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Toxic properties of stem bark extracts of *Myrica spathulata* Mirb. (Myricaceae), a medicinal plant

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Abstract

Toxic activity has been demonstrated in extracts of *Myrica spathulata* Mirb. (or *Morella spathulata*) stem bark, a Myricaceae endemic to Madagascar. A purification process involving ethanol precipitation, dialysis and n-butanol fractionation yielded a partially purified extract (E3) from a hot aqueous crude extract (CE). The purification yield from CE was 29.87%. The active principles were thermostable, soluble in water, ethanol, but not in butanol, precipitable by neutral lead acetate and did not pass through the dialysis membrane (15,000 Da). Phytochemical screening of the partially purified extract (E3) revealed the presence of tannins, phenolic compounds, deoxyoses, leucoanthocyanins and unsaturated sterols. Mice administered intraperitoneally E3 at a lethal dose of 1087.8 mg/kg developed symptoms suggestive of damage to the nervous system and kidneys. The LD_{50} (24 h) was estimated at between 875.99 mg/kg and 877.8 mg/kg. *In vitro*, active ingredients lysed sheep red blood cells. CE was also toxic to tadpoles (LC_{50} 24 h = 1.89 mg/ml). Fish *Cyprius carpio* were highly sensitive even at very low concentrations (0.12 µg/ml). CE had no effect on *Culex quinquefasciatus* mosquito larvae. It inhibited the germination of various seeds, and the growth of young seedlings of *Oryza sativa* (Monocotyledon) and *Phaseolus vulgaris* (Dicotyledon). Both CE and E3 were active against *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Keywords: *Myrica spathulata*; Myricaceae; Toxicity; Hemolytic activity; Germination inhibition; Antimicrobial activity

1. Introduction

The genus *Myrica* Linn. (Myricaceae) comprises 97 species of small trees and aromatic shrubs averaging 20 to 25 feet in height [1]. This genus is widely distributed in both the temperate and sub-tropical regions with maximum species diversity in Africa and Boreal America.

Many species of *Myrica* possess a number of therapeutic virtues that have long been known and exploited by traditional medicine in several countries. For example, in China, *Myrica rubra* [2], is used in the treatment of mouth and gums related problems, cold, flu asthma and laryngitis. In India, *Myrica esculenta* [3], the bark of the plant boiled in water has various health benefits, such as curing diarrhea.

In addition, several bioactive compounds present in *Myrica* species show interesting activities with potential as natural treatments for a variety of ailments [4]. For example, myricanol from *M. rubra* is associated with anti-tumor properties, myricitrin from *M rubra, M. adenophora*, *M. cerifera* and *M. esculenta*, is recognized for its anti-diabetic effects. Quercitrin

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(*M. rubra, M. adenophora*), has protective potential for the nervous system and liver. Betulin from *M. cerifera* has anti-inflammatory and anti-cancer properties.

In Madagascar, according to the literature, the decoction of *Myrica spathulata* stem bark is used as a mouthwash for certain types of toothache [5]. On the east coast of Madagascar, infusions or decoctions of the leaves and bark of this same plant are also used to relieve pain and inflammation that occur during sickle-cell crises [6]. High content of phenolic and bioactive components in the leaf and bark extracts of this plant, associated with antioxidant, analgesic and anti-inflammatory properties was reported [7].

As part of our laboratory's program to explore the possible toxicity of Malagasy medicinal plants, in the present work we have undertaken research into the chemical composition and toxicity of *Myrica spathulata* bark extracts on various organisms. The toxicity of this part of the plant had already been demonstrated in preliminary experiments on mice.

2. Materials and methods

2.1. Plant material

2.1.1. Myrica spathulata Mirb *(*synonym*: Morella spathulata)*

The plant is a shrub or tree up to 12 m high, grows in Madagascar's eastern coastal forest, on coastal sands and hills [8] (figure 1). It has several vernacular names, including Andravola (Alaotra), Laka, Tsilakana and Silaka (Betsimisaraka) and Sarindriaka (Betsileo). The plant material was collected in the Amparafana coastal forest, Mahatsara commune, Mahanoro region, in April.

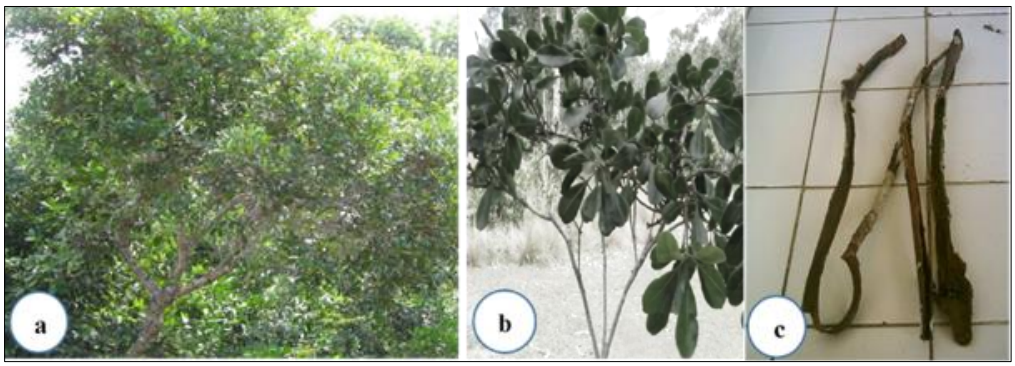

Figure 1 *Myrica spathulata*: a) whole plant b) leafy twigs c) stem bark

The stems were peeled, then the resulting bark was washed and sun-dried for 24 h. The dry material was then ground using a mortar. The resulting powder was sieved and stored at room temperature. It constituted the study material.

2.1.2. Plant seeds

Seeds from 9 plants belonging to different families were tested (table 1).

Table 1 List of plant seeds tested

Plant families	Monocotyledons	Dicotyledons	Common name
Poaceae	*Zea mays*		Maize (corn)
	Oryza sativa		Rice
Apiaceae		*Daucus carotta*	Carrot
Brassicaceae		*Brassica sp*	Tissam white
Compositeae		*Lactuca sativa*	Lettuce
Cucurbitaceae		*Cucumis sp*	Cucumber
Fabaceae		*Phaseolus vulgaris*	Beans
Solanaceae		*Pisum sativum*	Pea
		Lycopersicum esculentum	Tomato

2.2. Animals

2.2.1. Mice

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

2.2.2. Tadpoles

Legless tadpoles of the *Ptychadena mascareniensis* frog were caught on the day of the test in rice fields near the Antananarivo University Campus.

2.2.3. Fishes

The 2-month-old carp *Cyprius carpio* alvins were supplied by an approved fish farmer in Manjakandriana. These alvins were adapted in an aerated aquarium for a few days before testing.

2.2.4. Mosquito larvae

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

2.2.5. Microbial strains

GRAM-negative and GRAM-positive bacteria from the National Center for the Application of Pharmaceutical Research (CNARP) were used.

2.3. Methods used to prepare the different extracts

2.3.1. Hot aqueous extraction

Stem bark powder was suspended with distilled water in a 1:10 (w/v) ratio. The suspension was refluxed at 60 °C for 3 h under magnetic stirring. After cooling, the suspension was left to macerate at +4 °C for 24 h. After an hour's agitation, the macerate was filtered through 4 layers of gauze to remove the cakes. The filtrate was centrifuged for 30 min at 10,000 rpm. The resulting supernatant was evaporated to a 1:1 (w/w) ratio, i.e. 1 g powder to 1 ml extract, then centrifuged for 15 min at 12,000 rpm to remove the precipitate formed during evaporation.

2.3.2. Precipitation with ethanol

A volume of absolute ethanol was added dropwise to the same volume of extract to be treated. The mixture was magnetically stirred and left to stand at +4 °C for 15 min. The precipitate formed was removed by centrifugation at 12,000 rpm for 15 min. The supernatant was evaporated to dryness and the pellet recovered in distilled water.

2.3.3. Dialysis

The extract to be treated was introduced into a dialysis membrane with a filtration threshold of 15,000 Da. The counter-dialysis liquid was distilled water under magnetic stirring: 100 times the volume of extract to be dialyzed. The treatment was repeated several times.

2.3.4. n-Butanol fractionation

A volume of extract to be purified and the same volume of organic solvent, n-butanol, were introduced into a separating funnel. After manual stirring, the mixture was left to settle completely. The two phases were collected separately. The aqueous phase was again treated with n-butanol. The two organic phases were combined, and the n-butanol was evaporated after addition of distilled water.

2.4. Methods used to study effects on animals

2.4.1. Effect on mouse

The acute toxicity of the extracts was assessed on 25 ± 2 g mice by intraperitoneal (i.p.) administration of a constant volume of 0.3 ml per mouse. For each test solution, a batch of 4 mice was used. Another batch of 4 mice received saline (0.3 ml per mouse) as a control.

The LD_{50} (24 h) of each extract was determined on mice by calculation and graphical methods [9]. Five doses of the extract, in geometric progression of reason r =1.09, ranging from 770.64 mg/kg (0% mortality) to 1087.8 mg/kg (100% mortality), were injected intraperitoneally into batches of 5 mice. A batch of 5 mice served as a control. Results were interpreted using the Néné-BI *et al.* (2008) [10] scale.

2.4.2. Hemolytic test

Fresh citrated sheep blood was mixed volume by volume with physiological saline. The solution was centrifuged at 3,000 rpm for 5 min. The supernatant was discarded and the pellet was again mixed with saline and centrifuged as before. This washing operation was repeated 3 times. The pellet from the third wash was the 100% red cell suspension. It was diluted 2 times to obtain a 50% suspension from which a 2% red cell suspension was prepared. All dilutions were made using Phosphate Buffered Saline (PBS). The 2% red cell suspension was dispensed into 48 wells of a U-bottom microplate, at a rate of 50 µl per well. The test extract was also diluted to 2 mg/ml. The resulting solution was then cascade diluted (dilution coefficient 0.5, final volume 50 µl). The composition of the medium in each well is shown in table 2.

Table 2 Composition of the medium for the hemolytic test

Wells n°	**1(C+)**	**2(C^-)**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Test extract 2 mg/ml (µl)	0	0	50	25	12.5	6.25	3.125	1.56	0.78	0.39	0.196	0.097
PBS (µl)	0	50	0	25	37.5	43.75	46.87	48.44	49.22	49.61	49.805	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 extract concentration (mg/ml)	0	0	2	1	0.5	0.25	0.125	0.0625	0.0312	0.015	0.0078	0.0039
Final volume of mixture (µl)	100	100	100	100	100	100	100	100	100	100	100	100

C+ : positive control; C- : negative control

2.4.3. Effect on carp alvins and frog tadpoles

Batches of animals of the same age were placed in crystallizers each containing a total volume of 200 ml of rainwater and extract to be studied at different concentrations. The experiment lasted 24 h.

The graphical method of linear regression [11] was used to determine the LC_{50} (24 h) or lethal concentration that killed 50% of the animals tested in 24 h.

2.4.4. Effect on mosquito larvae

Eight batches of 10 larvae were placed in crystallizers each containing a final volume of 200 ml of rainwater and the extract to be tested at different concentrations. After 24 h, dead and morbid larvae were counted. Larvae were dead if they did not move when touched in the cervical region with a needle. Morbid larvae could not dive or surface when the water is agitated. For each concentration, the percentage mortality was calculated as the sum of dead and morbid larvae.

2.5. Methods used to study effects on plants

2.5.1. Effect on seed germination

Two batches of 10 seeds of each species were tested. After soaking in water for 24 h (48 h for beans), the soaked seeds were decontaminated with 10% bleach, then rinsed with water. For each species, seeds were germinated in Petri dishes lined with cotton soaked in water (control) or the extract to be studied (10 seeds per dish). The Petri dishes were then placed in the dark. Seed germination was observed 48 h later.

2.5.2. Effect on young seedling growth

Nine batches of 10 seeds were soaked in water for 48 h. Two batches were then soaked in the extract to be studied of determined concentration, and the other 7 in water. Soaking lasted 48 h. After washing, the 2 batches soaked in crude extract were divided as follows: 1 batch was germinated in a Petri dish on cotton soaked in the extract to be tested at

the same concentration, the other batch on cotton soaked in water. The remaining 7 batches were placed in Petri dishes on water-soaked cotton. They were sprayed with different concentrations of the test solution. Epicotyls and hypocotyls were observed and measured every 2 days for 2 weeks. They were sprayed with CE at different concentrations.

2.6. Methods used to study effects on microorganism growth

All the materials and methods used for antimicrobial assay were detailed in a previous study [12]. The results were interpreted using the scale of Ponce *et al.* (2003) [13] and Celikel *et al*. (2008) [14]: bacteria were not considered sensitive for an inhibition zone diameter (IZD) ≤ 8 mm; sensitive for 9 ≤ IZD ≤ 14 mm; very sensitive for IZD of 15 ≤ IZD ≤ 19 mm and extremely sensitive for IZD ≥20 mm.

3. Results

Hot aqueous extraction from 50 g of bark powder yielded a clear, dark-brown, pungent-tasting, toxic crude extract (CE) with a pH of 4.75.

The various purification steps used for CE purification are summarized in figure 2.

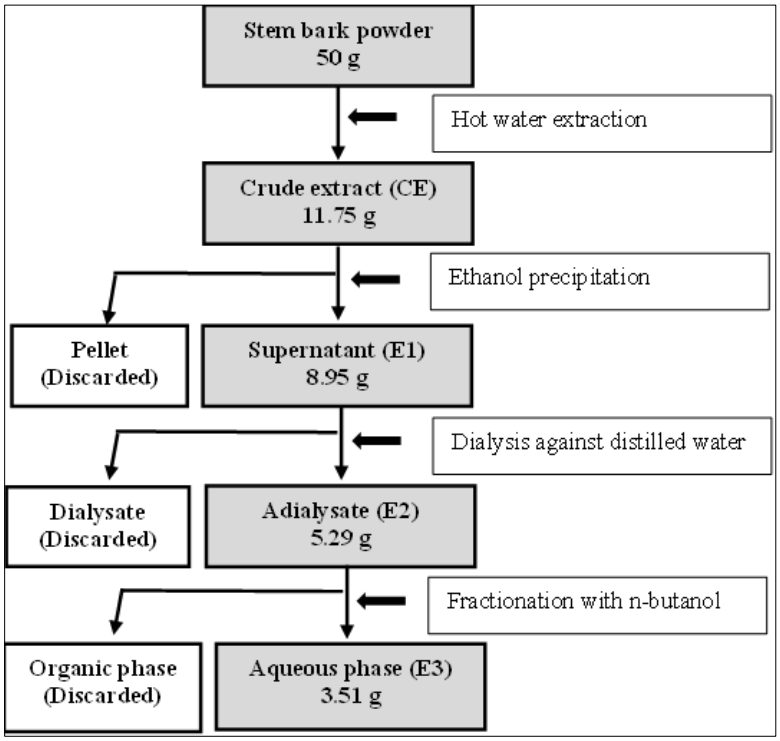

Figure 2 Diagram summarizing the extraction and the purification stages of active principles

A quantity of 11.75 g of CE gave 3.51 g of E3, corresponding to a purification yield of 29.87%.

The evolution of the homogeneity of the toxic extracts obtained at the different purification stages is shown in figure 3. The crude extract contained eight major bands, while the E3 extract contains only two bands.

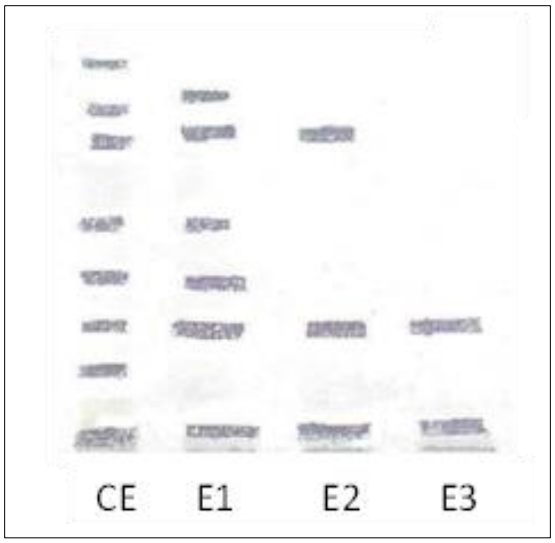

Figure 3 Thin layer chromatography of extracts obtained at the various purification stages

Solvent: Butanol/Acetic acid/Water (60/60/20, w/w); Developer: Sulfuric vanillin reagent

Phytochemical screenings carried out on CE and E3 are presented in table 3.

Table 3 Results of the phytochemical screening of CE and E3 extracts

Chemical families	**Tests**	**Results**	
		CE	**E3**
Saponin	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 (unsaturated sterols)	+	+
Anthraquinones	Bornträger	-	-

CE and E3 contained the same components, including tannins, polyphenols, deoxyoses, flavonoids, leucoanthocyanins, and unsaturated sterols, with the exception of flavonoids, which were eliminated during purification. In both cases, saponins, alkaloids and anthraquinones were absent.

3.1. Effects of extracts on animals

3.1.1. On mice

Ten minutes after injection of extracts (CE or E3) at a lethal dose of 1087.8 mg/kg, fatigue was observed in mice. After 1 h, the mice lost their balance and could no longer walk. Exophthalmos accompanied by palpitations was observed 1 h 30 min after injection. After 4 h, fatigue was total and the lower limbs began to feel paralysed. Convulsions appeared a few minutes before death which occured after 6 h.

At the sublethal dose of 770.64 mg/kg, 15 min after injection, mice showed fatigue with piloerection. After 3 h, a loss of balance with exophthalmos was observed. They remained motionless for a few hours. After 6 h, the mice recovered gradually.

The LD_{50} of CE by the intraperitoneal route was estimated at 875.99 mg/kg and 877.8 mg/kg weight by calculation and graphical methods respectively.

3.1.2. On sheep red blood cells

CE and E3 had the same effects on sheep red blood cells (table 4). These effects varied according to concentration: total hemolysis (100%) was observed at concentrations ≥ 0.031 mg/ml; partial hemolysis at 0.015 mg/ml and at concentrations ≤ 0.008 mg/ml, no hemolysis.

Table 4 Effects of different concentrations of CE and E3 on sheep red blood cells

Concentration (mg/ml)	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.008	0.004
CE	+++	+++	+++	+++	+++	+++	+++	+	-	-
E3	+++	+++	+++	+++	+++	+++	+++	+	-	-

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

3.1.3. On carp alvins

Six CE concentrations ranging from 0.12 µg/ml to 3.9 µg/ml were tested. At all the concentrations tested, CE caused alvins to die within 24 h.

3.1.4. On frog tadpoles

Nine CE concentrations, in geometric progression of reason 1.19, were tested on 9 batches of 10 tadpoles. According to the results obtained (table 5), the crude extract had a toxic activity on frog tadpoles. A dose effect was observed. The LC_{50} was evaluated at 1.89 mg/ml.

Table 5 Effects of different concentrations of CE on frog tadpoles

Batch	Concentrations		Number of tadpoles		% of deaths
	mg/ml	log C	Dead	Survivors	
1	0.3	- 0.096	0	10	0
2	0.1	-1	2	8	20
3	1.2	0.079	3	7	30
4	1.4	0.146	3	7	30
5	1.7	0.230	4	6	40
6	2	0.301	6	4	60
7	2.4	0.380	6	4	60
8	2.9	0.462	8	2	80
9	3.5	0.544	10	0	100

3.1.5. On mosquito larvae

Eight CE concentrations ranging from 0.015 mg/ml to 2 mg/ml were tested on mosquito larvae. No mortality or morbidity was observed after 24 h.

3.2. Effects of extracts on plants

3.2.1. Effects of CE on seed germination

Seeds were germinated in the presence of CE at a concentration of 1 mg/ml. According to the results (table 6), CE did not affect bean seed germination (0% inhibition) but in other seeds, inhibition ranged from 10% (*Cucumis sp*.) to 90% (*Lactuca sativa*).

Table 6 Effects of CE at 1 mg/ml on the germinative capacity of a few seeds

Plant family	Species	% germination	% inhibition
Apiaceae	*Daucus carotta*	30	70
Brassicaceae	*Brassica sp*	60	40
Compositeae	*Lactuca sativa*	10	90
Cucurbitaceae	*Cucumis sp*	90	10
Fabaceae	*Phaseolus vulgaris*	100	0
Fabaceae	*Pisum sativum*	70	30
Solanaceae	*Lycopersicum esculentum*	20	80
Poaceae	*Oryza sativa*	80	20
Poaceae	*Zea mays*	70	30

3.2.2. Effects of CE on seedling growth

Given the effects of CE on seed germination, the experiments aimed to evaluate the effects of CB on the growth of young seedlings. They were carried out on *Oryza sativa* and *Phaseolus vulgaris* seedlings whose seeds germinated at 80% and 100% respectively in the presence of CE at 1 mg/ml. A range of CE concentrations from 0.45 to 14.69 mg/ml was tested. The results are presented in table 7.

Table 7 Effects of different CE concentrations on epicotyl and hypocotyl growth in *Oryza sativa* and *Phaseolus vulgaris* after 14 days of treatment

		Control (water)	CE (mg/ml)					
			0.45	0.91	1.83	3.67	7.34	14.69
Oryza sativa	Epicotyl (cm)	5.4	2.4	1.38	0.92	0.9	0.7	0.1
	Inhibition (%)	0	55.55	74.44	82.96	83.33	87.03	98.14
	Hypocotyl (cm)	6.2	5.9	2.6	3.4	3.2	1.7	1.9
	Inhibition (%)	0	4.83	58.06	45.16	48.38	72.58	69.35
Phaseolus vulgaris	Epicotyl (cm)	250	210	106	24	6.9	3.9	3.4
	Inhibition (%)	0	16	70.24	90.4	97.24	98.44	98.64
	Hypocotyl cm)	86	98	56	22	20.8	17.1	8.62
	Inhibition (%)	0	0	34.88	74.41	75.81	80.11	89.97

Epicotyl and hypocotyl of both plants were sensitive to the tested concentrations of CE. In both cases, epicotyl was more sensitive than hypocotyl: at the highest CE concentration (14.69 mg/ml), epicotyl growth inhibition in *O. sativa* and *P.*

vulgaris was 98.14% and 98.64% respectively, while that of hypocotyl was 69.35% and 89% respectively. CE effects were dose-dependent.

3.3. Effects of CE and E3 on bacteria

CE (235.19 mg/ml) and E3 (90 mg/ml) were tested on *Escherichia coli, Klebsiella pneumoniae*, *Salmonella typhi* and *Staphylococcus aureus*. The results are presented in table 8.

Table 8 Activity of CE and E3 on test germs

Strains	GRAM	IZD (mm)		
		CE (4.70 mg/disk)	E3 (1.80 mg/disk)	Neomycin (30 µg/disk)
Escherichia coli	-	7	8	27
Klebsiella pneumoniae	-	16	11	25
Salmonella typhi	-	8	9	21
Staphylococcus aureus	+	25	21	25

Staphylococcus aureus was the most sensitive to both extracts: IZD as high as that of neomycin. *Klebsiella pneumoniae* was sensitive to E3 and very sensitive to CE. *Salmonella typhi* was sensitive to E3 but insensitive to CE. *Escherichia coli,* on the other hand, was insensitive to both extracts.

4. Discussion

The purification yield of CE was 29.87%.

The same chemical families were found in CE and E3, with the exception of flavonoids eliminated during purification. Our results differed slightly from those reported by Debray and Jacquemin (1971) [5] obtained with stem bark harvested in June. For example, saponins were absent from bark harvested in April.

According to the data from the various techniques tested, whether retained or not for different reasons, the active ingredients were thermostable, precipitable by neutral lead acetate, soluble in polar solvents (ethanol) but insoluble in n-butanol. The molecular weights of the active ingredients could be high, up to 15,000 Da, as they did not cross the dialysis membrane unless they interfere with it.

Both crude and purified extracts of *Myrica spathulata* stem bark have been shown to be toxic to animals, plants, and bacteria.

In mice, toxicity was assessed only by intraperitoneal administration, since according to Fioccardi *et al.* (2024) [7], leaf and bark extracts of the plant administered orally did not induce unusual behavior or mortality, and LD_{50} values were greater than 2000 mg/kg body weight. Apparently, plants of the *Myrica* genus were not toxic when taken orally, as the methanolic extract of *M. salicifolia*, at a dose of 2000 mg/kg, also caused no mortality and no visible signs of intoxication [15].

Intraperitoneal administration caused intoxication characterized mainly by fatigue, paralysis of the lower limbs, imbalance and absence of urine. All these symptoms suggested damage to the nervous system and kidneys LD_{50} values (24 h), ranging from 875.99 mg/kg to 877.8 mg/kg, showed that CE was moderately toxic to mice via the intraperitoneal route (500 mg ≤ LD50 ≤ 5 g/kg). Compared with the toxicity of Malagasy medicinal plants such as *Dodonaea madagascariensis*, a Sapindaceae, (LD_{50} = 36.12 mg/ml) [16] and *Pittosporum ochrosiaefolium*, a Pittosporaceae, (LD_{50} = 46.69 mg/ml) [17], the toxicity of *Myrica spathulata* stem bark was much lower. The level of toxicity may vary according to the phenological stage of the plant.

CE and E3 lysed sheep red blood cells and had the same effect on them. The hemolytic power of both extracts was very high: concentrations ≥ 0.031 mg/ml caused 100% hemolysis. The HD_{50} (concentration that caused 50% hemolysis) would therefore be < 0.031 mg/ml. Compared with the hemolytic power of *Gambeya boiviniana*, which caused *in vitro* total hemolysis at 0.25 mg/ml [18], that of *Myrica spathulata* was significantly higher (0.031 mg/ml). High hemolytic power corresponds to high cytotoxicity, as observed with *Gambeya boiviniana* extract, which induced histological

lesions characterized by vascular congestion and hemorrhages in various organs [18]. The hemolytic properties of an agent are attributable to its interaction with the sterols in the erythrocyte membrane, which lead to an increase in membrane permeability and movement of ions: Na^+ and H_2O enter, K^+ leaves, the membrane bursts, allowing haemoglobin to leave [19]. Both extracts did not contain saponins, secondary metabolites well known for their high hemolytic power. Hemolysis could certainly be due to other molecules that also have hemolytic properties, such as polyphenols [20, 21].

The active principles in *Myrica spathulata* stem bark also had effects on cold-blooded animals. Carp alvins were highly sensitive even to very low concentrations (0.12 µg/ml). In tadpoles, the LC_{50} (24 h) was estimated at 1.89 mg/ml.

The higher sensitivity of fish to the effects of the crude extract compared to tadpoles could be due to the fact that the active principles entered the bloodstream more easily via the gills.

However, mosquito larvae, were insensitive to the 2 mg/ml CE concentration.

Seed sensitivity to CE (1 mg/ml) was highly variable (from 0% to 90%). The inhibition of germination could be due to the destruction of embryos or the inactivation of the enzymes required for germination by the toxic principles. The absence or low sensitivity of certain seeds could be due to the fact that their coat were impermeable to toxic principles.

Regarding the growth of young plants, at concentrations of 1.83 mg/ml or higher, the growth of *Oryza sativa* and *Phaseolus vulgaris* epicotyls and hypocotyls was inhibited. For both plants, the epicotyl was clearly more sensitive than the hypocotyl.

In microorganisms, CE and E3 affected the growth of all 4 strains tested, with a more marked effect on *Staphylococcus aureus.* This bacterium is considered to be one of the most dangerous human pathogens, responsible for numerous diseases ranging from moderate skin infections to fatal illnesses [22]. The 2 extracts were as effective as the reference antibiotic neomycin. This strong activity against *Staphylococcus aureus* was very interesting, but given the high cytoxicity of the extracts, they should only be used to treat skin infections caused by this bacterium.

Work is underway to improve purification processes to examine seasonal variations in the composition and content of compounds produced by the plant, isolate toxic principles and elucidate their chemical nature, carry out more research on toxicity and assess their antimicrobial properties on other microorganisms.

5. Conclusion

In conclusion, the results obtained demonstrated the toxicity of *Myrica spathulata* extracts on various living organisms. They provided a scientific argument for the traditional use of bark extract as a mouthwash. They added to the scientific data on *Myrica spathulata* and draw attention to the precautions to be taken when using the plant to avoid harmful effects on health.

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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