Original Research Article

Saba senegalensis (A.DC) Pichon (Apocynaceae): Treatment of Loperamide-induced constipation in mice

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ABSTRACT

This study aimed to assess the laxative properties of an aqueous extract of *Saba senegalensis* leaves, an African medicinal plant, in mice. To this end, assessment tests were carried out on the laxative activity of *Saba senegalensis* and its effect on the transit of activated charcoal. As regards the evaluation of its laxative activity, four batches of five mice, non-constipated and constipated with loperamide, were formed. Batch 1 served as a control, while batch 2 received Forlax, a reference osmotic laxative. Batches 3 and 4 were treated orally with *Saba senegalensis* with concentrations of 150 and 300 mg/Kg BW. Parameters such as the number of constipated and non-constipated mice that defecated were recorded, as were the moisture content and appearance of the faeces. As for the effect of activated charcoal on transit, batches of non-constipated mice treated as above were given 0.2 mL of activated charcoal sixty minutes after treatment. Then, thirty minutes later, the intestine of the sacrificed mouse was sampled and the transit of the activated charcoal was assessed.

The laxative activity assessment test revealed that *Saba senegalensis* with 150 mg/Kg BW had no significant effect in non-constipated mice. However, it moistened the stools of constipated mice ($P < 0.001$). With 300 mg/Kg BW, *Saba senegalensis*, like Forlax, increased the number of non-constipated mice defecating and relieved constipation in loperamide-treated mice. It also softened the stools ($P < 0.01$-0.0001) and modified their appearance. The intestinal transit test showed that *Saba senegalensis* with 150 and 300 mg/Kg BW caused a dose-dependent increase in the intestinal transit of activated charcoal ($P < 0.001$), as did Forlax ($P < 0.001$).These findings support the *Saba senegalensis* laxative properties and its use in traditional medicine to treat constipation.

Keywords: laxative, Saba senegalensis, loperamide, constipation, mouse

1. INTRODUCTION

Constipation, a gastro-digestive disorder with a prevalence ranging between 3 and 15%, affects more women than men (Saheed and Omotayo 2005, Suares and Ford 2011). The use of laxatives as a means of treating this condition is less popular today, given their side-effects such as cramps, pain and atony of the intestine. For these reasons, the African population has opted for plants as an alternative (Kagnou et al. 2020). However, if medicinal plants are only used in the traditional way, patients are at risk of intoxication and overdose. They therefore need to be subjected to pharmacological research in order to assess their toxicity and identify their properties and mechanisms of action. The interest shown in *Saba senegalensis*, a plant in the Ivorian pharmacopoeia with multiple virtues, is part of this context.

Originating in tropical Africa, *Saba senegalensis* is a liana-like plant belonging to the Apocynaceae family (Sarr et al. 2018)].

In traditional medicine, *Saba senegalensis* is used to treat wounds and gastro-digestive disorders. Its roots are used to treat female sterility and skin lesions. The powdered bark of the dried root is applied to wounds to heal them (Burkill 2000). According to pharmacological data, *Saba senegalensis* leaves are used to treat dysentery, food poisoning and urinary schistosomiasis; the leaves can also be used to treat amoebic dysentery and vomiting (Guissou 2014, Sarr et al. 2015) have shown that *Saba senegalensis* leaves have haemostatic and antiseptic properties. In addition, it is reported to be anthelmintic (Belemlilga et al. 2014) and potentially anti-diabetic (Dosso et al. 2020).

Despite all these pharmacological studies, very few have focused on gastro-digestive disorders. In order to address this lack of data, this study was initiated with the aim of evaluating the laxative effect of *Saba senegalensis* leaves in mice.

2. MATERIAL AND METHODS

2.1 Planting material

The material used consists of fresh *Saba senegalensis* leaves harvested in June 2021 in Korhogo. These leaves were identified by an expert from the National Centre for Floristics at the Félix HOUPHOUET-BOIGNY University in Côte d'Ivoire.

2.2 Animal material

Male and female mice of the species *Mus musculus* weighing between 20 and 30 g were used for testing the laxative effect of the aqueous extract of *Saba senegalensis* leaves. These effects were also evaluated on the transit of activated charcoal. They came from the animal supply facility at the Institut Pasteur in Adiopodoumé on the road to Dabou (Côte d'Ivoire). They were transported to the animal supply facility at Peleforo GON COULIBALY University, where they were reared at an average temperature of 28°C with a relative humidity of 70% and were given a diet of pellets and water.

2.3 Aqueous extract of *Saba senegalensis* leaves preparation

The method used to prepare the aqueous extract of *Saba senegalensis* leaves is the one described by (Guédé-Guina et al. 1997)]. The leaves of *Saba senegalensis* were dried in the shade at a temperature ranging from 26°C to 30°C and then ground to a powder using a grinder. A quantity (150.5 g) of *Saba senegalensis* leaf powder was macerated in 2 litres of distilled water for 24 hours on an AGIMATIC-N type magnetic stirrer operating at room temperature. At the end of maceration, the solution was filtered through cotton wool and 3 mm diameter wattman paper. The filtrate obtained was placed in crystallisers and then placed in an oven at 60°C for complete dehydration. The mass at the bottom of the crystallisers was scraped off and the powder obtained constituted the dry extract used to prepare the aqueous extract of *Saba senegalensis* leaves.

2.4 Calculation of extraction yield

For a given quantity of *Saba senegalensis* leaf powder, the quantity of extract obtained after cold maceration in distilled water is evaluated. The extraction yield is calculated as the ratio of the quantity of dry extract obtained to the quantity of leaf powder used.

2.5 Calculation of the minerals in the aqueous extract of *Saba senegalensis* leaves

The minerals in the aqueous extract of *Saba senegalensis* leaves were calculated using the AAS 20 VARIAN air-acetylene flame atomic absorption spectrophotometer. To do this, the aqueous sample of *Saba senegalensis* leaves is sprayed onto a flame and the light produced is used to measure the minerals.

2.6 Laxative effect of *Saba senegalensis* in non-loperamide treated mice

This study was carried out using the modified method of (Diurno et al. 1996). Twenty (20) mice were divided into four homogeneous groups of five mice each. They were kept fasting for 16 hours prior to the experiment. The different groups were treated as follows:

Batch 1: distilled water (10 mL/kg BW)
Batch 2: Forlax (10 mg/kg BW)
Batch 3: *Saba senegalensis* (150 mg/kg BW)
Batch 4: *Saba senegalensis* (300 mg/kg BW).

The animals were kept in individual cages with floors lined with white paper and the number of dry and watery excreta was counted every four hours for a period of eight hours. The number of treated animals releasing faeces was recorded. The percentage of wet faeces was also assessed as the ratio of the number of wet faeces to the total number of faeces. Faeces consistency was also assessed using the slightly modified (scale) (Heaton et al. 1992).

2.7 Laxative effect of *Saba senegalensis* in loperamide-treated mice

The modified method of (Diurno et al. 1996) was used on batches of mice treated with loperamide, an anti-diarrhoeal, inducing a state of constipation in the animal. Twenty (20) constipated mice were divided into four (4) groups with five (5) mice per group:

Batch 1: Loperamide (5 mg/Kg BW) and distilled water (10 mL/kg),
Batch 2: Loperamide (5 mg/Kg BW) and Forlax (10 mg/kg BW)
Batch 3: Loperamide (5 mg/Kg BW) and *Saba senegalensis* (150 mg/kg BW)
Batch 4: Loperamide (5 mg/Kg BW) and *Saba senegalensis* (300 mg/kg BW).

The mice were kept in individual cages with floors lined with white paper and the number of dry and aqueous excreta was counted every four hours for a period of eight hours. The number of treated mice releasing faeces was recorded. The percentage of wet faeces was also calculated. The consistency of the faeces was also recorded (Heaton and Radvan 1992).

The method described by (Wolfgang et al. 2002) was used. This involved assessing the time taken for activated charcoal to pass through the gastrointestinal tract of mice, after a predefined time.

2.8 Gastrointestinal motility test

The method described by (Wolfgang and Bernward 2002) was used. This involved assessing the time taken for activated charcoal to pass through the gastrointestinal tract of mice, after a predefined time. Twenty (20) mice were divided into four (4) batches of five (5) mice each. Eighteen hours before the start of the experiment, the mice were only given access to water but no food. They were then force-fed with the test products via a feeding tube.

Batch 1 (negative control): distilled water (10 ml/kg BW)
Batch 2 (positive control): Forlax 10 mg/kg (BW).
Batch 3: *Saba senegalensis* (150 mg/kg BW)
Batch 4: *Saba senegalensis* (300 mg/kg BW).

Sixty (60) minutes after force-feeding of these products, 0.2 mL of activated charcoal was administered to the treated mice. Thirty (30) minutes later, the mice were sacrificed and the abdominal wall was incised. The intestines were isolated and tied from the pyloric sphincter to the ileocaecal junction. The distance from the pyloric sphincter to the ileocaecal junction (total length) was measured. The distance between the pyloric sphincter and the activated charcoal front was also measured and evaluated as a percentage of the total length.

3. RESULTS AND DISCUSSION

3.1 Extraction yield

To establish a link between dry extract and *Saba senegalensis* leaf powder, the extraction yield was determined. This study showed that for one quantity (150.5 g) of *Saba senegalensis* leaf powder, 44.12 g of dry extract was obtained. This corresponds to an extraction yield of 29.32%.

3.2 Mineral content

Qualitative and quantitative mineralogical analysis of *Saba senegalensis* aqueous extract revealed its mineral content (Table 1). The aqueous extract of *Saba senegalensis* is a source of magnesium (10.716 ± 0.560 mg/L), phosphorus (11.000 ± 0.573 mg/L), iron (9.764 ± 0.500 mg/L), calcium (21.691 ± 0.701 mg/L) and potassium (11.191 ± 0.587 mg/L), with a predominance of calcium. It contains small amounts of manganese (2.335 ± 0.450 µg/L), nickel (16.471 ± 0.678 µg/L), copper (7.994 ± 0.321 µg/L) and molybdenum (22.278 ± 0.698 µg/L).

3.3 Laxative effect of aqueous extract of *Saba senegalensis* leaves in loperamide-untreated (non-constipated) mice

Table 2 shows the results of the test for the laxative effect of aqueous extract of *Saba senegalensis* leaves in loperamide-untreated mice.

At 150 mg/Kg BW, *Saba senegalensis* had similar effects to those of the control, treated with distilled water. Aqueous extract of *Saba senegalensis* had no significant effect ($P > 0.05$) at this concentration. At 300 mg/Kg BW, it increased the number of mice defecating, modified the appearance of stools and softened them significantly ($P < 0.0001$), compared with the control. These effects are similar to those of Forlax, an osmotic laxative, used at 10 mg/kg BW. Administered in two doses, the aqueous extract of *Saba senegalensis* leaves increased the number of mice defecating, modified stool appearance and softened stools, in a dose-dependent manner.

Table 1. Mineral content of aqueous extract of *Saba senegalensis* leaves

Mineral	Average concentration
Magnesium	10.716 ± 0.560 mg/L
Phosphorus	11.000 ± 0.573 mg/L
Sodium	1.311 ± 0.321 mg/L
Iron	9.764 ± 0.500 mg/L
Potassium	11.191 ± 0.587 mg/L
Calcium	21.691 ± 0.701 mg/L
Manganese	2.335 ± 0.450 µg/L
Nickel	16.471 ± 0.678µg/L
Copper	7.994 ± 0.321 µg/L
Molybdenum	22.278 ± 0.698 µg/L

Table 2. Laxative effect of aqueous extract of *Saba senegalensis* leaves in non-constipated mice

Treatments	Number of mice having defecated	Type of stool	Stool moisture (%)	
			0-4 hour(s)	4-8 hours
Batch 1 Distilled water (10 mL/Kg BW)	1/5	1	0	25
Batch 2 Forlax (10 mg/Kg BW)	5/5	3 et 4	65.00 ± 2,88****	85.00 ± 5,77***
Batch 3 SAB (150 mg/Kg BW)	1/5	1	0	25
Batch 4 SAB (300 mg/Kg BW)	4/5	2 et 3	37.50 ± 2,517 ****	56.25 ± 1,15**

*Each value represents the mean ± SEM (standard error of the mean) n = 3 ; (**P < 0.01) : highly significant laxative effect (****P < 0/0001) : very highly significant laxative effect Legend: SAB : Aqueous extract of Saba senegalensis , Type 1 : type 1: hard stool in the form of beads; type 2: molded stool in the form of chains; type 3: soft stool in the form of chains; type 4: completely liquid stool.*

3.3 Laxative effect of aqueous extract of *Saba senegalensis* leaves in loperamide-treated (constipated) mice

Table 3 shows the results of the laxative test with *Saba senegalensis* in mice previously treated with Loperamide (constipated).

At 150 mg/kg BW, *Saba senegalensis* moistened stools by 25% (P < 0.0001) without altering their appearance. With this dose, the effect of *Saba senegalensis* aqueous extract is significant compared with the control. At 300 mg/kg BW, *Saba senegalensis* relieved constipation in three-fifths of mice treated with loperamide (5 mg/kg BW). It significantly softened stools (P < 0.001) and modified their appearance. These effects are comparable to those of Forlax (10 mg/kg BW), an osmotic laxative. However, Forlax softened and modified stools to a greater extent than the aqueous extract of *Saba senegalensis* used at a dose of 300 mg/kg BW. When given in two doses, the aqueous extract of *Saba senegalensis* leaves induced constipation in mice, modified stool appearance and softened stools, in a dose-dependent manner.

3.4 Effect of aqueous extract of *Saba senegalensis* leaves on intestinal transit of activated charcoal in mice

Table 4 shows the results of the effect of the aqueous extract of *Saba senegalensis* leaves on the intestinal transit of activated charcoal.

With a dose of 150 mg/Kg BW, *Saba senegalensis* had a non-significant effect (P > 0.05) on the intestinal transit of activated charcoal, compared with the control, treated with distilled water. However, at 300 mg/kg BW, *Saba senegalensis* significantly increased the intestinal transit of activated charcoal (P < 0.0001). This increase in transit is similar to that induced by Forlax, an osmotic laxative of reference, used at 10 mg/Kg BW. Administered in two doses, aqueous extract of *Saba senegalensis* leaves increases intestinal transit of activated charcoal in a dose-dependent manner in mice.

Table 3. Effect of laxative aqueous extract of *Saba senegalensis* leaves in mice treated with loperamide (constipated)

Treatments		Number of mice having defecated	Type of stool	Stool moisture (%)	
				0-4 hour(s)	4-8 hours
Batch 1 Lop (5 mg/Kg BW) + Distilled water (10 mL/Kg BW)		0/5	-	0	0
Batch 2 Lop (5mg/Kg BW) + Forlax (10 mg/Kg BW)		3/5	3 et 4	50.00 ± 5,77****	83.33 ± 1,73****
Batch 3	Lop (5 mg/Kg BW) + SAB1(50 mg/Kg BW)	1/5	1	0	25.00 ± 2,88***
Batch 4	Lop (5 mg/Kg BW) + SAB (300 mg/Kg BW)	3/5	1 et 2	16.66 ± 3,46**	33.33 ± 1,73***

*Each value represents the mean ± SEM (standard error of the mean) n = 3 ; (**P < 0.01) : highly significant laxative effect ; (***P < 0.001) : significative highly significant laxative effect; (****P < 0.0001) : significative very highly significant laxative effect; Legend: Lop : Loperamide, SAB : Aqueous extract of Saba senegalensis , Type 1: hard stool, broken up into beads; type 2: molded stool in the form of chains; type3: soft, broken-up stool; type 4: entirely liquid stool*

4. DISCUSSION

The aim of this study was to evaluate the laxative properties of *Saba senegalensis*, a plant used in Ivorian pharmacopoeia.

The extraction yield of *Saba senegalensis* leaves by cold maceration in distilled water was 29.32%. This value shows that the powder used to prepare the aqueous extract of *Saba senegalensis* is three times lower than the extract obtained. Furthermore, this yield is different from those obtained by (Coulibaly et al. 2019), working with *Hugonia platysepala*. This result suggests that extraction yields depend on the plant and the solvent used. The mineralogical composition of the aqueous extract of *Saba senegalensis* leaves revealed the presence of calcium, potassium, magnesium, phosphorus, iron, sodium, manganese, nickel, copper and molybdenum.

The results of the in vivo study showed that administration of *Saba senegalensis* aqueous extract to healthy mice with a concentration of 150 mg/Kg BW had no significant effect. However, at 300 mg/Kg BW, *Saba senegalensis,* like Forlax, an osmotic laxative, softened stools ($P < 0.01$ - 0.0001), altered their appearance and increased the number of healthy mice defecating. These results indicate that the aqueous extract of *Saba senegalensis* possesses a laxative effect similar to Forlax, consisting of polyethylene glycol (PEG) capable of retaining water in the intestinal lumen via hydrogen bonds (Katelaris and Naganathan 2016).

In loperamide-treated mice, *Saba senegalensis* at 150 mg/Kg BW moistens stools ($P < 0.001$). With 300 mg/Kg BW, it also softens stools ($P < 0.01$-0.0001) and lifts loperamide-induced constipation in mice. Indeed, loperamide is an agonist of the mû (μ) and delta (δ) opioid receptors of the enterocyte. It induces a decrease in cyclic adenosine monophosphate (cAMP) production in intestinal epithelial cells, which reduces electrolyte hypersecretion, leading to constipation (Huijghebaert et al. 2003). Saline laxatives, notably potassium, sodium and magnesium salts, stimulate jejunal secretion and inhibit the reabsorption of water and electrolytes, thereby relieving constipation and softening stools (Dumont et al. 2010). *Saba senegalensis* could also act through this mechanism, especially as it contains sodium, phosphorus and magnesium.

From this point of view, *Saba senegalensis* mechanism of action is close to that of *Mareya micrantha*, which, compared with sodium pico-sulfate, is capable of activating the chlorine channel in the large intestine (Méité et al. 2010).

As regards the activated charcoal intestinal transit test, *Saba senegalensis* was found to induce a dose-dependent increase in activated charcoal passage ($P < 0.001$). This effect of *Saba senegalensis* could be partly explained by the presence in its extract of calcium, known for its ability to regulate intestinal transit and its pivotal role in muscle contraction processes (Rodwell et al. 1990). These results confirm the idea that *Saba senegalensis* has a laxative effect. They are also in line with those of (Dosso et al. 2022), who obtained an increase in contraction of rabbit duodenum under the effect of *Saba senagalensis* aqueous extract. According to their findings, like *Mareya micrantha*, *Saba senegalensis* aqueous extract contains muscarinic cholinomimetic substances. These muscarinic agonists could easily explain the increased transit of activated charcoal in mice. It has been established that certain cells in the intestine and stomach are rhythmogenic and influenced by chemical stimuli such as acetylcholine and its agonist. Stimulated by this neuromediator or muscarinic agonists, they increase their contraction frequency and impose it on the entire muscle layer, thus accelerating intestinal transit (Elaine 1999)]. These myostimulant properties of *Saba senegalensis* and *Mareya micrantha* (Dosso et al. 2022, Traoré et al. 2004) testify to the similarity between their mechanisms of action.

5. CONCLUSION

This study enabled minerals to be quantified and the laxative effect of *Saba senegalensis* to be tested in vivo. Mineral assays revealed the presence of calcium, potassium, magnesium, phosphorus, iron, sodium, manganese, nickel, copper and molybdenum, with a predominance of calcium. With regard to in vivo tests, the aqueous extract of *Saba senegalensis* leaves moisten the stools of constipated mice in a dose-dependent manner. It softens the stools of non-constipated mice, relieves constipation in those treated with loperamide and increases the number of mice defecating. It also facilitates intestinal transit. These results attest to the laxative effect of *Saba senegalensis*, justifying its use in digestive disorders.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

The study was approved by the animal ethical committee of the Department of Biochemistry – Genetics, UFR Biological Sciences, Peleforo GON COULIBALY University, BP 1328 Korhogo – Ivory Coast.

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Table 4. Effect of aqueous extract of *Saba senegalensis* leaves on intestinal transit of activated charcoal in mice.

Treatment		Intestinal transit (%)
Batch 1 Distilled water (10 mL/kg BW.)		30.45 ± 1,97
Batch 2 Forlax (10 mg/kg PC.)		78.37 ± 4,59***
Saba senegalensis	Batch 3 (150mg/kg BW)	41.78 ± 3,52
	Batch 4 (300 mg/kg BW)	87.36 ± 3,65****

*Each value represents the mean ± SEM (standard error of the mean) n = 3; (***P < 0.001): highly significant effect, (****P < 0.0001): very highly significant effect*