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(RESEARCH ARTICLE)

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Phytochemical screening and evaluation of the anti-diarrheal activity of *Syzygium emirnense* (Baker) Labat and G.E. Schatz (MYRTACEAE)

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Abstract

Syzygium emirnense (Myrtaceae), a plant endemic to Madagascar, is used in traditional medicine to treat diarrhea. Phytochemical screening of its leaves revealed the presence of tannins, polyphenols, steroids and irridoids, as well as the absence of alkaloids, flavonoids and saponins. The use of three different extraction methods, including reflux decoction, first boiling decoction and halving decoction, yielded extracts RFM114a, RFM114b and RFM114c respectively. The anti-diarrheal properties of these extracts were assessed by an antibiogram test (1mg/ml) against the bacterial strains responsible for diarrhea (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella enterica, Clostridium perfringens, Proteus mirabilis and Listeria monocytogenes*). No antibacterial effect was observed. On the other hand, an *in vivo* intestinal transit test was carried out on mice previously induced with diarrhea by castor oil, by measuring the displacement of the colored meal. All three extracts inhibited diarrhea at all doses tested (100mg/kg and 300mg/kg) compared with the untreated control ($p < 0.05$). At 300mg/kg, the RFM114a extract was the most active.

These results suggest that *Syzygium emirnense* leaf decoction is effective against secretory and motor diarrhea, but not against bacterial diarrhea.

Keywords: *Syzygium emirnense;* Endemic; Phytochemical screening; Anti-diarrheal; Decoction

1. Introduction

Diarrhea is the passage of more than three loose or liquid stools per day. This is the second leading cause of death among children under five worldwide every year [1].

The demographic and health survey carried out in Madagascar revealed that 9% of children under 5 years old have contracted diarrhea [2]. Thus, in developing countries like Madagascar, the fight against diarrhea remains a priority, because it is one of the factors that promote malnutrition [3]. To reduce the incidence of diarrhea, several methods have been adopted in both adults and children, including: oral rehydration therapy, use of probiotics, and resorting of

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medicinal plants. The latter practice is strongly influenced by limited access to healthcare and poverty, making it a fundamental part of the population's medical treatment and an alternative to modern medicine [4].

In Madagascar, 80% of plants are endemic [5]. Thanks to this richness, the use of plants in traditional medicine is very widespread. Among them, *Syzygium emirnense* (Baker) Labat & G.E. Schatz, an endemic and medicinal plant of Madagascar belonging to the Myrtaceae family, is widespread on the island and known by the vernacular name of « rotranala ». Due to this great distribution, its leaves are used as a remedy to treat scabies, toothaches and diarrhea [6].

Severals studies carried out on the *Syzygium* genus have demonstrated a range of biological properties, including antinociceptive, anti-inflammatory [7,8], anticancer [9–11], antibacterial [12–14], insecticide [15] as well as antioxidant [16–20]. However, to our knowledge, no research on the antidiarrheal properties of *Syzygium emirnense* is currently available. In this context, this document has been prepared to highlight this information for the first time, taking into account the mode of use by local population.

2. Materials and methods

2.1. Materials

2.1.1. Collection and preparation of the plant

Leaves of *Syzygium emirnense* (Baker) Labat & G.E. Schatz were collected and identified by botanists from the National Center for Pharmaceutical Research Applications (CNARP) in Tampolo in the Fenerive-Est District, Analajirofo Region, Madagascar in July 2021. A reference herbarium coded RFM114 has been deposited in the Centre's herbarium. The leaves were dried in a dark place and then ground. The powder obtained was stored until it was used.

2.1.2. Microbial strains

In this study, strains referenced ATCC responsible for diarrhea and food poisoning were used. Four were Gram-positive: *Staphylococcus aureus* (ATCC 11632), *Bacillus cereus* (ATCC 14579), *Listeria monocytogenes* (ATCC 19114), *Clostridium perfringens* (ATCC 13124). Three were Gram-negative: *Escherichia coli* (ATCC 8739), *Salmonella enterica* (ATCC 13076), *Proteus mirabilis* (ATCC 13047).

2.1.3. Experimental animals

The *Swiss* breed mice weighing approximately 30g used for the tests came from the CNARP Pharmacodynamics Department animal house. They were fasted and allowed to acclimatize to laboratory conditions before each test.

2.2. Methods

2.2.1. Extraction

Methanolic extraction

An extraction by methanol exhaustion was carried out on 50 g of *S. emirnense* powder (w/v; 1/10). After dry evaporation of the solvent with a rotative evaporator, a methanolic extract was obtained.

Extraction using decoction

The ethnobotanical investigation carried out on the plant revealed that the decoction of its leaves is used against diarrhea, although no exact dosage and method of preparation has been determined. Thus, three decoction methods were adopted: reflux decoction (t=15min), generally used in the laboratory, decoction by first boiling (t≤ 5min) and decoction by reduction by half (t≥30min), the last two being the most commonly used by the local population. Each method is carried out from 100g of leaf powder, mixed with distilled water according to the ratio 1/10 (w/v). After decoction, the mixture is filtered and then concentrated to dryness in a ventilated oven at 40°C.

2.2.2. Phytochemical screening

Phytochemical screening is a method based on colorimetric reactions and precipitation using different reagents to identify the chemical families present in the plant.

It was carried out according to the method of FONG *et al*., (1977) [21], using a methanolic extract obtained from plant powder.

2.2.3. Antimicrobial activity test

The antibiogram disk diffusion method was used to assess the antimicrobial activity of the extracts. A young culture suspension of bacteria with an opacity equivalent to the Mac Farland 0.5 standard was prepared and spread on the surface of the agar in a Petri dish using a sterile swab. Ten microliters of the 1 mg/ml solution of each extract were impregnated onto empty standard antibiogram discs (6 mm in diameter) and then placed on the agar surface using sterile forceps. The preparation was incubated at 37 °C for 24 h. Gentamycin (10 µg/disc), a reference antibiotic, was used as a control under the same conditions. The test was performed in triplicate.

The result is read by measuring the diameter of the zone of inhibition or halo of inhibition according to the standards of Ponce *et al*. (2003) [22]. The halo diameter depends on the degree of sensitivity of the germs to the extracts or antibiotics (table 1).

Table 1 Interpretation of the diameter of the inhibition halo

Halo diameter	Germ sensitivity	Results
x<8 mm	Insensitive	-
9 mm<x<14 mm	Sensitive	+
15 mm<x<19mm	Very sensitive	++
x>20 mm	Extremely sensitive	+++

2.2.4. Intestinal transit test

To assess the ability of extracts to act on intestinal transit, the Andargie *et al.*'s test (2022) was adopted [23]. It involves measuring the distance travelled by a colored meal in the small intestine in the presence of an extract or reference product. To do this, male *Swiss* mice were randomly divided into 8 groups of 5 mice: 2 groups served as controls (Imodium and untreated), while the other 6 received the 3 decoction extracts at doses of 100 and 300 mg/kg, respectively. The mice were fasted overnight and allowed to acclimate to laboratory conditions.

Before the test, castor oil was administered orally at a rate of 1 ml per 100 g of the animal's body weight to induce diarrhea. One hour later, the extracts and Imodium were administered. Then, after a one-hour interval, the mice in each group were force-fed the colored meal consisting of a mixture of activated charcoal, gum arabic, and distilled water. Finally, one hour later, a dissection of the mice in each group preceded by general anesthesia with urethane was carried out to observe the effect of the extracts or products on the displacement of the colored meal by measuring the distance traveled in the small intestine.

The result is expressed as a percentage according to the formula:

$$\text{Displacement (\%)} = (\text{distance travelled by the meal} / \text{total length of the small intestine}) \times 100$$

And the inhibition of diarrhea is obtained by:

$$\text{Inhibition of diarrhea} = 100\% - \text{Displacement}$$

2.2.5. Statistical analysis

The results obtained are expressed as mean±standard deviation. Differences between batches in the intestinal transit test are determined by Student's t-test. The difference is considered significant if the *p*-value is less than 0.05.

3. Results

3.1. Yield

The three decoction methods adopted: reflux decoction, decoction by first boiling and decoction by halving yielded extracts RFM114a (34.34%), RFM114b (23,7%) and RFM114c (14,2%) respectively.

3.2. Chemical families

This technique revealed the presence of tannins, polyphenols, steroids, and irridoids in the plant's leaves. Alkaloids, flavonoids, and saponins were not detected. The results are presented in Table 2.

Table 2 Results of the phytochemical screening on *Syzygium emirnense*

Families	Test	Results
Alkaloids	Mayer	-
	Wagner	-
	Dragendorff	-
Tannins and polyphenols	1% gelatin	++
	1% salted gelatin	++
	with $FeCl_3$	++
	Hydrochloric vanillin	-
Triterpenes and steroids	Libermann-Bürchard	++
Unsaturated sterols	Salkowsky	++
Lactonic sterols	Badget	++
Irridoids	Trim et Hill	+
	Heated HCl	+
	Glycerol	+
Deoxyoses	Keller-Killiani	+
Cardenolides	Pesez	-
Quinones	Swendsen – Jensen	-
Flavonoids	Wilstatter	-
Leucoanthocyanes	with HCl35%	-
Saponins	Foam index	-
Coumarins	With sodium hydroxide	-

With: -: negative reaction, chemical family not detected or in trace amounts; +: precipitation or weak coloration, chemical family in small quantities; ++: precipitation or marked coloration, chemical family in significant quantities; +++: precipitation or abundant coloration, chemical family in abundant quantities

3.3. Antimicrobial activity test

The results obtained are recorded in Table 3. The comparison with the standards of Ponce *et al*., (2003) [22], show that the extracts did not cause any inhibition of strains growth with halo diameters of less than 8 mm.

Table 3 Inhibition halo diameter of extracts tested

Souches	RFM114a	RFM114b	RFM114c	Gentamycine (10µg/disque)
S. aureus	8±0	7.5±0	0±0	13±1
B. cereus	6±0	6±0	0±0	14±0
E. coli	6±0	6±0	6±0	12±0
S.enterica	0±0	0±0	0±0	13±0
C. perfringens	0±0	0±0	0±0	11±0
L. monocytogenes	0±0	0±0	0±0	12±0
S. mirabilis	0±0	0±0	0±0	12±0

3.4. Effects on intestinal transit

After general anesthesia with urethane, the total length of the small intestine and the distance covered by the colored meal were measured. The displacement was calculated and the results obtained are shown in Table 4 and Figure 1.

According to these results, the RFM114a b and c extracts inhibited the displacement of the colored meal compared with the untreated control. A significant difference was also demonstrated ($p<0.05$). RFM114a at a dose of 300 mg/kg showed an activity comparable to Imodium drug control, which was 73.2±3.1% ($p>0.05$). This means that of the extracts tested, RFM114a (300mg/kg) was the most active.

Table 4 Summary of intestinal transit results

Extracts/Products	Length of small intestine (cm)	Length covered by the coloured meal (cm)	Displacement of coloured meal (%)	Inhibition of diarrhoea (%)
Untreated control	54.2±7.5	41±7.2	75.4±2.8	**24.6±2.8**
Imodium (10mg/kg)	57.2±1	15.2±2	26.8±3	**73.2± 3** ***a1***
RFM114a (100mg/kg)	52±5.7	25±1.4	48.5±8	**51.5±8** ***a1b1***
RFM114a (300mg/kg)	51.2±2.1	13±2	25.4±3.6	**74.6±3.6** ***a1b2***
RFM114b (100mg/kg)	50.5±3.5	34.3±3.2	67.8±1.6	**32.2±1.6** ***a1b1***
RFM114b (300mg/kg)	46±1.4	22.5±9.2	49.2±2.5	**50.8±2.5** ***a1b1***
RFM114c (100mg/kg)	50.8±0.4	28.8±2.5	56.6±4.5	**43.4± 4.5** ***a1b1***
RFM114c (300mg/kg)	46±1.4	22.5±1.4	48.3±2.6	**51.7±2.6** *a1b1*

mean± standard deviation (n=5); analysis performed with Student's t test; a: comparison with non untreated control; b: comparison with treated; 1: $p<0.05$ the difference is considered significant; 2: $p>0.05$ no significant difference is found.

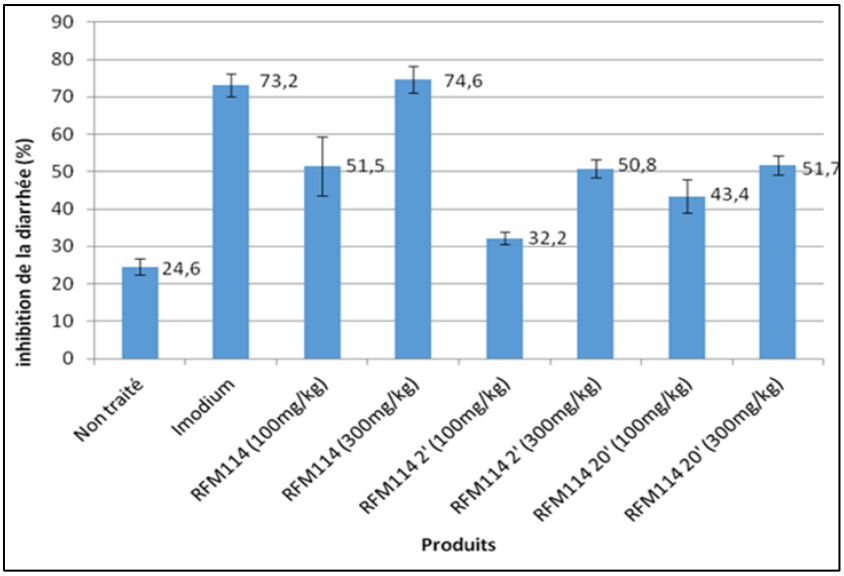

Figure 1 Inhibition of diarrhea by extracts at different doses

4. Discussion

The plant continues to be one of the main sources of active compounds, as it has long been used as a remedy to treat almost all human diseases. This study assesses the anti-diarrhoeal activity of *Syzygium emirnense.* An in-depth study of the therapeutic properties of a plant, from a biological point of view, helps to optimize its use in traditional medicine. Determinate of the secondary metabolites present in the plant, by phytochemical screening, provides an insight into the plant's potential therapeutic and physiological properties.

Syzygium emirnense leaves revealed the presence of tannins, polyphenols, triterpenes, sugars and irridoids, while alkaloids, flavonoids and saponins were absent. The composition of secondary metabolites varies according to the plants. In the Myrtaceae family for example *Myrtus communis* [24] and *Syzygium aquem* [25] alkaloids are also absent, while flavonoids, tannins and polyphenols are predominantly represented. In contrast, the work carried out by Yahaya *et al*, (2024) [26] on *Syzygium samaragens*e showed that all families of alkaloids, flavonoids, saponins, tannins and polyphenols compounds were present.

Tests carried out on a number of bacterial strains responsible for diarrhoea and food poisoning (*Staphylococcus aureus, Bacillus cereus, Escherichia coli, Salmonella enterica, Clostridium perfringens, Proteus mirabilis, Listeria monocytogenes*) showed that none of the aqueous extracts of *Syzygium emirnense* leaves had inhibitory effect on their growth. Similar results were observed with *Syzygium cumini* leaves [27], of the extracts tested (methanolic, ethanolic and aqueous), the aqueous extract was inactive against tested bacteria. The lack of antibacterial activity in aqueous extracts of *Syzygium emirnense* could be linked to the absence of flavonoids, alkaloids and saponins. Research by several authors has shown that these compounds are largely responsible for the antimicrobial activity of a plant [28–34].

On the other hand, the ability of a compound to diffuse in an agar medium affects the size and shape of inhibition zones in antimicrobial susceptibility tests. Compounds with high diffusibility create larger, more distinct zones, while those that diffuse poorly present smaller, more irregular zones. This could be due to the molecular weight and properties of the compound: small molecules generally diffuse faster than large ones, and some compounds may present inherent limitations to diffusion due to their chemical structure or interactions with the agar matrix. All these characteristics could directly affect the visual results of antibiograms, influencing data interpretation and the accuracy of sensitivity determination [35–38].

Diarrhea occurs when there is an imbalance between secretion and reabsorption in the gastrointestinal tract and/or when there is damage to the mucosa [39]. This has been observed in particular with the use of castor oil as a diarrhea precursor in *in-vivo* tests of intestinal transit. Castor oil induces primarily secretory diarrhea, which stimulates intestinal secretion of electrolytes and water via prostaglandin-mediated pathways. It also has a secondary motor effect, increasing intestinal motility and reducing absorption time [40]. Its active compound, ricinoleic acid, disrupts intestinal

permeability and stimulates peristalsis, leading to excessive secretion of water and electrolytes into the intestinal lumen. [24]. The inhibition or regulation of this disruption is an indicator of anti-diarrheal activity.

The three extracts obtained from the decoction of *Syzygium emirnense* leaves significantly inhibited the displacement of the colored meal compared with the control. The strongest effect was seen with the RFM114a extract at a dose of 300mg/kg. This suggests that the plant can reduce or even inhibit the effects of castor oil by modulating intestinal peristalsis [41]. It could be due to the presence of tannins [42–44] which exert an astringent effect by reinforcing the protection of the deep layers of the gastrointestinal mucosa, making it more impervious to irritants and micro-organisms [45].

As no standardized traditional preparation method was identified, three decoction methods were tested: reflux decoction (15 min), decoction by first boiling (≤5 min) and decoction by halving (≥30 min). All showed antidiarrheal activity, but the extract from the reflux decoction was the most active. This result could be explained by the extraction time: too short a time may prevent complete extraction of the active ingredients, while too long a time may lead to their degradation [46].

Thus, the two methods most commonly used by the Malagasy population - decoction by first boiling (RFM114b) and decoction by halving (RFM114c) - also showed an inhibition of diarrhoea, with no significant difference between them. This suggests that, whatever the traditional method of preparation adopted, the therapeutic result remains similar.

5. Conclusion

The present work has demonstrated, for the first time, the anti-diarrheal activity of *Syzygium emirnense* leaves, traditionally used in medicine. The results showed that the plant was effective against secretory and motor diarrhea, but had no activity against diarrhea of microbial origin. In addition, the duration of the decoction proved to be a determining factor, significantly influencing the efficacy of the extract. In order to gain a better understanding of the overall therapeutic potential of *Syzygium emirnense*, Further studies involving other plant organs will be undertaken, as well as studies of antimicrobial activity in liquid media, to ensure that diffusibility in solid media is not a limiting factor in the dynamic interaction between the compounds tested and bacteria.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest

Statement of ethical approval

All the tests on animal followed the directive of Institut Pasteur of Madagascar guidelines

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