illustrate the results.

Table 2 Comparison of the haemolytic effects of CE and E2 at different concentrations on sheep erythrocytes

Concentration (µg/ml)	0.9	1.9	3.9	7.8	15.6	31.25	62.5	125	250	500
CE	-	±	±	±	+	+	+	+	+	+
E2	-	-	-	-	-	±	±	+	+	+

+ Total hemolysis; ±: Partial hemolysis; -: No hemolysis



TH: Total hemolysis; PH: Partial hemolysis; NH: No hemolysis

Figure 4 Haemolytic activity of CE (a) and E2 (b) at different concentrations on sheep erythrocytes

3.5. Effects of extracts on cold-blooded animals

3.5.1. Effects on frog tadpoles

Four concentrations of CE ranging from 27 to 48 µg/ml (reason 1.2) were tested on 4 batches of ten frog tadpoles. Then, seven concentrations of E2 ranging from 17 to 40.3 µg/ml (reason 1.15) were evaluated on seven batches of ten tadpoles. The results showed a dose-response relationship for the two extracts tested, with respective LC_{50} values of 35 µg/ml for CE and 25 µg/ml for E2. These results are presented in Tables 3 and 4.

Table 3 Effects of CE on frog tadpoles

Concentration (C) in µg/ml	log C	Death (%)
27	1.41	0
33	1.51	10
41	1.60	80
48	1.68	100

Table 4 Effects of E2 on frog tadpoles

Concentration (C) in µg/ml	log C	Death (%)
17	1.23	0
20	1.30	10
23	1.36	60
26	1.41	70
30.3	1.48	70
35	1.54	90
40.3	1.6	100

3.5.2. Effects on carp alvins

Six CE concentrations ranging from 1 to 33.1 µg/ml (reason approximately 2) were tested. A batch of 6 carp alvins was used for each concentration. Then, 8 concentrations of E2 ranging from 1.11 to 4.1 µg/ml (reason 1.2) were evaluated on eight batches of 6 carp alvins. The effects of CE and E2 on carp alvins obeyed the « all-or-nothing » rule. A concentration of 4.1 µg/ml for CE and 2.37 µg/ml for E2 caused the death of all the alvins (100%), while a concentration below these thresholds produced no effect (0%). These results are presented in Table 5 and 6**.**

Table 5 Effects of CE on carp alvins

Concentration (C) in µg/ml	log C	Death (%)
1	0	0
2	0.3	0
4.1	0.61	100
8.2	0.91	100
16.5	1.21	100
33.1	1.51	100

Table 6 Effects of E2 on carp alvins

Concentration (C) in µg/ml	log C	Death (%)
1.11	0.04	0
1.13	0.05	0
1.16	0.06	0
1.97	0.29	0
2.37	0.37	100
2.84	0.45	100
3.41	0.53	100
4.1	0.61	100

3.5.3. Effects on mosquito larvae

A CE concentration of 1.389 mg/ml was tested on a batch of 10 mosquitos' larvae. The results showed that *Culex quinquefasciatus* larvae were insensitive to CE at this concentration. At higher concentrations, the experiment could not be carried out due to the strong coloration of the extract which made the medium opaque and the larvae difficult to count.

3.6. Effects of extracts on plant seed germination

The results of the study (Table 7) showed that CE (1 mg/ml), compared to control seeds watered with tap water, had different effects on germination of different seed species. Total inhibition of germination (100%) was observed for lettuce, tomato, zucchini, basil and onion. Partial inhibition was noted for bean (50%), carrot (50%), black nightshade (90%), and tissam white (20%). No inhibition of germination was observed in pea, cowpea, rice and maize.

Table 7 Effects of CE at 1 mg/ml on vegetable seeds after 48 h

Plant families	Species	Common names	GP (%)	IP (%)
Fabaceae	*Phaseolus vulgaris*	Bean	50	50
	Pisumsativum	Pea	100	0

	Vigna indicate	Cowpea	100	0
Asteraceae	*Lactuca sativa*	Lettuce	0	100
Solanaceae	*Solanum nigrum*	Black nightshade	10	90
	Solanum lycopersicum	Tomato	0	100
Cucurbitaceae	*Cucurbita pepo*	Zucchini, courgette	0	100
Apiaceae	*Daucus carota*	Carrot	50	50
Lamiaceae	*Ocimum basilicum*	Basil	0	100
Brassicaceae	*Brassica sp.*	Tissam white	80	20
Liliaceae	*Allium cepa*	Onion	0	100
Poaceae	*Oryza sativa*	Rice	100	0
	Zea mays	Maize	100	0

GP (%): germination percentage; IP (%): Inhibition percentage

3.7. Effects of extracts on microorganisms

The results showed variability in the response of bacteria to the extracts studied. According to Ponce *et al*. [26], on the one hand, *Serratia marcescens*, *Salmonella typhi, Pasteurella multocida, Candida albicans, Alkalescens dispar, Staphylococcus aureus, Streptococcus spp*, *Listeria welshimeri, Gardnerella vaginalis* and *Neisseria gonorrhoeae* were insensitive to both CE and E2. On the other hand, *Shigella sonnei, Esherichia coli* and *Listeria ivanovii* were very sensitive to CE and sensitive to E2. IZD varied from 6 mm to 19 mm for CE and from 6 mm to 10 mm for E2. *Listeria seeligeri* was very sensitive to CE but resistant to E2. The results are presented in Table 8.

Table 8 Effects of CE and E2 on the microbial strains tested

Strains	**Gram**	**CE (2.778 mg/disk)**	**E2 (1.04 mg/disk)**
Serratia marcescens	-	6	6
Salmonella typhi	-	6	6
Shigella sonnei	-	18	9
Pasteurella multocida	-	6	6
Alcalescens dispar	-	6	6
Escherichia coli	-	19	9
Neisseria gonorrhoeae	-	6	6
Staphylococcus aureus	+	6	6
Streptococcus spp.	+	6	6
Listeria welshimeri	+	8	8
Listeria seeligeri	+	16	8
Listeria ivanovii	+	18	10
Gardnerella vaginalis	Gram-variable	6	6
Candida albicans	Yeast	6	6

4. Discussion

The extraction and purification techniques used were guided by toxicity tests on mice and TLC.

Several extraction techniques were tested, but the cold aqueous extraction method was chosen for the preparation of the crude stem bark extract (CE) due to its greater toxicity on mice, resulting in a shorter survival time compared to other crude extracts. This aqueous extraction was also simple, inexpensive and compatible with the traditional use of the plant, which was usually used in the form of a decoction.

From 40 g of stem bark powder, cold extraction gave a yield of 0.34%. After purification, the yield of toxic principles was 0.13% of the starting powder. The crude extract obtained was black in colour, syrupy in consistency, bitter in taste and heat resistant, with a pH of 6.25.

The poorly performing methods that were not selected but provided additional data on the physico-chemical properties of the toxic ingredients. These showed an affinity for polar solvents such as water and ethanol. They were precipitable by neutral lead acetate indicating the presence of acid groups or a high molecular weight [27]. In addition, they were adsorbed by activated charcoal, suggesting the presence of aromatic rings in their structure [17, 28]. The toxic principles did not diffuse through the dialysis membrane. Consequently, their molecular weight was greater than 15,000 unless they interfered with the membrane.

Studies on the acute toxicity of E2 were conducted to assess its safety. I.p. administration of E2 induced symptoms of intoxication characterised mainly by intermittent abdominal torsion, dilation of auricular capillaries, enophthalmos, bristling hairs, motor hypoactivity, intermittent diarrhoea and respiratory difficulty. These reactions observed in mice suggested that the toxin(s) caused central nervous system depression. The LD_{50} (24 h) was between 396.12 mg/kg and 398.56 mg/kg b.w. With such an LD_{50}, E2 was moderately toxic according to the Hodge and Sterner scale [29] but very toxic (LD_{50} between 50 and 500 mg/kg) according to the Nene Bi *et al*. scale [21].

Oral administration of the extract did not however, cause any symptoms of intoxication at the dose used (628.8 mg/kg b.w.). This could be due to the destruction of the toxic principles by the acidity of the digestive tract, their enzymatic inactivation or their non-absorption by the intestinal wall [30]. Apparently, E2 was not orally toxic in mice, but further studies will be needed to confirm these observations. Other studies have also highlighted the low oral toxicity of plant extracts from the *Uapaca* genus. This was the case for *U. togoensis* stem bark extract, whose LD_{50} was found to be greater than 5000 mg/kg b.w. and for which no mortality was recorded during the 48 h observation period [11]. The same was true of the ethanolic extract of *U. pilosa* leaves, with no deaths observed after 14 days of observation [31].

When tested intraperitoneally on mice, CE was more toxic than the hot aqueous extract of *Myrica spathulata* Mirb stem bark (Myricaceae) with an estimated LD_{50} between 875.99 mg/kg and 877.8 mg/kg b.w. [32]. However, CE was less toxic than the hydroethanol extract of *Gambeya boiviniana* leaves (Sapotaceae) with an estimated LD_{50} between 118.15 mg/kg and 122.39 mg/kg b.w. [22].

CE and E2 caused lysis of sheep erythrocytes *in vitro.* Compared with the haemolytic power of other plant extracts (Table 9), that of CE was significantly higher. The effect of E2 was also important (≥ 125 µg/ml), but was significantly smaller than that of CE (≥ 15.6 µg/ml). Some of the active ingredients present in CE may have been eliminated or quantitatively reduced during the purification steps.

Table 9 Concentration of crude extract of various plants from which total haemolysis of sheep erythrocytes was obtained

Crude extract	Plant species	Family	Concentration ≥	Reference
Leaf	*Deinbollia boinensis*	Sapindaceae	125 µg/ml	[33]
Leaf	*Gambeya boiviniana*	Sapotaceae	256 µg/ml	[22]
Stem bark	*Myrica spathulata*	Myricaceae	31 µg /ml	[32]
Stem bark	*Uapaca thouarsii*	Phyllanthaceae	15.6 µg/ml	The authors

Saponins are among the compounds implicated in the haemolytic effects of plants, but given their low quantity in *Uapaca thouarsii*, other secondary metabolites, including leucoanthocyanins, may also have haemolytic effects by interacting with cell membranes [34].

The extracts were also toxic to frog tadpoles and carp alvins. CE (LC_{50} = 35 µg/ml) and E2 (LC_{50}= 25 µg/ml) were significantly more toxic than the aqueous extract of *Myrica spathulata* (LC_{50} = 1.89 mg/ml) [33]. However, they are less

toxic than extracts of *Albizia androyensis* (LC_{50} = 3.85 µg/ml) or *Albizia bernieri* (LC_{50} = 7.36 µg/ml) [35]. Therefore, it is important to take precautions when using these extracts to minimise the risks to the environment.

The inhibition of seed germination by CE could be explained by a number of mechanisms, such as inhibition of the enzymes required for germination, alteration of the permeability of seed cell membranes, thus preventing the absorption of water and nutrients required for germination, the production of toxic substances that damage embryonic seed cells, an allelopathic effect or a change in the hormonal balance of the seeds [36, 37]. CE could be used to control the germination of certain undesirable or invasive plant species.

CE and E2 showed variable antibacterial activity, particularly against *Shigella sonnei, E. coli, Listeria ivanovii* and *Listeria seeligeri*. *Shigella sonnei* and *E. coli* are common pathogens responsible for diarrhoea [38, 39]. The traditional use of the plant to treat diarrhoea seemed well-founded. However, the use of this plant requires a great deal of caution and in-depth scientific research.

5. Conclusion

The results obtained, although still preliminary, showed the presence of compounds in the bark of *Uapaca thouarsii* that are toxic to various organisms. Given the number of uses of *Uapaca thouarsii* in traditional medicine in different regions of Madagascar, and the relatively broad action spectrum of toxic compounds, it is important to take precautions to protect health and the environment.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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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