World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

Check for updates

Chemical and toxicological research on extracts from the leaves of *Pittosporum senacia* Poir. (Pittosporaceae), a medicinal plant endemic to Madagascar

Eliane Razafintsalama Vahinalahaja [2, 3], Maholy Pricille Ratsimiebo [1, 2], Herizo Lalaina Andriamampianina [1, 2], Mihajasoa Stella Razanatseheno [1, 2], Lovarintsoa Judicaël Randriamampianina [1, 2], Danielle Aurore Doll Rakoto [1, 2], Hanitra Ranjàna Randrianarivo [1, 2] and Victor Louis Jeannoda [1, 2, *]

[1] *Laboratory of Applied Biochemistry to Medical Sciences (LABASM), Fundamental and Applied Biochemistry Department (DBFA), Faculty of Sciences, University of Antananarivo, P.O. Box 906, Antananarivo 101, Madagascar.*
[2] *Life and Environment Sciences Doctoral School (SVE), University of Antananarivo, P.O. Box 906, Antananarivo 101, Madagascar.*
[3] *National Research Centre for Pharmaceutical Application (CNARP), P.O. Box 702, Antananarivo 101, Madagascar.*

World Journal of Advanced Research and Reviews, 2025, 27(02), 059-074

Publication history: Received on 22 June 2025; revised on 29 July 2025; accepted on 01 August 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.27.2.2823

Abstract

A toxic activity has been observed in the extracts of *Pittosporum senacia* leaves, a Pittosporaceae from Madagascar. A bitter crude extract (CE) was obtained by hot aqueous extraction. A purification procedure from CE comprising ethanol precipitation, dialysis and fractionation with n-butanol yielded a partially purified extract (E3). The active ingredients, which had a bitter taste, were thermostable, precipitable by neutral lead acetate, absorbed by activated charcoal, and soluble in water, ethanol, and n-butanol. A phytochemical screening undertaken on E3 revealed the presence of phenolic compounds, unsaturated sterols, triterpenes and saponins. Mice injected with E3 at the lethal dose of 37.5 mg/kg by intraperitoneal (i.p.) route developed symptoms suggesting an attack of the central nervous system. The LD_{50} was estimated between 26.05 and 27.41 mg/kg of body weight (b.w.). E3 provoked tissue lesions which were mainly characterized by hemorrhages in the heart, lungs and liver, and by vessel congestion in the brain, intestine and kidneys. *In vitro*, the active principles caused the lysis of sheep red blood cells. CE was also toxic to carp alvins (LC_{50} = 27.54 µg/ml) and frog tadpoles (LC_{50} = 28.11 µg/ml). CE inhibited the germination of diverse plant seeds. E3 and CE were active on *Staphylococcus aureus* with a Minimum Inhibitory Concentration (MIC) of 1.2 mg/ml and a Minimum Bactericidal Concentration (MBC) of 9.6 mg/ml for CE, and a MIC of 5.9 mg/ml and a MBC of 11.9 mg/ml for E3. CE exhibited a bacteriostatic activity (MBC/MIC= 8) and E3 a bactericidal activity (MBC/MIC= 2.01).

Keywords: *Pittosporum Senacia*; Toxicity; Tissue Lesions; Haemolytic Property; Cold-Blooded Animals; Germination Inhibition; Antimicrobial Properties

1. Introduction

Medicinal plants are a valuable source of bioactive compounds that have been used in traditional medicine for thousands of years [1]. Madagascar is a hotspot of plant biodiversity, and many of its endemic species are used for therapeutic purposes without prior scientific validation [2]. Among these plants are species of *Pittosporum* from the Pittosporaceae family.

The *Pittosporum* genus comprises about 160 species growing wild in tropical and subtropical regions [3]. In Madagascar, *Pittosporum* is represented by 11 species, 9 of which are endemic.

* Corresponding author: Victor Louis Jeannoda

Several species of *Pittosporum* are widely used as medicinal plants for the treatment of various diseases. Different Malagasy species are also widely used as medicinal plants. They have anti-inflammatory, antimicrobial and antispasmodic activities [4, 5].

At LABASM, a research programme on Pittosporaceae has been undertaken: *Pittosporum verticillatum* [6, 7], *Pittosporum ochrosiaefolium* [8] and other species are currently being studied.

This study focused on *Pittosporum senacia*, another species of Pittosporaceae used in traditional medicine. This choice was motivated by several reasons, including the lack of studies on this plant other than botanical ones, its use in traditional medicine, the wide diversity of biological properties of its congeners around the world, its widespread availability in Madagascar and, above all, the positive results of preliminary toxicity tests on mice using extracts from its leaves and bark.

The investigations aimed to characterise the secondary metabolites present in *Pittosporum senacia* leaf extracts and to assess their potential toxicity through *in vitro* tests. The objective was to provide a scientific basis for the safe use of this species in a therapeutic context, while contributing to the inventory of Malagasy plant resources with medicinal or toxicological value.

2. Materials and methods

2.1. Plant materials

2.1.1. Pittosporum senacia

Pittosporum senacia is a small tree, 5 to 6 m tall (Figure 1). In Madagascar, it grows in coastal rainforests, from sea level up to 300 m altitude, but rarely up to 1,200 m altitude. The plant was harvested in the Andasibe region (142 km from Antananarivo) in January, when it was in its vegetative stage. In this region, it is commonly known as Maimbovitsika and Ambovitsika.

(Source: the authors)

Figure 1 *Pittosporum senacia*: the whole plant (a) and leaves (b)

2.1.2. Plant seeds

The seeds used came from the National Center for Applied Research for Rural Development (FOFIFA/CENRADERU) (Table 1).

Table 1 Plants whose seeds were used for germination assays

Plant families	Liliopsida	Magnoliopsida	Common name
Poaceae	*Zea mays*		Maize, corn
	Oryza sativa		Rice
Apiaceae		*Daucus carota*	Carrot
		Petroselinum crispum	Chinese parsley
Brassicaceae		*Brassica sp*	Tissam white
Amaranthaceae		*Beta vulgaris subsp. maritima*	Beetroot
Asteraceae		*Lactuca sativa*	Lettuce
Cucurbitaceae		*Cucurbita pepo*	Pumpkin, zucchini
Fabaceae		*Pisum sativum*	Bean
		Phaseolus vulgaris	Pea
Lamiaceae		*Ocimum basilicum*	Basil
Solanaceae		*Solanum nigrum*	Black nightshade
		Solanum lycopersicum	Tomato

2.2. Animals

2.2.1. Mice

White *Mus musculus* OF1 mice from the Pasteur Institute of Madagascar (IPM) breeding farm were used in the experiments. Five-week-old male or female mice weighing 25 ± 2 g were selected.

2.2.2. Tadpoles

Legless tadpoles (*Ptychadena mascareniensis*) were captured on the day of the test in rice fields located around the Ankatso University Campus.

2.2.3. Fishes

Carp alvins (*Cyprinus carpio*) were supplied by approved private fish farmers. Before testing, these alvins must be kept in an aerated aquarium for one week.

2.2.4. Mosquito larvae

Mosquito larvae (*Culex quinquefasciatus*) were collected on the day of testing from stagnant water located on the campus of the University of Antananarivo.

2.2.5. Microbial strains

The germs used, including two Gram-positive bacteria, four Gram-negative bacteria and one yeast, were obtained from the Joseph Ravoahangy Andrianavalona Hospital Laboratory (HJRA). They were isolated from human urine or pus (Table 2).

Table 2 List of germs used

Germs	Gram	Origin	
		Urine	Pus
Candida albicans		x	
Streptococcus pyogenes	+		x
Staphylococcus aureus	+		x
Klebsiella pneumoniae	-	x	
Pseudomonas aeruginosa	-		x
Escherichia coli	-	x	
Enterobacter gergoviae	-	x	

2.3. Methods used to prepare the different extracts

All purification steps were guided by toxicity tests on mice and homogeneity tests using thin-layer chromatography.

2.3.1. Leaf powder preparation

The fresh leaves were dried away from direct sunlight for one week. They were then ground using a Philips Cucina HR1731/6 blender. The resulting fine powder was stored in jars at room temperature.

2.3.2. Hot aqueous extraction

The plant powder was suspended in distilled water at a ratio of 1:10 (w/v). The mixture was heated under reflux for 2 h on a hot plate with magnetic stirring, at the boiling point of distilled water. After cooling, the mixture was left to macerate overnight at 4 °C. The macerate was filtered through four layers of gauze. The resulting filtrate was then centrifuged at 16,000 g for 20 min. The pellet was discarded and the supernatant volume was reduced by evaporation using a rotary evaporator at a ratio of 1:1 (1 ml per 1 g of starting material). The resulting solution constituted the crude extract (CE).

2.3.3. 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 resulting residue recovered in distilled water.

2.3.4. Dialysis

The extract to be treated was introduced into a dialysis membrane with a filtration threshold of 15,000 Da. The counter-dialysis liquid used was distilled water, the volume of which was 500 times greater than that of the extract to be dialyzed. This liquid was constantly kept under magnetic stirring. This process was repeated several times.

2.3.5. Activated charcoal treatment

Treatment with activated charcoal was carried out using the Jeannoda method [9].

2.3.6. Precipitation with neutral lead acetate

The precipitation method using neutral lead acetate was adapted from the procedure described by Rasoatahina *et al.* (2024) [10].

2.3.7. n-Butanol fractionation

The same volume of extract and 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 mice

Assessment of acute toxicity

Acute toxicity in mice was assessed by intraperitoneal (i.p.) injection of 0.3 ml of test extract per 25 g of mouse. For each test, a batch of 3 mice was used. Another batch of 3 mice of the same weight, receiving 0.3 ml of a physiological solution (0.9% NaCl), served as a control.

The dose that killed 50% of mice in 24 h or LD_{50} was determined by calculation and graphical methods [11**]**. Seven doses of the extract, in geometric progression of reason r =1.113, ranging from 20.03 mg/kg to 37.5 mg/kg b.w., were injected intraperitoneally into 7 batches of 6 mice. A batch of 6 mice injected with 0.3 ml of saline solution served as a control.

Histopathological examination

Mice that have developed symptoms of intoxication were sacrificed. Hearts, livers, kidneys, brains, lungs and stomachs were rapidly removed and immersed in fixative fluid (BOUIN solution: picric acid 5 ml, formol 40% 20 ml, glacial acetic acid 5 ml). After 48 h, each organ was fragmented into thin 5 mm thick sections using a scalpel. More details of the method used were given in our previous article [10].

2.4.2. Haemolytic test on sheep red blood cells

In the presence of a haemolytic substance, red blood cells were lysed, releasing hemoglobin and turning the supernatant red. Intact red blood cells sedimented. The method used was that described in our previous article [10]. The composition of the medium used for the test is presented in Table 3.

Table 3 Composition of the medium for the haemolytic test

Well number	**C^+**	**C^-**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Test extract 1 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	49.80	49.90
2% red blood 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 concentration of extract (mg/ml)	0	0	1	0.5	0.25	0.125	0.065	0.031	0.015	0.007	0.003	0.001
Final volume of the mixture (µl)	100	100	100	100	100	100	100	100	100	100	100	100

C+: Positive control (total haemolysis); C-: Negative control (no haemolysis)

2.4.3. Effect on carp alvins and frog tadpoles

Seven alvins or legless tadpoles were selected and placed in crystallisers containing 200 ml of rainwater. Different quantities of the extract to be studied were added in order to obtain different concentrations in geometric progression. The experiment lasted 24 h. The LC_{50} (24 h) was determined by testing various concentrations of the extract, and the results were analyzed using the graphical method described by Boyd (1966) [12**].**

2.4.4. Effect on mosquito larvae

Five batches of 20 stage three larvae were used. These larvae were placed in crystallisers containing 200 ml of spring water with various concentrations of the extract to be tested. A further batch placed in water served as a control [13].

2.5. Methods used to study effects on seed germination

For each seed species, two batches of seeds were soaked in tap water for 48 h in darkness at 30°C. The first batch was then germinated on water-soaked cotton wool, while the second was germinated on cotton wool impregnated with the extract at a specific concentration. The results were observed 72 h after soaking.

2.6. Methods used to study effects on microorganisms' growth

2.6.1. Antibiogram test

All the materials and methods used for antimicrobial assay were detailed in a previous study [14]. The results were interpreted using the scale of Ponce *et al.* (2003) [15] and Celikel *et al.* (2008) [16]: bacteria were considered not sensitive for an inhibition zone diameter (IZD) ≤ 8 mm; sensitive for 9 ≤ IZD ≤ 14 mm; very sensitive for 15 ≤ IZD ≤ 19 mm and extremely sensitive for IZD ≥ 20 mm.

2.6.2. MIC and MBC determination

The method used was based on the tube dilution technique described in the CLSI guidelines (2012) [17]. It involved the use of Mueller-Hinton broth as the sole medium, without resorting to microplates. Composition of tubes used for MIC determination are presented in Table 4.

This approach allowed the determination of the MIC, defined as the lowest concentration showing no visible turbidity, and the MBC identified as the lowest concentration with no bacterial growth after subculturing on Mueller-Hinton agar. The tubes and agar plates were then inspected for turbidity or microbial growth with the unaided eye. The culture was incubated at 37°C for 24 h. Interpretation of the results was based on the standards proposed by Dalmarco *et al*. (2010) [18]. The extract was considered bactericidal when the MBC/MIC ratio was ≤ 4, and bacteriostatic when the ratio was > 4 [19].

Table 4 Composition of tubes used for MIC determination

Test tube number										
C+	C-	1	2	3	4	5	6	7	8	9
Inoculum volume (ml)										
0	0	1	1	1	1	1	1	1	1	1
Culture medium volume (ml)										
2	1	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Extract volume (ml)										
0	1	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Final mixture volume (ml)										
2	2	2	2	2	2	2	2	2	2	2

C+: Positive control; C-: Negative control

3. Results

3.1. Preparation of the various extracts.

The various steps used to obtain the different extracts are summarized in Figure 2.

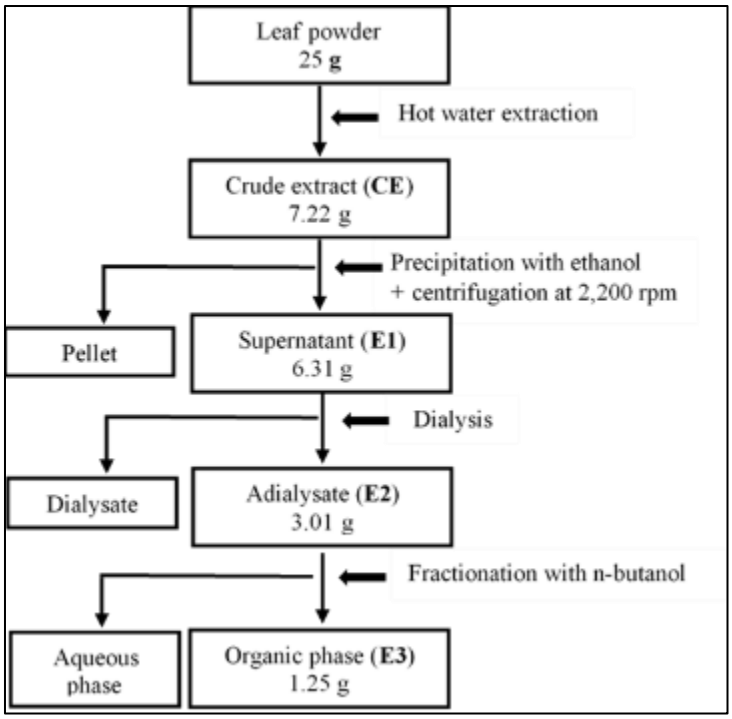
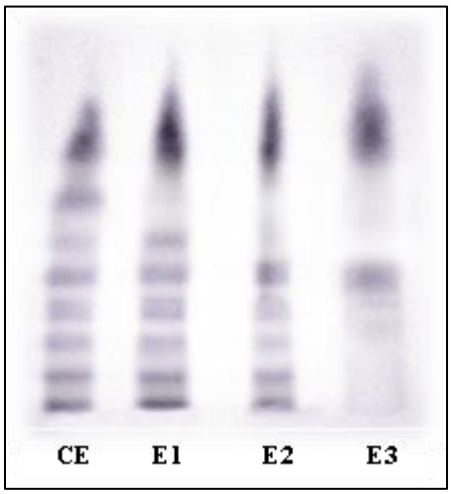


Figure 2 Diagram summarizing the extraction and the purification stages of active principles

The hot water extraction of 25 g of leaf powder produced a dark brown, bitter-tasting crude extract (CE) with a pH of 6.5 that was toxic to mice.

The extraction yield was 28.88%, whereas the purification yield of E3 from CE was 17.31%.

The evolution of the homogeneity of the toxic extracts obtained at the different purification stages is shown in Figure 3.



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

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

3.2. Phytochemical screening

Phytochemical screening results of CE and E3 are presented in Table 5.

Table 5 Phytochemical screening results of CE and E3

Chemical families	Tests	Results	
		CE	E3
Alkaloids	Mayer	-	-
	Wagner	-	-
	Dragendorff	-	-
Flavonoids	Wilstater	-	-
Leucoanthocyanins	Bate-Smith	-	-
Tannins	Gelatin 1% test	-	-
	Salted gelatin test	-	-
Polyphenols	Ferric chloride test	+	+
Unsaturated sterols	Salkowski	+	+
Triterpenes	Liebermann-Burchard	+	+
Steroids		-	-
Anthraquinones	Bornträger	-	-
Deoxyoses	Keller-Kiliani	+	-
Iridoids		-	-
Saponins	Foam test	+	+

+: Positive test; -: Negative test

CE contained polyphenols, unsaturated sterols, triterpenes, deoxyoses, and saponins. The E3 fraction contained the same phytochemical compounds with the exception of deoxyoses.

3.3. Effects on animals

3.3.1. Effects on Mice

Symptoms of intoxication

Intraperitoneal administration of E3 at a dose of 37.5 mg/kg b.w. immediately caused agitation in the mice. After 10 min, they were breathing deeply, with a respiratory rate ranging from 90 to 110 breaths per min., and remained immobile. Thirty min. later, earlobes were turned backwards and became hyperemic. After 50 min, the mice moved only rarely and dragged their hind legs. After 1 h, exophthalmos and cyanosis of the tail appeared. A gradual decrease in respiratory rate was then observed. After 2 h 30 min, clonic convulsions appeared, becoming increasingly severe and leading to the death of the animal.

The sublethal dose of 20.03 mg/kg caused the same symptoms as the lethal dose, with the exception of convulsions. The mice remained motionless for several hours and exhibited enophthalmos. A gradual remission was observed after 10 h.

LD50 value

The LD_{50} of CE by the i.p. route was estimated at 26.05 mg/kg b.w. and 27.41 mg/kg b.w. by calculation and graphical methods respectively.

3.3.2. Histopathology

The effects of the E3 at the tissue level were studied at a lethal dose of 37.5 mg/kg of mice by i.p. route. The main lesions on each organ are summarized in the Table 6 and illustrated in the Figure 4.

Table 6 Histopathological lesions caused by E3 at a dose of 37.5 mg/kg in mice

Organs	**Lesions observed (magnification x 400)**
Brain	- Congestion in the parenchyma - Dilated capillaries
Lungs	- Interalveolar haemorrhagic areas - Dilated bronchiolar veins
Liver	- Dilated sinusoidal capillaries - Haemorrhagic areas
Intestin	- Haemorrhagic areas on the mucous membrane - Congestion in the muscular layer
Heart	- Haemorrhagic areas - Congestion in the myocardium
Kidneys	- Intertubular haemorrhagic areas - Intertubular congestion

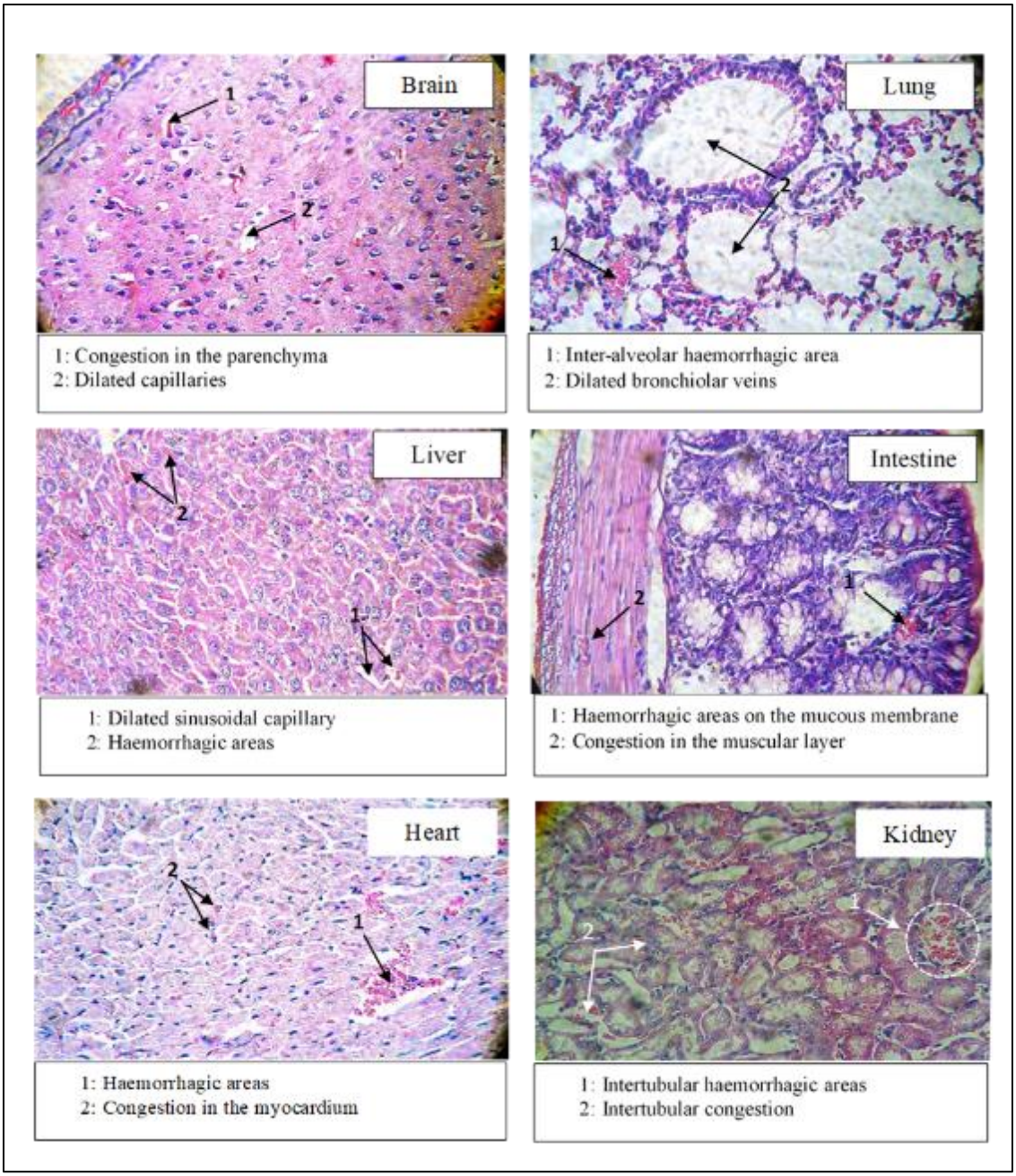


Figure 4 Histological lesions in the brain, lungs, liver, intestine, heart and kidneys due to i.p. administration of E3 at a dose of 37.5 mg/kg body weight (magnification x 400)

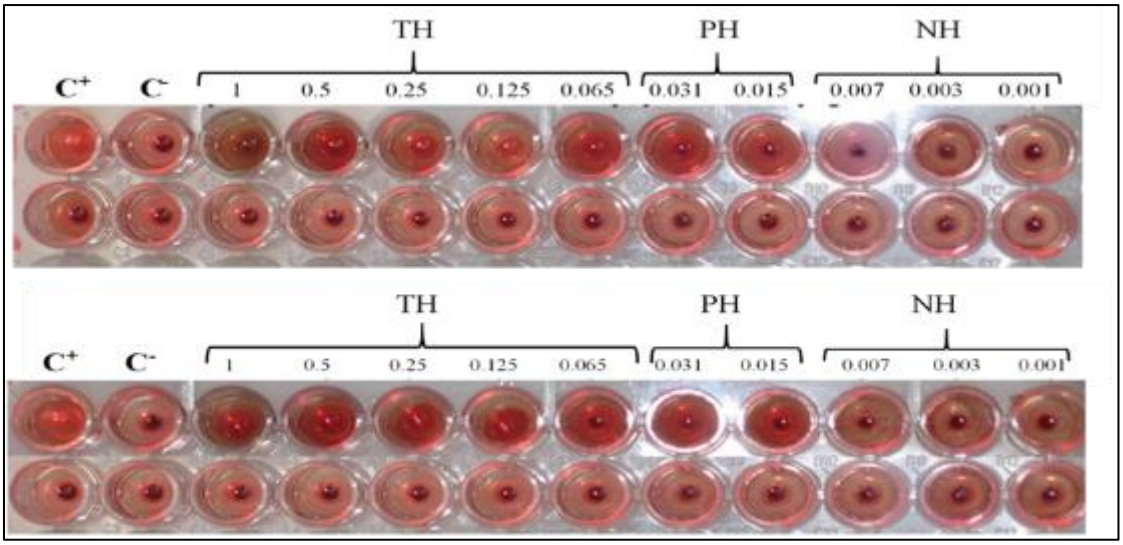
3.3.3. Effects on sheep red blood cells

A concentration-dependent hemolytic activity was observed for both CE and E3. While total haemolysis occurred at 0.065 mg/ml for CE and 0.125 mg/ml for E3, no haemolytic effect was detected at concentrations ≤ 0.007 mg/ml. The results of the haemolytic tests are presented in Table 7 and Figure 5.

Table 7 Effects of different concentrations of CE and E3 on sheep red blood cells

Concentration (mg/ml)	1	0.5	0.25	0.125	0.065	0.031	0.015	0.007	0.003	0.001
CE	++	++	++	++	++	+	+	-	-	-
E3	++	++	++	++	+	+	+	-	-	-

++: Total haemolysis; +: Partial haemolysis; -: No haemolysis.



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

Figure 5 Effects of different concentrations of CE (a) and E3 (b) on sheep red blood cells

3.3.4. Effects on frog tadpoles

Seven concentrations of CE with a geometric progression of r = 1.05 ranging from 23.87 µg/ml to 32 µg/ml, were tested on 7 batches of 7 frog tadpoles. According to the results (Table 8), CE was toxic to frog tadpoles with a dose effect. Toxicity ranged from 0% at 23.87 µg/ml to 100% at 32 µg/ml. The LC_{50} (24 h) was estimated at 28.11 µg/ml.

Table 8 Effects of different concentrations of CE on frog tadpoles

Concentration (c) in µg/ml	Log C	Number of frog tadpoles		% of dead
		dead	survival	
32	1.505	7	0	100
30.46	1.483	6	1	85.71
29.01	1.462	5	2	71.42
27.63	1.441	3	4	42.85
26.32	1.420	2	5	28.57
25.06	1.398	1	6	14.28
23.87	1.377	0	7	0

3.3.5. Effects on carp alvins

Seven concentrations of CE with a geometric progression of r = 1.05 ranging from 25.74 to 29 µg/ml were tested on 7 batches of 6 carp alvins. According to the results (Table 9), CE was toxic to carp alvins. Toxicity ranged from 0% at 25.74 µg/ml to 100% at 29 µg/ml. The LC_{50} (24 h) was estimated at 27.54 µg/ml.

Table 9 Effects of different concentrations of CE on carp alvins.

Concentration (C) in µg/ml	Log C	Number of alvins		% of dead
		dead	survival	
29	1.462	6	0	100
28.43	1.453	5	1	83.33
27.87	1.445	4	2	66.66
27.32	1.436	2	4	33.33
26.78	1.427	1	5	16.67
26.25	1.419	1	5	16.67
25.74	1.410	0	6	0

3.3.6. Effects of CE on mosquito larvae

Five concentrations of CE ranging from 1.64 to 2 mg/ml were used. No mortality or morbidity was observed after 24 h.

3.4. Effects on seed germination

The sensitivity of seeds to CE was variable (Table 10).

Table 10 Effects of CE (1 mg/ml) on seed germination

Plant family	Species	Common name	Germination rate (%)	Inhibition rate (%)
Apiaceae	*Daucus carota*	Carrot	40	60
	Petroselinum crispum	Chinese parsley	20	80
Brassicaceae	*Brassica sp*	Tissam white	100	0
Amaranthaceae	*Beta vulgaris subsp. maritima*	Beetroot	40	60
Asteraceae	*Lactuca sativa*	Lettuce	100	0
Cucurbitaceae	*Cucurbita pepo*	Pumpkin, zucchini	60	40
Fabaceae	*Phaseolus vulgaris*	Bean	100	0
	Pisum sativum	Pea	80	20
Lamiaceae	*Ocimum basilicum*	Basil	20	80
Solanaceae	*Solanum nigrum*	Black nightshade	0	100
	Solanum lycopersicum	Tomato	80	20
Poaceae	*Zea mays*	Maize, corn	80	20
	Oryza sativa	Rice	100	0

Of the 13 seed species tested, 4 (tissam white, lettuce, beans, rice) were insensitive and germinated normally, while 1 (black nightshade) did not germinate at all. Germination inhibition was also observed for the other species, ranging from 20% to 80%.

3.5. Effects of CE and E3 on microorganisms

The effects of CE at 76.8 mg/ml and E3 at 47.5 mg/ml on the seven strains using the solid medium disc method are presented in the Table 11.

Table 11 Effects of CE and E3 on microorganisms

Germs	Gram	IZD (mm)	Sensitivity	
			CE	E3
Candida albicans		6	-	-
Streptococcus pyogenes	+	6	-	-
Staphylococcus aureus	+	10	+	+
Klebsiella pneumoniae	-	6	-	-
Pseudomonas aeruginosa	-	6	-	-
Escherichia coli	-	*6*	-	-
Enterobacter gergoviae	-	6	-	-

-: non sentive; +: sensitive

Of the seven pathogenic strains tested, only *Staphylococcus aureus* was sensitive.

The MIC and MBC of CE and E3 against *Staphylococcus aureus* were determined. CE exhibited a MIC of 1.2 mg/ml and a MBC of 9.6 mg/ml, while E3 showed a MIC of 5.9 mg/ml and a MBC of 11.9 mg/mL (Table 12). The MBC/MIC ratio for CE was greater than 4, corresponding to a bacteriostatic effect on this strain while the ratio was less than 4 for E3 indicating a bactericidal effect.

Table 12 Determination of CE and E3 MIC in liquid medium on *Staphylococcus aureus*

Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Final concentration of extract (mg/ml)									
CE	76.8	38.4	19.2	9.6	4.8	2.4	1.2	0.6	0.3
Turbidity									
CE	+	+	+	+	+	+	+	-	-
Final concentration of extract (mg/ml)									
E3	47.5	23.8	11.9	5.9	2.9	1.8	0.7	0.3	0.2
Turbidity									
E3	+	+	+	+	-	-	-	-	-

+: Absence of turbidity (no microbial growth); –: Presence of turbidity (microbial growth)

4. Discussion

Several extraction and purification methods guided by toxicity tests and thin-layer chromatography were tried. The purification process comprising precipitation with ethanol, dialysis, followed by fractionation with n-butanol, yielded a partially purified toxic extract from a crude extract obtained by hot aqueous extraction. Although the other techniques tested were not selected, they provided additional information on the physicochemical properties of the toxic principles.

The toxic principles were thermostable compounds, soluble in polar solvents such as water, ethanol, methanol, and organic solvents such as n-butanol. They were precipitable by heavy metal salts such as neutral lead acetate. These compounds did not pass through the dialysis membrane, suggesting that their molecular weight could be greater than 15,000 Da, unless they interfered with the membrane. The fact that they were strongly adsorbed onto activated charcoal suggested that they might contain one or more aromatic rings in their structure.

The main chemical groups found in leaf extract of *Pittosporum senacia* were polyphenols, triterpenes, unsaturated sterols and saponins, but saponins appeared to be the main group. Saponins were found to be responsible of the toxic activity of a number of *Pittosporum* species including *Pittosporum viridiflorum* [20], *Pittosporum tobira* [21] and

Pittosporum manii [22]. From extracts of *Pittosporum verticillatum* bark powder, subspecies *verticillatum* (Malagasy species), Manase (2013) [23] isolated triterpene saponins, including three new ones, which exhibited cytotoxic properties.

Unlike extracts from *Pittosporum ochrosiaefolium* leaves, another medicinal plant of Madagascar, extracts from *Pittosporum senacia* leaves did not contain alkaloids, flavonoids, or tannins.

The set of symptoms developed by mice following intraperitoneal administration of CE suggested that the toxic principles affected several organs, but particularly on the nervous system.

E3 exhibited lytic activity against sheep red blood cells. This confirmed the saponosidic nature of its active principles. The haemolytic power of saponosides is attributed to their interaction with cholesterol in the erythrocyte membrane [24, 25].

The LD_{50} of E3 was estimated to be between 26.05 mg/kg and 27.41 mg/kg. This value was close to that of substances considered to be highly toxic (25 mg/kg) [26]. Compared to toxins already studied at LABASM, such as *Ocotea madagascariensis*, whose LD_{50} ranged between 265.99 and 272.25 mg/kg [27], E3 was significantly more toxic. However, it was much less toxic than the methanolic extract of *Albizia greveana* seeds (LD_{50} = 1.13-2.30 mg/kg) [28]. Compared to the LD_{50} of leaf extracts from other Malagasy species, evaluated under almost identical conditions (animal, route of administration) at LABASM, CE was more toxic than *Pittosporum ochrosiaefolium* (LD_{50} = 46.69 mg/kg) [8] and *Pittosporum verticillatum* (LD_{50} = 43.65–46.4 mg/kg) [6]. Comparison with the toxicity of foreign *Pittosporum* species was not easy, as their LD_{50} was sometimes evaluated on other animals and by other routes of administration, and concerned other parts of the plant.

CE was highly toxic to frog tadpoles (LC_{50} = 28.11 µg/ml) and carp alvins (LC_{50} = 27.54 µg/ml). This high toxicity was probably due to the presence of saponins. These compounds are well known to be toxic to cold-blooded animals. Many plants containing saponins are used as poison for fishing in several countries. Several species of *Pittosporum* are traditionally used for poison fishing in certain regions of the world, including *Pittosporum ferrugineum* [29] and *Pittosporum arborescens* [30].

Unlike *Pittosporum ochrosiaefolium* (2015) [8], CE has no effect on mosquito larvae.

In plants, germination of most vegetable seeds tested was inhibited by CE at 1 mg/ml, with inhibition rates ranging from 0% (*Lactuca sativa, Phaseolus vulgaris, Brassica sp., Oryza sativa*) to 100% (*Solanum nigrum*). Intermediate effects were observed in species such as *Petroselinum crispum* (80%), *Beta vulgaris* subsp. *maritima* (60%) and *Cucurbita pepo* (40%). The inhibition could be due to the destruction of the embryo (direct effect) and/or the inactivation of enzymes involved in germination (indirect effect).

Of the seven microorganisms that were tested, only *Staphylococcus aureus* was sensitive to both CE and E3. However, CE was more active (MIC = 1.2 mg/ml) than E3 (MIC = 5.9 mg/ml). Extracts from the leaves of other *Pittosporum* species exhibited broader activity spectra and they were active against certain microorganisms that were insensitive to CE and E3. For example, extracts from the leaves of *Pittosporum angustifolium* were effective against *Klebsiella pneumoniae* and *Candida albicans* [31, 32], while ethanol extract from *Pittosporum viridiflorum* leaves was effective against *Escherichia coli* [33].

5. Conclusion

Although the results of this study were still preliminary, they have improved our knowledge of *Pittosporum senacia* by providing initial information on the chemical nature and toxicity of the active compounds present in its leaves.

Given the wide range of effects of toxic compounds, it is important to take precautions to protect health and the environment.

Future work will focus on plant material harvested at other stages of the plant's life cycle; the isolation, determination of the structure of the active ingredients and the study of their mechanism of action and the effects of extracts on other microbial strains.

Compliance with ethical standards

Acknowledgments

We sincerely acknowledged the Pasteur Institute of Madagascar (IPM) for providing us with the experimental animals, the National Center for Applied Research for Rural Development (FOFIFA / CENRADERU) for plant seeds and the Joseph Ravoahangy Andrianavalona Hospital Laboratory (HJRA) for microorganisms.

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 Pasteur Institute of Madagascar.

References

[1] Das D, Khatun R, Sengupta S, Bhattacharya M. Bioactive compounds – a new era of therapeutic medicines. Journal of Plant Sciences. 2025; 13(3): 112-21.

[2] Fioccardi A, Donno D, Razafindrakoto ZR, Tombozara N, Henintsoa S, Mahitasoa E, Torti V, Solofoniaina M, Rosso L, Gamba G, Andrianjara C, Ramanitrahasimbola D, Beccaro GL. Assessing a "Least-Concern" Red List tree species from Madagascar used in traditional medicine: *Morella spathulata* (Myricaceae) phyto-compounds and anti-inflammatory properties. Plants. 2024; 13(20): 2899.

[3] Cufodontis G. Pittosporaceae. Paris: National Museum of Natural History; 1955. 43 p. (Flora of Madagascar and the Comoros; 92).

[4] Boiteau P. Traditional medicine and pharmacopoeia: a compendium of Malagasy medicinal substances. Madagascar: Agency for Cultural and Technical Cooperation; 1986.

[5] Rabesa Z. Alaotra pharmacopoeia. Madagascar: Edition Fanantonana]; 1986.

[6] Arijaona M. Partial purification and characterisation of toxic principles in *Pittosporum verticillatum* leaves (Pittosporaceae). [Master's thesis]. Madagascar: University of Antananarivo; 2005.

[7] Rakotondravaly M.D. Partial purification and characterization of toxic principles from the stem bark of *Pittosporum verticillatum*. [Master's thesis]. Madagascar: University of Antananarivo; 2015.

[8] Ratsimiebo MP, Ramanitrahasimbola D, Rajemiarimoelisoa CF, Razafindrakoto ZR, Randrianarivo HR, Rakoto DAD, Jeannoda VL. Toxicity study of *Pittosporum ochrosiaefolium* Bojer (Pittosporaceae), a medicinal plant of Madagascar. Journal of Plant Sciences. 2015; 3(6): 349–57.

[9] Jeannoda VLR. Chemical, biological and toxicological studies of the convulsant principle of the Connaraceae of Madagascar. [PhD dissertation]. Strasbourg, University Louis Pasteur of Strasbourg, 1986.

[10] Rasoatahina V, Razanatseheno MS, Ratsimiebo MP, Ranjàna HR, Randrianarivo L, Randriamampianina LJ, Rakoto DAD, Jeannoda VL. Phytochemical and toxicological studies of leaf extracts of *Gambeya boiviniana* Pierre (Sapotaceae), a medicinal plant. GSC Biological and Pharmaceutical Sciences. 2024; 29(2): 420-30.

[11] Reed L and Muench HA. Simple method of estimating fifty per cent point. American. Journal of Hygiene. 1938; 27: 493-497.

[12] Boyd WC. Fundamentals of immunology, 4th ed. New York: Wiley and Sons; 1966.

[13] WHO (1970). Insecticide resistance and vector control. 17th Report of the WHO Expert committee on insecticides. Series and technical reports n°443. Geneva, 306.

[14] Randriamampianina LJ, Razafintsalama VE, Rakoto DAD, Randrianarivo HR, Jeannoda VL. Antimicrobial activity of seed extracts from *Albizia bernieri* E. Fourn. (Fabaceae). Journal of Pharmacy and Biological Sciences. 2017; 12(3):72-9.

[15] Ponce AG, Fritz R, Del Valle C, Roure SI. Antimicrobial activity of essential oils on the native microflora of organic Swiss chard. Lebensmittel-Wissenschaft und-Technologie. 2003; 36: 679-84.

[16] Celikel N, Kavas G. Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech Journal of Sciences. 2008; 26(3):174-81.

[17] CLSI. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 9e éd. Wayne (PA) : Clinical and Laboratory Standards Institute; 2012.

[18] Dalmarco JB, Dalmarco EM, Koelzer J, Pizzolati MG and Fröde TS. Isolation and identification of bioactive compounds responsible for the anti-bacterial efficacy of *Lotus corniculatus* var Sâo Gabriel. International Journal of Green Pharmacy. 2010; 4(2): 108-14.

[19] Chamandi G, Olama Z, Holail H. Antimicrobial effect of propolis from different geographic origins in Lebanon. International Journal of Current Microbiology and Applied Sciences. 2015; 4(4): 328-42.

[20] Seo Y, Berger JM, Hoch J, Neddermann KM, Bursuker I, Mamber SW, Kingston DGI. A new triterpene saponin from *Pittosporum viridiflorum* from the Madagascar rainforest. Journal of Natural Products. 2002; 65(1):65-8.

[21] D'acquarica I, Di Giovanni MC, Gasparrini F, Misiti D, D'arrigo C, Fagnano N, Guarnieri D, Iacono G, Bifulco G, Riccio R. Isolation and structure elucidation of four new triterpenoid estersaponins from fruits of *Pittosporum tobira* Ait. Tetrahedron. 2002; 58:10127-36.

[22] Nyongbela KD, Lannang AM, Ayimele GA, Ngemenya MN, Bickle Q, Efange S. Isolation and identification of an antiparasitic triterpenoid estersaponin from the stem bark of *Pittosporum mannii* (Pittosporaceae). Asian Pacific Journal of Tropical Disease. 2013; 3(5):389-92.

[23] Manase MJ. Chemical and biological study of saponins isolated from three Malagasy species belonging to the Caryophyllaceae, Pittosporaceae and Solanaceae families. [PhD dissertation]. France: University of Bourgogne; 2013.

[24] Bruneton J. Elements of phytochemistry and pharmacognosy. Paris: Technique and Documentation – Lavoisier; 1987.

[25] Bruneton J. Pharmacognosy, phytochemistry, medicinal plants. Paris: Lavoisier; 1993.

[26] Ramade E. Ecotoxicology. Paris: Masson; 1979.

[27] Randriamahavalisoa F. Chemical and biological study of toxic extract from *Ocotea madagascariensis* leaves (Lauraceae). [Master's thesis]. Madagascar: University of Antananarivo; 2003.

[28] Randrianarivo HR, Razafindrakoto AR, Ratsimanohatra HC, Randriamampianina LJ, Rajemiarimoelisoa CF, Ramamonjisoa L, Ramanitrahasimbola D, Rakoto DAD, Jeannoda VL. Toxic effects of seed methanolic extracts of endemic *Albizia* species (Fabaceae) from Madagascar on animals. Journal of Life Sciences. 2014; 8(8):676-89.

[29] Lucidcentral. 2025. *Pittosporum ferrugineum*-Rainforest. In: Australian Tropical Rainforest Plants – Species Profiles. Lucidcentral. [https://apps.lucidcentral.org/rainforest/text/entities/pittosporum_ferrugineum.htm] (cited 2025 Jul 20).

[30] Secretariat of the Pacific Community (SPC). Fisheries in the economies of Pacific Island countries and territories. Nouméa: SPC; 2009.

[31] Blonk B, Cock IE. Interactive antimicrobial and toxicity profiles of *Pittosporum angustifolium* Lodd. extracts with conventional antimicrobials. J Integr Med. 2019; 17(4): 261-72.

[32] Phan ADT, Chaliha M, Hong HT, Tinggi U, Netzel ME, Sultanbawa Y. Nutritional value and antimicrobial activity of *Pittosporum angustifolium* (Gumby Gumby), an Australian indigenous plant. Foods. 2020; 9(7): 887.

[33] Swamy TA, Obey JK, Kipngetich TE, Edwin M. In vitro antibacterial activity of ethanolic extract of Pittosporum viridiflorum leaves against laboratory strains of selected microorganisms. International Journal of Bioassays. 2014; 3(10): 3388-94.