WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 11, Issue 5, 26-40. Research Article ISSN 2277– 7105

MYOSTIMULANT AND HYPOTENSIVE EFFECTS OF AN AQUEOUS EXTRACT OF *SABA SENEGALENSIS* (APOCYNACEA) LEAVES IN RABBITS

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Article Received on
22 Feb. 2022,

Revised on 16 March 2022,
Accepted on 06 April 2022

DOI: 10.20959/wjpr20225-23791

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ABSTRACT

Saba senegalensis, is a plant used for its multiple therapeutic virtues, in traditional medicine. It could be used in the treatment of constipation and hypertension, which is constantly gaining ground. Therefore, this study aimed to evaluate the myostimulant and hypotensive effects of an aqueous extract of the leaves of this plant, in rabbits. The myostimulant effect of the aqueous extract of the leaves of *Saba senegalensis* was evaluated using a fragment of rabbit intestine, 3 cm long, suspended between a hook and a writing pen, then kept alive in an organ tank containing glucosed and oxygenated Mac Ewen. A LUDWIG mercury manometer was used to evaluate the effect of this extract on blood pressure in an anesthetized rabbit whose carotid artery was exposed and intubated. The results of this work indicate that the aqueous extract of the leaves of *Saba senegalensis* for concentrations ranging from 2.10^{-1} mg/ml to 8.10^{-1} mg/ml increases the amplitude of rhythmic contractions of the rabbit intestine by 130 ± 3.7% to 244.29 ± 5.29% (P<0.05-0 .001). Similarly, baseline tone varies from 8 ± 1.53 mN to 10 ± 2.6 mN. These myostimulatory effects are inhibited by atropine, a muscarinic antagonist. Moreover, the aqueous extract of this plant with an ED_{50} of 6, 11mg/Kg bw, decreases the arterial pressure of rabbits from 7, 4% to 18, 33%. These data indicate that the aqueous extract of *saba senegalensis* with myostimulant and hypotensive effects, contains cholinomimetic compounds of musarinic type. In view of these effects, this plant could be used in the treatment of constipation and hypertension, in traditional medicine.

KEYWORDS: *Saba senegalensis*, myostimulant, hypotension, rabbit, cholinomimetic.

INTRODUCTION

For thousands of years, plants have served as part of humanity's medicine. Despite the remarkable progress of modern medicine, 80% of the world's inhabitants continue to use them for their primary care.[1] They are an undeniable source of discoveries of new molecules and contribute to more than 30% of the development of future drugs.[2] In view of this contribution and with a view to improving it, the WHO has drawn up a strategy for the development and evaluation of traditional medicine in the period 2002-2005.[3] In the context of this work, the ethnobotanical data produced by Ake, 1991, which gives an overview of the Ivorian floristic heritage, indicate that the Ivory Coast is home to more than 1421 species of medicinal plants.[4] Among these is *Saba senegalensis*, a plant native to tropical Africa, which can reach more than 40 meters in height. Absent in the Sahelian zone, it is found from Senegal to Nigeria.[5] This angiosperm of the dicotyledonous class and the Apocynaceae family is a strong woody liana with a white latex.[6] These stems intertwine, or more often, climb on the surrounding trees with tendrils. In traditional medicine, it is known for its healing effects by direct application of the latex of its fruit on lesions. The decoction of its leaves or trunk bark is used in the treatment of dysentery, constipation, food poisoning, urinary schistosomiasis and vomiting.[7,8] Pharmacological studies have shown that *Saba senegalensis* has anthelmintic[8], anti-inflammatory, analgesic and antioxidant properties.[9] In addition, the leaves of this plant would be potentially anti-diabetic, according to Dosso et al, 2020.[10] Recognized as having multiple therapeutic virtues, this natural substance could probably be used in the treatment of constipation and high blood pressure. Perceived as a subjective feeling, constipation is a disorder of intestinal transit. High blood pressure, not a pathology, is caused by an increase in blood flow and or peripheral resistance. If constipation is considered by the common man as a trivial evil, high blood pressure is to be feared because it is considered a major factor in cardiovascular accidents. More than a quarter of the world's population suffers from it. 150 million people will be affected by this disease in 2025.[11] It is gaining ground in Africa, including in Côte d'Ivoire, where the prevalence is 8 to 12%.[12] The treatment of this pathology is expensive and synthetic drugs have for the most part side effects, hence the choice of plants as an additive route.

The aim of the present study is to enrich the pharmacological data of *Saba senegalensis*, by evaluating the effects of the aqueous extract of its leaves on the isolated duodenum and the blood pressure of rabbits.

MATERIALS AND METHODS

Material

Plant material

The leaves of *Saba senegalensis* were harvested in December 2019 in Korhogo, a town north of Côte d'Ivoire. The plant was identified by the Botany Laboratory of the UFR Biosciences of the Felix Houphouët Boigny University from a herbarium of the National Center of Floristics. The leaves were dried in the shade between 25 and 28°C, then crushed and reduced to a powder from which the aqueous extract was made.

Animal material

Mice of the species *Mus musculus* (Muridae) male and female weighing between 20 and 30 g were used for the toxicological and pharmacological tests. Fed with granules supplied by the company Ivograin, they were reared at an average temperature of 28°C with a relative humidity of 70%, at the pet shop of the Pasteur Institute of Ivory Coast, located on the road to Dabou, Km17 Adiopodoumé (Abidjan, Ivory Coast).

The rabbits used belong to the species *Oryctolagus cuniculus* (Leporidae). They come from different breeding farms around Abidjan (Ivory Coast). Also, they were acclimatized for a few days in the Vivarium of the Normal Superior School (ENS), Abidjan Ivory Coast, in order to regulate and harmonize their physiological state before the experiments. Only rabbits weighing 2 kg or more are used.

Experimental device for recording rhythmic contractions of the isolated rabbit intestine

The experimental device includes a water bath containing an isolated organ tank filled with Mac Ewen type physiological solution, contained in vials placed 40 cm above the apparatus. The liquids contained in vials pass through polyvinyl catheters and then coils that allow these solutions to warm up and maintain a temperature of 38°C. This liquid supply is controlled by a multiway selector valve. The insulated tank is emptied through a drain located at the bottom of the equipment.

Experimental device for the recording of arterial pressure in rabbits

The experimental device used to record blood pressure is a LUDWIG manometer, consisting of a U-shaped tube with mercury in both arms.

Pharmacodynamic substances

Acetylcholine (Ach), atropine (Atr) [Sigma chemical compagny, USA], are the pharmacological substances used during this work.

Physiological solution

The normal physiological Mac Ewen solution of pH 7.4 used, contains in (mM) NaCl.122; KCl. 4.9; $CaCl_2$. 2, 52; $NaPO_4H_2$. 1.18; $NaHCO_3$. 15.5; $MgCl_2$. 1.2 and glucose 5.5.

METHODS

Extraction

Two hundred grams (200) of crushed leaves of *Saba senegalensis* are dried at a temperature of 27°C. They are ground into powder and mixed for 24 hours in four liters of distilled water. The solution obtained is filtered using cotton wool and Wattman paper. The same operation is repeated. Distilled water is added to the pellet, mixed for 2 hours and filtered as well. The filtrates are collected in a flask and oven-dried at 40°C. The resulting powder, perfectly soluble in water, is used as a crude extract of *Saba senegalensis*.

Phytochemical study of the aqueous extract of *Saba senegalensis*

The main chemical groups of pharmacological interest, namely Alkaloids, Saponins, Flavonoids and Phenols, were searched, according to standard screening tests also used by Nene Bi et al. (2008).[13] These qualitative methods are based on specific chemical reactions.

Acute toxicity study by gavage

Acute oral toxicity was performed on 9 mice grouped in 3 batches of 3 mice. These mice have weights between 20 and 30 g. This study is conducted according to the guidelines of the Organization for Economic Cooperation and Development (OECD 423).[14] After 16 hours of fasting, batch1 received the initial dose of 2000 mg/kg bw. No deaths were recorded after 24 hours. Batch 2 and 3 received doses of 3000 and 5000 mg/Kg bw respectively. After administration of the extract, the mice were again deprived of food for three to four hours to observe their behavior during this time. The experiment is repeated twice. Animals are observed individually at least once during the first 30 min and every 24 h for 14 days.

Method for recording rhythmic contractions of the isolated rabbit duodenum

After fasting for 24 hours, the rabbit was stunned. Following a median laparotomy, a 3 cm long intestinal fragment is removed. Using a wire passed through the wall of the duodenum fragment, a knot is made at one end of the fragment allowing it to be hooked inside the isolated organ tank containing glucose Mac Ewen, oxygenated and maintained at 38°C. The other end is connected by a wire to the writing pen whose nib is in contact with a smoked cylinder subjected to a constant speed rotation.

Method for recording rabbit blood pressure

The rabbit is anesthetized by intraperitoneal injection of Thiopental at 1 g/kg bw. Its carotid artery is exposed and intubated with a catheter connected to the mercury manometer. Changes in the rabbit's carotid pressure are transmitted to the mercury column. They are transcribed with a writing pen on a cylinder covered with paper coated with lampblack and rotating at constant speed.

Statistical analysis

The statistical analysis of the values and the graphical representation of the data were performed using GraphPad Prism 9 software (San Diego, California, USA). The statistical difference between the results was performed using analysis of variance (ANOVA). All values are presented as mean ± SEM (Standard Error on the Mean).

Ethical Statement

All these experiments have been approved by the Scientific Ethics Committee of Biology of the UFR Biosciences of the Felix Houphouët Boigny University with regard to the guidelines 2010/ 63/ UE.

RESULTS

Phytochemical study of the aqueous extract of *Saba senegalensis*

The phytochemical screening of the aqueous extract allowed to highlight in this extract the presence of Sterols, Tannins, flavonoids, polyphenols and finally of saponoside. Alkaloids and quinone compounds are absent in the extract (Table 1).

Table 1: Chemical composition of the aqueous extract of *Saba senegalensis* leaves (EAqSs).

Compounds	Terpenoids (Libermann test)	Phenols (ferric chloride test)	Flavonoids (Alkanie reagent test)	Saponin (Foam test)	Quinonic compounds (Borntraeger reagent test)	Alkaloids (Bouchardat reagent test)	Tannins (Stiasny réagent test)
EAqSs	+	+	+	+	-	-	+
(+): presence of the chemical group **(-): Absence of the chemical group**							

Acute toxicity study by gavage

Administration of EAqSs did not cause any deaths in mice with doses of this extract up to the maximum dose of 5000 mg/kg bw The LD_{50} is greater than 5000 mg/kg bw.

Dose-response effect of aqueous extract of *Saba senegalensis* leaves (EAqSs) on rhythmic contractions of rabbit duodenum

Figure 1 represents the typical effects of EAqSs on the rhythmic contractions of the rabbit duodenum.

EAqSs at doses ranging from 2.10^{-1} mg/ml to 8.10^{-1} mg/ml causes an increase in the amplitudes of rhythmic contractions in the rabbit duodenum ranging from 130 ± 3.7% to 244,29 ± 5.29% (P<0.05-0 .001). At the same time, basal tone ranged from 8 ± 1.53 mN, to 10 ± 2.6 mN. At 8.10^{-1} mg/ml EAqSs, the recorded increase in amplitude has the appearance of a contracture.

The mean values obtained after several experiments (n=3), allowed us to plot a histogram (Figure 2).

For concentrations between 2.10^{-1} mg/ml and 8.10^{-1} mg/ml, the increases in amplitudes of rhythmic contractions induced by EAqSs are significant.

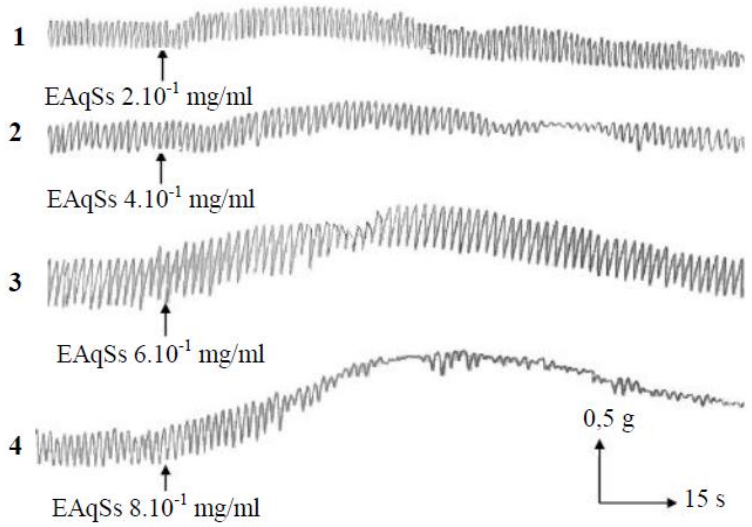

Figure 1 : Dose-response effect of aqueous extract of *Saba senegalensis* leaves on rhythmic contractions of isolated rabbit duodenum.

From 1 to 4: Effect of EAqSs at 2.10^{-1} mg/ml (1); Effect of EAqSs at 4.10^{-1} mg/ml (2); Effect of EAqSs at 6.10^{-1} mg/ml (3); Effect of EAqSs at 8.10^{-1} mg/ml (4) on rhythmic contractions of isolated rabbit duodenum. EAqSs increases contractions in a concentration-dependent manner.

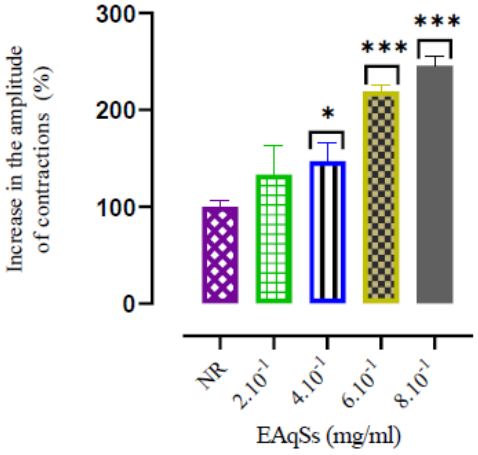

Figure 2: Increase in the amplitude of contractions in isolated rabbit duodenum as a function of the concentration of *Saba senegalensis* extract.

Values express percentages of maximum increase in rhythmic contractions of rabbit duodenum compared with normal recording (NR) (Mean±*P<0 .05; **P<0 .01; ***P<0 .001).

Dose-response effect of acetylcholine (Ach) on contractile activity of isolated rabbit duodenum.

Figure 3 represents the typical dose-response effect of acetylcholine on the contractile activity of isolated rabbit duodenum.

Acetylcholine, at doses ranging from 10^{-5} to 10^{-2} mg/ml, causes a dose-dependent increase in the amplitude and baseline tone of rhythmic contractions in isolated rabbit duodenum. For acetylcholine concentrations of 10^{-5} and 10^{-4}mg/ml, the increases recorded are 100.55 ±7.5% and 111.38 ± 6.97% for contraction amplitudes and 0.56 ± 0.32 mN and 14.5 ± 0.8 mN for baseline tone, respectively. At doses of 10^{-3} and 10^{-2} mg/ml Ach causes contractures with amplitudes ranging from 222.06 ± 3.6% to 255.55 ± 5.9%. The basic tone is between 16.8 ± 0.7 mN and 19.16 ± 0.7 mN.

These series of experiments were performed several times (n=3). The mean values obtained were used to plot the histogram in Figure 4.

The amplitude increases induced by Ach are significant from the concentration 10^{-4} mg/ml.

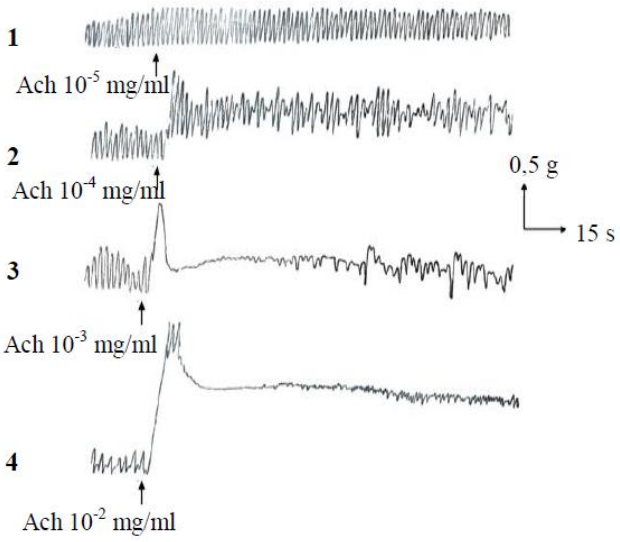

Figure 3 : Dose-response effect of acetylcholine on rhythmic contractions of isolated rabbit duodenum.

From 1 to 4: Effect of Ach at 10^{-5} mg/ml (1); Effect of Ach at 10^{-4} mg/ml (2); Effect of Ach at 10^{-3} mg/ml (3); Effect of Ach at 10^{-2} mg/ml (4) on rhythmic contractions of isolated rabbit duodenum. Ach increases contractions in a concentration-dependent manner.

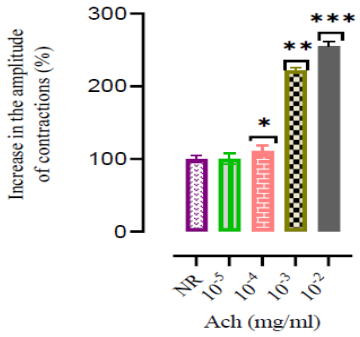

Figure 4: Increased amplitude of contractions in isolated rabbit duodenum as a function of acetylcholine concentration.

Values express percentages of maximum increase in rhythmic contractions of rabbit duodenum compared with normal recording (NR) (Mean ± *P<0 ,05; **P< 0 ,01; ***P< 0, 001).

EAqSs-atropine interaction

The presence of atropine at doses between 10^{-5} mg/ml and 10^{-2} mg/ml, strongly inhibits the rhythmic contraction of the gut induced by EAqSs at 6.10^{-1} mg/ml (figure). These inhibitions ranged from 57, 09% to 59, 47% (Figure 5).

These experiments were conducted several times (n=3). The average values obtained, allowed to plot the histogram 4 in figure 6.

The decreases in contraction of the rhythmic amplitudes of the rabbit duodenum induced by EAqSs at 6.10^{-1} mg/ml in the presence of atropine are significant for concentrations of the latter situated between 10^{-5} mg/ml and 10^{-2} mg/ml.

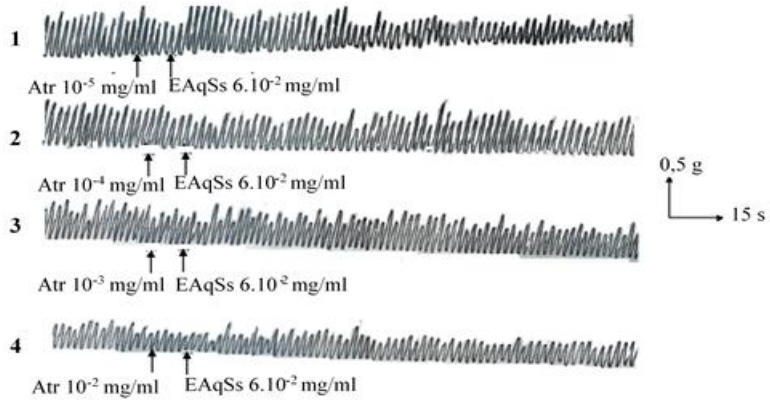

Figure 5. Effect of aqueous extract of *Saba senegalensis* leaves on rabbit duodenal contractions in the presence of atropine.

Atr-EAqSs interaction: Effect of EAqSs at 6.10-1 mg/m from 1 to 4: Effect of Atr at 10-5 mg/ml (1); Effect of Atr at 10-4 mg/ml (2); Effect of Atr at 10-3 mg/ml (3); Effect of Atr at 10-2 mg/ml (4)

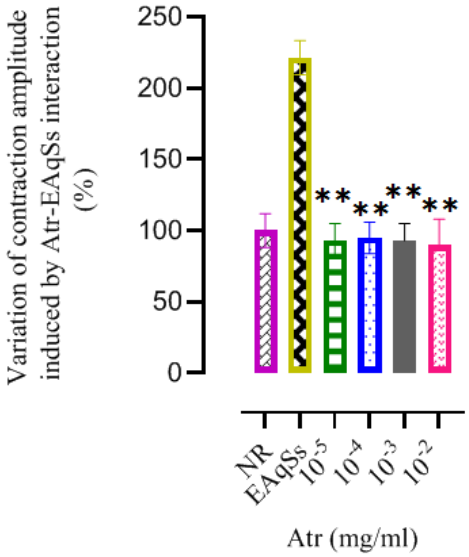

Figure 6: Variation in the amplitude of rhythmic contractions in the rabbit duodenum as a function of EAqSs concentration in the presence of atropine

Values express percent changes in rabbit duodenal rhythmic contractions induced by Atr-EAqSs (6.10^{-1} mg/ml) interaction compared with that of treatment with EaqSs at 6.10^{-1} mg/ml) (Mean±*P<0 ,05; **P<0 ,01; ***P<0 ,001).

Atr inhibits the myostimulatory effect of EAqSs at 6.10^{-1} mg/ml

Dose-response effects of EAqSs on rabbit blood pressure

Figure 7 shows the cumulative dose effects of EAqSs on rabbit blood pressure.

EAqASs at a dose of 5 mg/kg bw causes a transient hypotension of 8 mmHg, which is equivalent to a decrease in normal blood pressure of 7.4%. This hypotension lasts 0.22 seconds. At the doses of 7 mg/kg bw and 10 mg/kg bw, EAqSs induces sustained hypotensions with values of 18.4±0.516 mmHg and 26.55±0.4113 mmHg respectively, these correspond to decreases of 10.22% and 14.75% of normal blood pressure respectively. At a dose of 12 mg/kg bw, EAqSs induced arterial hypotension with a value of 33±0.298 mmHg, corresponding to a decrease of 18.33% of normal pressure.

The mean values obtained after several experiments (n=3), allowed to plot the curve of blood pressure decrease as a function of EAqSs dose (Figure 8).

The 50% effective dose of our extract obtained from this plot is 6.11 mg/kg bw.

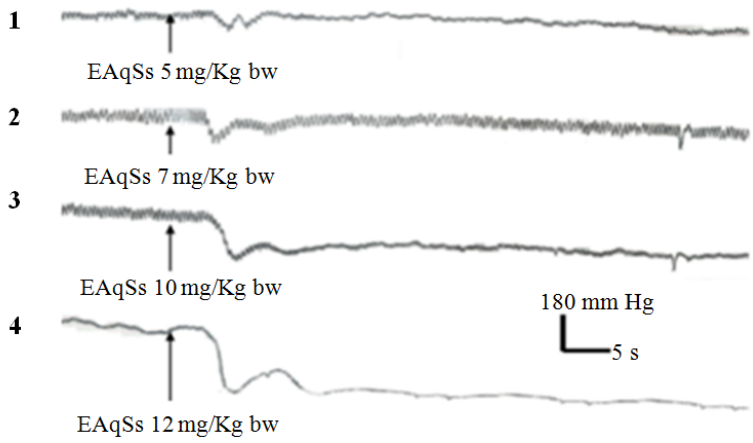

Figure 7: Dose-response effect of aqueous extract of *Saba senegalensis* leaves on rabbit blood pressure.

From 1 to 4: Effect of EAqSs at 5 mg/kg bw (1); Effect of EAqSs at 7 mg/kg bw (2); Effect of EAqSs at 10 mg/kg bw (3) and Effect of EAqSs at 12 mg/kg bw (4) on rabbit blood pressure. EAqSs lowers blood pressure

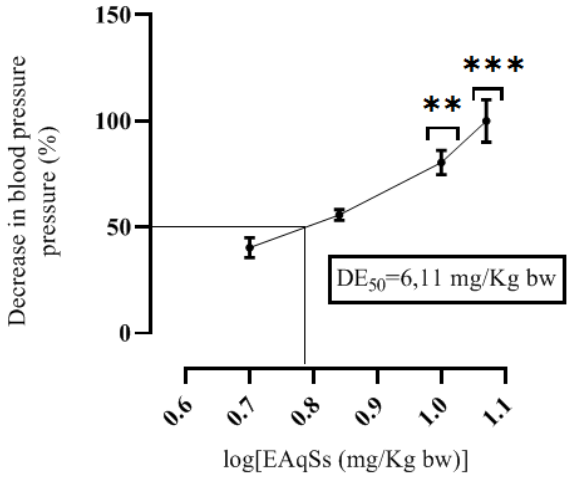

Figure 8: Dose-dependent decrease in blood pressure in rabbits with EAqSs.

Values expressing percentages of maximum blood pressure decrease compared with the control (Mean±SEM;* p<0.05; p<0.01; *** p<0.001). EAqSs induces dose-dependent hypotension, ED_{50}=6.11 mg/Kg bw**

DISCUSSION

The phytochemical study of the aqueous extract of *saba senegalensis* leaves showed that this plant contains tannins, sterols, terpenes, phenols, flavonoids and saponosides. However, quinones and alkaloids are absent in this extract. These results are consistent with those of Yougbaré-Ziébrou et al, (2016).[15]

The presence of these pharmacological compounds of interest in the aqueous extract of *saba senegalensis* leaves could explain the traditional use of this plant in the treatment of multiple pathologies including constipation and arterial hypertension.[16,4] Indeed, it is known that tannins have antibacterial activities.[17] Sterols and terpenes would be antipyretic and analgesic.[18] Flavonoids and phenols would have antihypertensive and laxative effects.[19- 20]

According to the OECD classification[14], EAqSs with an LD_{50} greater than 5000 mg/Kg bw is classified as category 5. Therefore, this plant substance by gavage is non-toxic in mice. These results corroborate those of Nacoulma, (1996)[21], who showed the harmlessness of dried fresh leafy stems of *Saba senegalensis.*

Pharmacological data, indicate that the aqueous extract of the leaves of *Saba senegalensis* (Apocynaceae) for concentrations ranging from 2.10^{-1} mg/ml to 8.10^{-1} mg/ml causes a dose-dependent increase in the amplitude and baseline tone of rhythmic contractions of the isolated rabbit duodenum. These results suggest that aqueous extract of *Saba senegalensis* leaves (EAqSs) contains myostimulatory compounds. The effects of EAqSs are similar to those of acetylcholine.[22] To confirm this, the effect of EAqSs was evaluated in the presence of atropine (Atr), a competitive inhibitor of muscarinic cholinergic receptors. For the same reason, the effect of EAqS was also evaluated on the blood pressure of rabbits.

The EAqSs-induced increase in the amplitude of rhythmic contractions in the rabbit duodenum is inhibited in a dose-dependent manner in the presence of atropine. At the level of blood pressure of rabbits, aqueous extract of *saba senegalensis* at doses ranging from 5 mg/kg bw to 12 mg/kg bw causes hypotension. These hypotensive effects of aqueous extract of *saba senegalensis* are dose-dependent and sustained. These data indicate that the aqueous extract of *saba senegalensis* leaves contains cholinomimetic substances of muscarinic types. Researchers having worked on *Mareya micrantha* (Euphorbiaceae) and *Mirabilis jalapa* L. (Nyctaginaceae) with more or less similar methods have reached the same conclusions.[23-24]

CONCLUSION

The non-toxic aqueous extract of the leaves of *Saba senegalensis* causes a dose-dependent increase in the amplitude of contraction and basic tone of the isolated rabbit intestine. Furthermore, it causes a decrease in rabbit blood pressure. This aqueous extract of the leaves of *Saba senegalensis* would thus contain cholinomimetic compounds of muscarinic type also confirmed by the inhibition of these myostimulant effects by atroipine. These myostimulant and hypotensive effects of the aqueous extract of *Saba senegalensis* linked to its molecular content of pharmacological interest, could militate in favor of its use in the treatment of constipation and arterial hypertension, in traditional medicine. However, further studies on hypertensive rats will be necessary to confirm without ambiguity this antihypertensive effect of *Saba senegalensis*.

DECLARATION OF INTEREST

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENT

We thank the team of the laboratory of animal physiology of the URF Biosciences of the Félix Houphouet-Boigny University who kindly accompanied and assisted us during the realization of the various pharmacological tests.

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