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(RESEARCH ARTICLE)

Evaluation of the toxic effects of *Albizia mahalao* Capuron extracts, a Fabaceae from Madagascar, on different organisms

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Abstract

This work was designed to study the toxic effects of *Albizia mahalao* extracts on various organisms and explore their possible uses to fight against noxious organisms. Methanolic extracts of leaves (LME), stem (SME) and root (RME) barks, alkaloids (Alk) and saponosides (Sap) from leaves were tested. All extracts were toxic to mice with LD_{50} values by intraperitoneal route ranging from 69.18 (RME) to 135.52 mg/kg body weight (Alk). By oral route, at doses 5 and 10 times higher than intraperitoneal LD_{100} those extracts were not toxic. LME, SME and RME were toxic to carp alvins, frog tadpoles but not to chicks and mosquito *Culex quinquefasciatus* larvae. LC_{50} on carp alvins varied from 63.78 (RME) to 86.89 µg/mL (SME) and LC_{50} on frog tadpoles from 68.43 (RME) to 153.4 µg/mL (SME). All the methanolic extracts inhibited the germination of 53.8 % of the seed plants tested with inhibition percentages ranging from 20 to 100 %. In previous study, the same *Albizia mahalao* extracts were found to be efficient against many pathogenic bacteria at low doses. The extracts non-toxicity by oral route allowed envisaging their use to treat some diseases associated with these bacteria.

Keywords: *Albizia mahalao*; Fabaceae; Toxicity; Alkaloids; Saponosides; Warm and Cold-blooded Animals; Seed Germination.

1. Introduction

Albizia is a very cosmopolitan botanical genus including about 150 species extensively distributed in tropical and subtropical regions. In Madagascar, 30 *Albizia* species are present 24 of which are endemic, 3 also occur elsewhere and 3 are introduced [1]. A research program on toxic plants endemic to Madagascar including *Albizia* species has been conducted in our laboratory. Several Malagasy *Albizia spp*. such as *A. divaricata, A. greveana, A. masikororum, A. tulearensis, A. viridis* and *A. bernieri* were already proved to be toxic to various organisms [2, 3, 4].

The antimicrobial activities we reported in a previous paper [5], showed the efficiency of the methanolic extracts and alkaloid fraction from *A. mahalao* against pathological bacteria strains for human like *Clostridium perfringens, Salmonella enterica* and *Shigella flexneri*. These results led us to pursue our investigations on *A. mahalao* in order to identify other biological properties that could serve useful purposes such as the control of noxious organisms.

The present work focused on the study of the toxicological effects of methanol extracts and total alkaloids and saponosides from leaves, stem and root bark of *Albizia mahalao* on various cold and warm-blooded animals and plant seed germination.

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2. Material and methods

2.1. Plant material

A. mahalao Capuron is a tree growing up to 5 m high (Figure 1).

Fresh leaves, root and stem barks of *A. mahalao* were collected from Tsimanampetsotsa (South west of Madagascar) with the following geographical coordinates: 24°02'19.13" latitude; 43°45'08.28" longitude; 7 m altitude.

Figure 1 *Albizia mahalao* (a) the whole plant; (b) flower; (c) fruit; (d) seeds (Source: The authors)

2.2. Plant seeds

The seeds of 11 plants belonging to 8 botanical families were used for germination tests (Table 1). They were purchased from an approved seed supplier.

Table 1 List of plants whose seeds were used for germination assays

Plant family	Species
Apiaceae	*Daucus carotta*
Asteraceae	*Lactuca sativa*
Brassicaceae	*Brassica chinensis*
	Brassica oleraceae
	Brassica sp
	Raphanus sativus
Cucurbitaceae	*Cucumis sp*
Fabaceae	*Phaseolus vulgaris*
	Pisum sativum
Papilionaceae	*Glycine max*
Solanaceae	*Lycopersicum esculentum*
Poaceae	*Oryza sativa*
	Zea mays

2.3. Animals

2.31 Warm-blooded animals

OF-1 strain Albino mice (*Mus musculus*), weighing 25 ±2 g, were provided by the Institut Pasteur de Madagascar (IPM) breeding farm.

Day-old chicks (*Gallus gallus domesticus*) were provided by an approved farm (AVITECH).

2.3.2 Cold-blooded animals

Mosquito larvae (*Culex quinquefasciatus*) were collected from ponds or puddles in the Antananarivo University site.

Carp alvins (*Cyprinus carpio*), Royal strain, 2-3 cm size, were furnished by an approved fish farmer in Ambohimangakely, Antananarivo, Madagascar.

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

Fishes and tadpoles were allowed to acclimatize to the aquarium conditions for one day after their arrival in laboratory.

2.4. Extracts preparation

The methanolic and alkaloids extracts used in this work were prepared by the method described in our previous paper [5]. The saponosides extract was obtained according to Randriamampianina *et al.* [3]. All these extracts are presented in table 2.

Table 2 List of *A. mahalao* extracts used

Extract name	Designation	Plant part
Leaf methanolic extract	LME	Leaf
Stem bark methanolic extract	SME	Stem bark
Root bark methanolic extract	RME	Root bark
Total saponosides	Sap	Leaf
Total alkaloids	Alk	Leaf

2.5. Toxicity determination

2.5.1 Toxicity test on warm-blooded animals

2.5.1.1 Acute toxicity test

Different administration routes were used for assessing acute toxicity on mice and chicks. For intraperitoneal (ip) and subcutaneous (sc) routes, 0.3 mL of extracts was injected per 25 ± 2 g of body weight. Concerning the oral or *per os* (po) route, 0.25 mL of extracts per 25 ± 2 g of body weight was administered by means of an intubation cannula with a curved distal.

2.5.1.2 LD_{50} (24 h) assessment on mice

Seven different doses of methanolic extracts, total alkaloids and saponosides of *A. mahalao* were injected by ip route on seven groups of five male mice. Another group receiving physiological serum served as control.

2.5.2 Toxicity test on cold-blooded animals

2.5.2.1 Acute toxicity test on fishes and frog tadpoles

Groups of five animals were placed in 500 mL crystallizers containing 250 mL of spring water. Different concentrations of methanolic extracts of *A. mahalao* were added. One group without extract served as a control.

2.5.2.2 LC_{50} (24 h) assessment

The LC_{50} (24 h) of each extract was determined on fishes and frog tadpoles using different concentrations. The tests were carried out in triplicate. Results were treated using the method of statistical analysis ANOVA with Graphpad Prism 7 software. Statistical values were expressed in 95 % confidence interval.

2.5.2.3 Acute toxicity test on mosquito larvae

Culex quinquefasciatus larvae in the third stage of development (size = 5 mm) were distributed in batch of 25 and deposited in 200 mL of tested extract at 2 mg/mL. After 24 h, larval mortality resulted in complete immobility (knock-down) of the larvae or their inability to surface or dive when their environment was agitated.

2.5.3 Seed germination assays

Seeds were sterilized by soaking in sodium hypochlorite solution (5%). After 1 min., they were immediately washed with distilled water. One lot of 10 seeds of each plant test was separately soaked in each of methanolic extracts at 1 mg/mL during 48 h in the dark and another one in distilled water served as control. Seeds were then transferred on to Petri dish lined with cotton soaked with extracts (tests) or with distilled water (control). Each treatment was carried out in three replicates. During 15 days, substrate was moistened every two days with extract (test) or water (control) and germinated seeds were counted. Results were expressed as germination percentage.

3. Results

3.1. Effects of extracts on warm-blooded animals

3.1.1 Effects on mice

At the doses used (252, 120, 168, 228 and 312 mg/kg body weight respectively for LME, RME, SME, Alk and Sap, all the extracts were toxic to mice by ip but not by po and sc routes. The developed symptoms were generally similar. The main symptoms visible before death were abdominal contortion, ataxia, piloerection, dyspnea, ear hyperemia, profuse salivation and clonic convulsions.

By po route, at doses 5 and 10 times higher than DL100 by ip route (Table 3), LME, RME and SME, caused only decreases in motor activity during 1 to 2 h. Complete remission was observed after 3 h. No mortality was recorded 24 h after force-feeding.

The values of LD50 24 h by ip route of the extracts ranged from 69.18 (RME) to 135.52 mg/mL (Alk).

Table 3 Lethal doses (LD) of methanol extracts, Alk and Sap on mice

Extracts	Lethal Dose (LD) (mg/kg body weight)		
	LD_0	LD_{50}	LD_{100}
LME	69.32	118.57	252
RME	46.78	69.18	120
SME	80.26	123.03	168
Alk	93.58	135.52	228
Sap	70.94	131.22	312

LD_0: Lethal Dose 0%; LD_{50}: Lethal Dose 50%; LD_{100}: Lethal Dose 100 %

3.1.2 Effects on chicks

At 2520 mg/kg (LME), 1200 mg/kg (RME) and 1680 mg/kg (SME) that were doses ten times higher than ip lethal doses in mice, all extracts were not toxic to chicks.

3.2. Effects of methanolic extracts on cold-blooded animals

3.2.1 Effects on carp alvins

Seven concentrations ranging from 41.94 µg/mL to 175 µg/mL in geometric progression having ratios of 1.25 (LME), 1.12 (RME) and 1.23 (SME), were tested on 7 lots of 5 alvins. The 3 methanolic extracts were toxic on carp alvins. A dose-dependent (p-value<0.05) increase in the effects was registered (Figure 2).

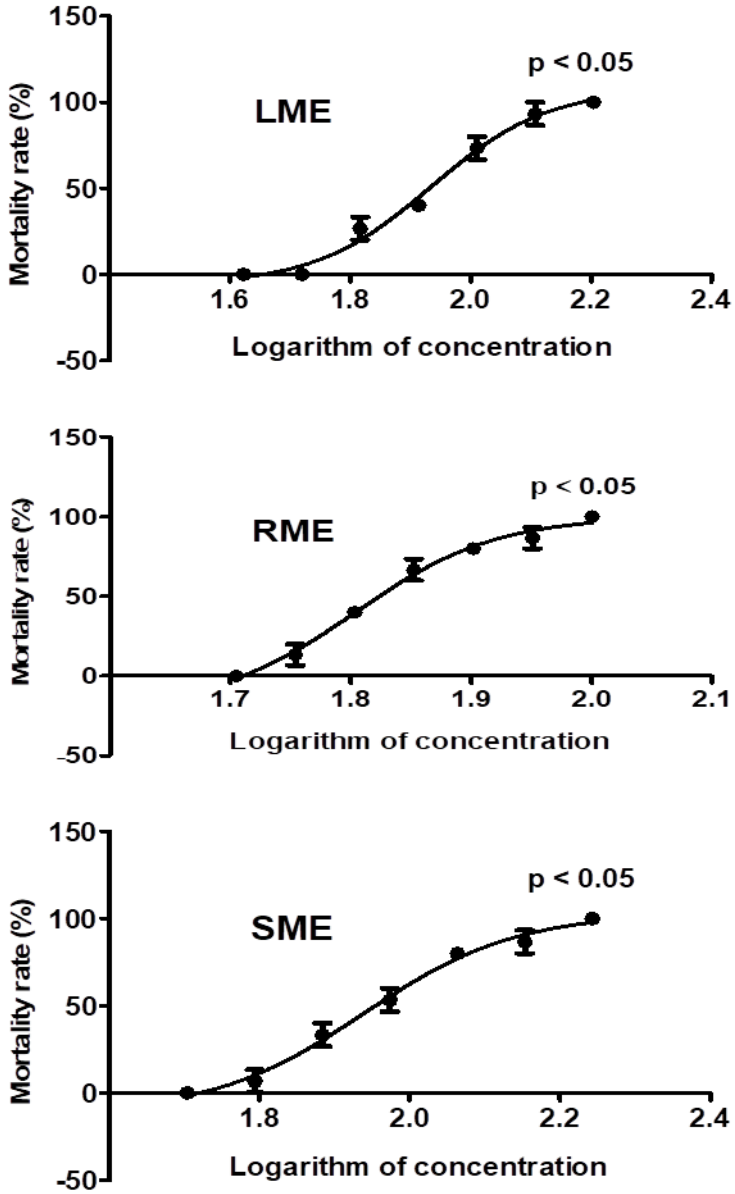

Figure 2 Concentration-effect curves of RME, SME and SME on carp alvins

The lethal concentrations (LC_0, LC_{50} and LC_{100}) were determined (Table 4). LC_0 varied from 41.94 µg/mL (LME) to 50.66 (µg/mL RME), LC_{50} from 63.78 µg/mL (RME) to 86.89 µg/mL (SME) and LC_{100} from 100 µg/mL (RME) to 175 µg/mL (SME).

Besides, statistical analyses showed that there was no significant difference between the effects of the three organs (p-value < 0.05)

Table 4 Lethal concentrations (LC) of methanolic extracts on carp alvins

Extracts	LC0 µg/mL)	LC50 (µg/mL)	LC100 (µg/mL)
LME	41.94	86.02	160
RME	50.66	63.78	100
SME	50.53	86.89	175

LC_0: Lethal concentration 0 %; LC_{50}: Lethal Concentration 50 %; LC_{100}: Lethal Concentration 100 %

3.2.2 Effects on apode frog tadpoles

Seven concentrations ranging from 50.66 µg/mL to 300 µg/mL in geometric progression having ratios of 1.16 (LME), 1.12 (RME) and 1.34 (SME), were tested on 7 lots of 5 frog tadpoles. The 3 methanolic extracts were also toxic on frog tadpoles. A dose-dependent (p-value < 0.05) increase in the effects was registered (Figure 3).

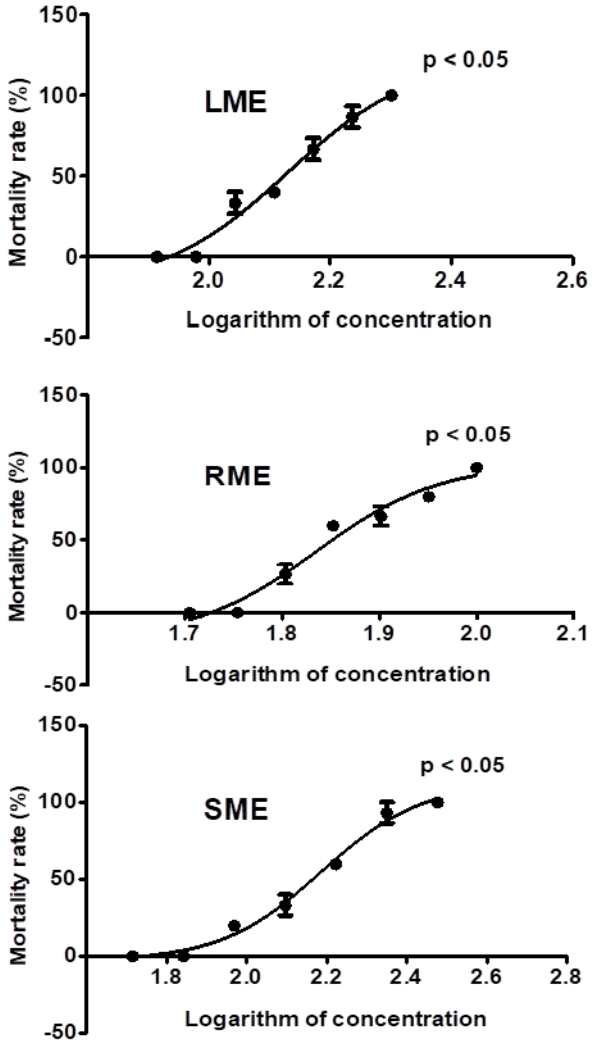

Figure 3 Concentration-effect curves of LME, RME and SME on frog tadpoles

LC_0 varied from 50.66 µg/mL (RME) to 82.08 µg/mL (LME), LC_{50} from 68.43 µg/mL (RME) to 153.4 µg/mL (SME) and LC_{100} from 100 µg/mL (RME) to 300 µg/mL for (SME) (Table 5).

Table 5 Lethal concentrations (LC) of methanolic extracts on frog tadpoles

Extracts	LC0 (µg/mL)	LC50	LD100
LME	82.08	135	200
RME	50.66	68.43	100
SME	51.81	153.4	300

LC_0: Lethal Concentration 0%; LC_{50}: Lethal Concentration 50%; LC_{100}: Lethal Concentration 100%

3.2.3 Effects on mosquito larvae

All tested extracts showed no effect on *Culex quinquefasciatus* larvae after 24 h: neither death nor morbidity of larvae was registered.

3.3. Effects of methanolic extracts on plant seed germination

At 1 mg/mL, 7 out of the 13 seed plants (53.8 %) were susceptible to 1 or 3 of the methanolic extracts (Table 6).

Table 6 Inhibition rates (%) of plant seed germination by the *A. mahalao* methanolic extracts (1 mg/mL)

Plant family	Species	LME	RME	SME
Apiaceae	*Daucus carotta*	20	40	86.67
Asteraceae	*Lactuca sativa*	0	50	100
Brassicaceae	*Brassica chinensis*	0	50	50
	Brassica oleraceae	0	0	13.34
	Brassica sp	0	0	0
	Raphanus sativus	0	0	0
Cucurbitaceae	*Cucumis sp*	90	100	100
Fabaceae	*Phaseolus vulgaris*	90	100	80
	Pisum sativum	0	0	0
Papilionaceae	*Glycine max*	0	0	0
Solanaceae	*Lycopersicum esculentum*	20	0	0
Poaceae	*Oryza sativa*	0	0	0
	Zea mays	0	0	0

The 3 extracts prevented selectively the seed germination of *Daucus carotta, Cucumis sp.* and *Phaseolus vulgaris* with inhibition percentages ranking from 20 % to 100 %. However, they had no effect on the seed germination of *Brassica sp, Raphanus sativus, Pisum sativum, Glycine max, Oryza sativa* and *Zea mays*.

4. Discussion

The toxicity of *A. mahalao* extracts on warm and cold-blooded animals and plants was well established.

On mice, ip LD_{50} (24 h) values ranged from 69.18 (RME) to 123.03 mg/kg (SME) for the methanolic extracts. The LD_{50} of total alkaloids (135.52 mg/kg) and total saponosides (131.22 mg/kg) were higher that means less toxic. That could be explained by the fact that there was a partial synergetic action between them in the methanolic extracts.

All the *A. mahalao* extracts were less toxic than seed methanolic extracts from other Malagasy *Albizia* species whose LD_{50} values were less than 40 mg/kg [2] (table 7).

Table 7 LD_{50} 24 h on mice of methanolic extracts from different Malagasy *Albizia* species

***Albizia* species**	**Methanolic extract of**	**LD_{50} 24 h**
A. greveana	Seeds	1.13-2.30
A. tulearіensis	Seeds	2.9-3.2
A. divaricata	Seeds	5.33-7.39
A. viridis	Seeds	6.72-8.04
A. masikororum	Seeds	16.5-16.81
A. androyensis	Seeds	35.27-41.55
A. aurisparsa	Seeds	36.30-38.76
A. mahalao	Leaf, stem and root barks	69.18-123.03

Compared with foreign *Albizia* species, the methanolic extracts of *A. mahalao* were also less toxic than seed extracts of *A. adianthifolia* (LD_{50} = 6 mg/kg) [6] but more toxic than aqueous extract of *A. zygia* stem bark (LD_{50} = 316.2 mg/kg) [7].

The *A. mahalao* extracts could be used to fight against noxious animals like harmful rodents (mice and rats). It is important to note that *Rhodocodon madagascariensis* bulbs (Hyacinthaceae), with a LD_{50} (24 h) of 170 mg/kg, have been a well-known rodenticide used in Madagascar [8, 9].

By po and sc routes, no visible signs and no mortality were observed on mice. This could be due either by enzymatic degradation of the bioactive principles in the digestive tract or their weak or non-absorption. The same results were observed on chicks by po route. However, by ip route, all the methanolic extracts showed no visible symptoms and no mortality on chicks which meant these animals were less affected by the tested extracts than mice.

On carp alvins, the *A. mahalao* methanolic extracts were by far less toxic ($63.78 \geq LC_{50} \leq 86.89$ µg/mL) than the seed methanolic extracts of several Malagasy *Abizia* species ($2.28 \geq LC_{50} \leq 15$ µg/mL) [2] (Table 8).

Table 8 LC_{50} 24 h on carp alvins of methanolic extracts from different Malagasy *Albizia species*

Albizia species	**Methanolic extract of**	**LC50 (µg/mL)**
A. greveana	Seeds	10
A. tulearіensis	Seeds	2.28
A. divaricata	Seeds	8.45
A. androyensis	Seeds	3.85
A. aurisparsa	Seeds	15
A. mahalao	Leaf, stem and root barks	63.78-86.89

On frog tadpoles, the *A. mahalao* methanolic extracts ($68.43 \geq LC50 \leq 153.4$ µg/mL) were less toxic than seed methanolic extracts from other Malagasy *Albizia* species tested ($4.50 \geq LC_{50} \leq 60$ µg/mL [10] (Table 9).

Table 9 LC_{50} 24 h on frog tadpole of methanolic extracts from different Malagasy *Albizia* species

Albizia species	**Methanolic extract of**	**LC_{50} (µg/mL)**
A. viridis	Seeds	4.50
A. masikororum	Seeds	4.55
A. bernieri	Seeds	10.49
A. aurisparsa	Seeds	60
A. mahalao	Leaf, stem and root	68.43 – 153.4

The high toxic effects on alvins and tadpoles were probably due to saponins which were found in large amount in tested *A. mahalao* organs especially in leaves. The toxicity of these compounds to cold blooded animals has been well-known. It accounts for the use of many plants containing saponins as poison fishing in several countries [11].

LME, SME and RME had no toxic effects on *Culex quinquefasciatus* larvae. However, methanolic extracts of leaf and seed of *Albizia lebbeck* were reported to have ovicidal and adulticidal effects against *Culex quinquefasciatus*, *Aedes aegypti*, and *Anopheles stephensi* [12] and *Albizia amara* was highly toxic to *Anopheles stephensi* [13]. It would be interesting to test the *A. mahalao* methanolic extracts on these mosquitoes responsible for the transmission of several dreaded diseases particularly in developing countries having poor socio-economic conditions.

Preliminary experiments on plant seeds were conducted in view of a double objective, to test the effects of the *A. mahalao* extracts on vegetables and to find out if they could be used in the control of undesirable plants. Like other Malagasy *Albizia* already studied [14, [10], LME, SME and RME prevented the germination of some plant seeds but the inhibitory effect varied according to both the extract and the target plants. Studies on the seeds and seedlings of weeds and invasive plants are ongoing in our laboratory.

At 1 mg per disk, leaf extracts of *A. mahalao* inhibited the growth of various pathogenic bacteria such as *Clostridium perfringens, Staphylococcus aureus, Enterobacter aerogenes, Listeria monocytogenes, Vibrio fisheri, Shigella flexneri* and *Salmonella enterica* [5]. This dose was by far below the LD_0 (69.32 mg/kg) of LME which is a strong argument for the use of leaf extracts as efficient drug against these pathogenic bacteria. They might be employed as an alternative solution to chemical antibiotics. However, in-depth pharmacological studies were still needed to confirm this hypothesis.

Several chemical groups, particularly alkaloids and saponins, were found in LME, SME and RME [5]. These secondary metabolites were responsible for the toxicity of the *Albizia mahalao*. Their involvement in the toxicity of other Malagasy *Albizia* were already reported: Albodorine isolated from *A. odorata* was extremely toxic to mice with LD_{50} (24 h) of 9 mg/kg [15], *A. bernieri* seeds contained alkaloids and saponins which were toxic to several pathogenic microorganisms such as *Enterobacter cloacae, Streptococcus pyogenes, Yersinia enterocolitica, Candida albicans* [3]. Further chemical and biological investigations would be necessary to determine the number and originality of the bioactive principles.

5. Conclusion

The results obtained in this study demonstrated the toxicity of *A. mahalao* on various organisms and showed their possible use to fight against noxious organisms. These new data increased knowledge of the Malagasy *Albizia* properties.

Compliance with ethical standards

Acknowledgments

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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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