

# Original Research Article

## Study of the pharmacological properties of *Dichrostachys cinerea* hydroalcoholic extract on the contractility of *taenia coli* isolated from guinea pigs

### ABSTRACT

**Aims:** *Dichrostachys cinerea* is a plant use for his severales properties: for the treatment of abscesses, eczema, gonorrhoea, rheumatism, gingivitis, dental caries, epilepsy, as a diuretic and laxative; against otitis, umbilical hernia, malaria in children. No studies shows the laxative effect of this plant.

**Study design:** The objective of this work is to highlight the laxative properties by studying its effect on *taenia coli* isolated from guinea pigs.

**Place and Duration of Study:** Pharmacology department of the Institute of Pharmacopoeia and Traditional Medicine, between January 2022 and October 2022.

**Methodology:** Pharmacological properties of *Dichrostachys cinerea* hydroalcoholic extract (Edici) was investigated on preparation of *taenia coli* isolated from guinea pig (PTGP) whose fragments taken are kept in an isolated organ tank containing a physiological solution of the Mac Ewen type. Normal contractile activity is recorded using a rikadenki type graphic recorder and increasing doses of our extract are administered into the medium.

**Results:** The analysis of our extract by a specific  $\beta$  receptor antagonist, propranolol, is used. We observe that the addition of propranolol ( $10^{-5}$  M) to PTGP does not result in the suppression of contractions by Edici. We go from a contraction percentage of 13.33% when adding dose  $E_3$  to 40% for dose  $E_4$ . Then, the effect of phentolamine, an  $\alpha$ -adrenergic blocker, followed by the addition of increasing doses of Edici is observed on smooth muscle. The extract causes an increase in basic tone with an increase in the amplitude of contraction. We go from an increase of 29.5% for the concentration of  $E_1$  to 75% when adding the  $E_3$  dose. The addition of promethazine to our PTGP leads to a significant reduction in it.

**Conclusion:** Our extract causes an increase in the rhythmic contraction of *taenia coli* isolated from guinea pigs, which justifies its use against constipation.

**Comment [AS1]:** Mention plant name here.

**Comment [AS2]:** City, country?

**Comment [AS3]:** alcoholic

**Comment [AS4]:** Shouldn't the methodology be in the past tense?

**Keywords:** *Dichrostachys cinerea*, Laxative, Medicinal plant, *Taenia coli*

### 1. INTRODUCTION

The use of plants for relief has always been a human practice. Today this practice is still relevant in our developing countries. This so-called traditional medicine is recognized by international institutions. This is why the WHO has a department in charge of this type of medication. Several bioactive molecules isolated from plants are used in the composition of many drugs. This is the case of *Artemisia annua*, a plant used in China for more than two thousand years and which is today one of the essential ingredients in the development of treatments against malaria. This plant is now included on the WHO list of essential medicines. Other African plants are also experiencing renewed interest, such as the *African Plum* and *Sutherlandia frutescens*, which only grows in South Africa and is used as an adjuvant in AIDS treatments. It is therefore important that modern and traditional medicine collaborate together to enable the validation and improvement of traditional remedies and complement each other to advance research. Our study focuses on *Dichrostachys cinerea*, from the Leguminosae family. It is a shrub that can reach up to eight meters high, with bi

**Comment [AS5]:** Any reference?

**Comment [AS6]:** None of the statement is justified with a reference. Add references against each statement.

ending in thorns. The leaves are bipinnate, each pinnate bearing a gland. The 2.5 cm long pendulous flowers are composed of a yellow hermaphrodite upper part and a sterile lower part ranging from mauve to pink. The fruits are twisted, indehiscent pods, with an original decorative effect. This species, quite variable in its appearance, is found in a wide area from Africa to Australia. Like many legumes, this species is resistant to drought, plus it tolerates sea air well. It is commonly called Mimosa bell and in Gabon: Mbara y'orové, Mbar'iyala (Mpongwé); Mbara y'orové (Nkomi, orungu); ngamba (Eshira, Bavarama, Bapunu). The roots are used as diuretics, antivenoms, astringents, against rheumatism and kidney disorders [1], gonorrhoea, abscesses, orchitis, leprosy, childhood cough and dysentery; as for the leaves, they are used for the treatment of abscesses, eczema, gonorrhoea, rheumatism, gingivitis, dental caries, but also measles. The barks are tenifuges and antivenoms, and are used against dysentery, asthma and as a bactericide. The fruits are used against otitis, umbilical hernia, malaria in children [2]. The bark is used to treat dysentery. The roots are used against leprosy, syphilis, coughs, as an anthelmintic, purgative and strong diuretic; the leaves are used for epilepsy, as a diuretic and laxative [3, 4]. Many properties of this plant have been highlighted through various works. Aworet Samseny *et al.* [5] showed that the hydroalcoholic extract of this plant relaxed the trachea artery isolated from guinea pigs and Irié *et al.* [6] determined its mechanism of action on relaxation of the trachea. Kudi and Myint [7] indicate that decocting the aerial parts of the plant would induce a significant drop in the level of CD<sub>4</sub> lymphocytes. The plant also has anti-inflammatory properties [8, 3], good anti-ulcer activity [3]. This plant being used as a laxative [9, 10], no work highlights the laxative properties of this plant. The objective of this work is to verify the laxative effect of this plant by studying its effect on the preparation of *taenia coli* isolated from guinea pigs.

**Comment [AS7]:** Why half of the pharmacological properties have a reference while others don't?

**Comment [AS8]:** This sentence is self-contradictory. Rephrase it.

## 2. MATERIAL AND METHODS

### 2.1 Chemicals and apparatus

Propranolol (Prolabo), Phentolamine (Sigma), Promethazine (Acros Organics).

### 2.2 Plant material

The bark of *Dichrostachys cinerea* were collected at Essassa (December 2019, rain season) in Ntoun, Gabon. Plant material was authenticated by a botanist of Gabon National Herbarium (IPHAMETRA/CENAREST). Voucher specimens were deposited in this department. A voucher specimen (H.P Bouroubou 387, M.S.M Sosef n°: 894, M.SM 1097) were deposited in this department.

### 2.3 Preparation of *Dichrostachys cinerea* methanolic extract

The barks of *Dichrostachys cinerea*, dried at room temperature ( $27 \pm 2$  ° C), are powdered. The bark powder (100 g) stays in petroleum ether for 24 hours at room temperature ( $27 \pm 2$  ° C). The residue is dried and taken up in 500 ml of methanol with magnetic stirring for 24 hours. The mixture is filtered and the filtrate is concentrated in a Rotavapor to dryness at a temperature of 45 ° C. This marc obtained is then kept in dry, clean and sterile bottles.

**Comment [AS9]:** Methodology needs to be written in past tense.

### 2.4 Phytochemical screening

Phytochemical study of the methanolic extract was performed, using described classical procedures [11, 12]. Chemical groups characterized were alkaloids, tannins, flavonoids, polyphenols, saponins, sterols and triterpenes, glycosides, sugars.

### 2.5 Ultra-Violet-Visible (UV-VIS) spectrum analysis

A solution of 1 mg/mL in water of the decoction of *Dichrostachys cinerea* was centrifuged at 3000 rpm for 10 min and filtered through Whatmann No. 1 filter paper. The sample was then diluted 1:10 and scanned at wavelengths ranging from 280 to 600 nm using a DRAWELL UV-VIS spectrophotometer. The spectrum was recorded in triplicate.

### 2.6 Animal material

The animals used are male guinea pigs of the *Cavia porcellus* (Cavidea) type weighing  $446 \pm 30$  g and aged from 75 to 90 days. They come from the Pharmacopea and Traditional Medicine Institute animal facility in Sibang, Libreville (Gabon). They are fasted 12 hours before the start of the experiment. Animals were cared for and treated according to the principles for the care and use of laboratory animals for biomedical research approved by the ethical committee of Pharmacopea and Traditional Medicine Institute.

**Comment [AS10]:** It will be appropriate if the reference number of the letter of the ethical approval is shared.

## 2.7 Experimental apparatus

The recording device consists of a 10 ml isolated organ vessel, contained in a thermostatically controlled water bath and set at a temperature of 37 ° C. The biological preparation is immersed in an oxygenated isolated organ tank containing the physiological solution of the Mac Ewen type, glucose. The contractions are picked up by an F30 HSE type 372 strain gauge and transmitted by the HUGO SACHS amplifier. Contractile activity is observed using a RIKADENKI type chart recorder.

## 2.8 Recording of contractile activity of *tænia coli* isolated from guinea pigs

The guinea pig is sacrificed by cervical dislocation according to the method described by Price et al. [13]. Immediately, a midline laparotomy is performed on the animal. Several *tænia coli* strips are taken and are immediately stored in a physiological solution of the Mac Ewen solution (in mM: NaCl, 130; KCl, 5.6; CaCl<sub>2</sub>, 2.6; NaH<sub>2</sub>PO<sub>4</sub>, 0.91; NaCO<sub>3</sub>H, 11.9; MgCl<sub>2</sub>, 0.24; glucose, 11) type, glucose, oxygenated (95% O<sub>2</sub> + 5% CO<sub>2</sub>) and maintained at a temperature of 37 ° C. For mounting, a 3 mm fragment is used. A knot is made at each end of this segment to connect it to the tank and to the transducer. At the start of the experiment, the *tænia coli* fragment is subjected to a tension of 1 g and left to stand for 1 hour.

## 2.9 Stylus calibration

A 1 g mass is suspended from a hook and everything is connected to the strain gauge for calibration. The movement of the recording pen allows us to determine the tension exerted by the weight. For a force of 1 g or 10 mN, the stylus moves eight (8) cm.

## 2.10 Statistical analysis

The statistical analysis was carried out with the Instat software. All values in the text and illustrations are presented as mean ± SEM, with n representing the number of different preparations. Values of p < 0.05 were considered to indicate significant differences.

## 3. RESULTS AND DISCUSSION

### 3.1 Results

#### 3.1.1 Phytochemical screening and spectral analysis

Phytochemical study of *Dichrostachys cinerea* shows that the methanolic extract is rich in phenol compounds (phenols compounds and flavonoids). Tannins, alkaloids, sterols and triterpenes were also found in this extract. The qualitative profile of the UV-VIS spectrum of the aqueous extract of *Dichrostachys cinerea* was analyzed at wavelengths from 280 to 600 nm. The profile shows the presence of 5 distinct peaks at 330, 350, 480, 530 and 580 with absorbances of 0.23, 0.25, 0.019, 0.017 and 0.01 respectively (figure 1).

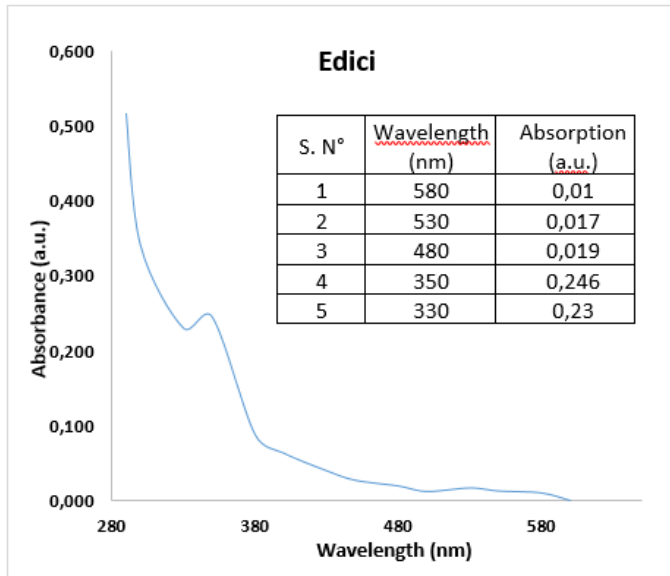


Figure 1: Qualitative profile of the UV-VIS spectrum of the aqueous extract of *Dichrostachys cinerea*

### 3.1.2 Effect of hydro-alcoholic extract of *Dichrostachys cinerea* on the contractile activity of *tænia coli* isolated from guinea pig

The addition of increasing concentrations of aqueous extract of *Dichrostachys cinerea* ( $3.2 \times 10^{-3}$  at 2 mg) causes an increase in the force of the contractions, followed by an increase in their frequency. The high concentrations of hydro alcoholic extract of *Dichrostachys cinerea*, E<sub>3</sub> (0.08 mg / ml) and E<sub>4</sub> (0.4 mg / ml) lead to an increase in basal muscle tone (figure 2).

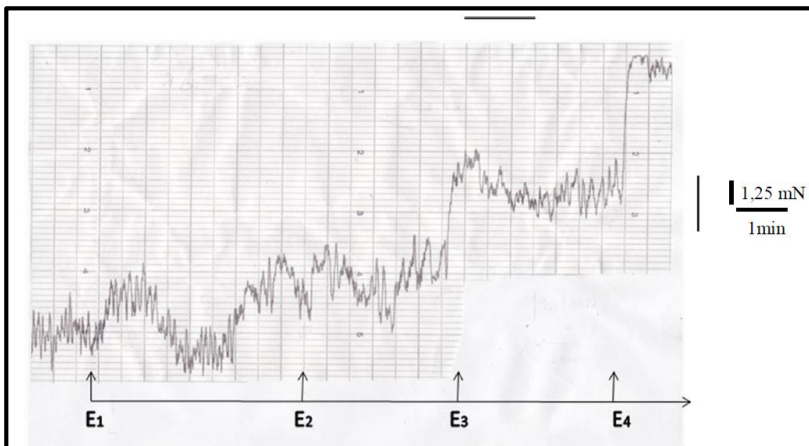
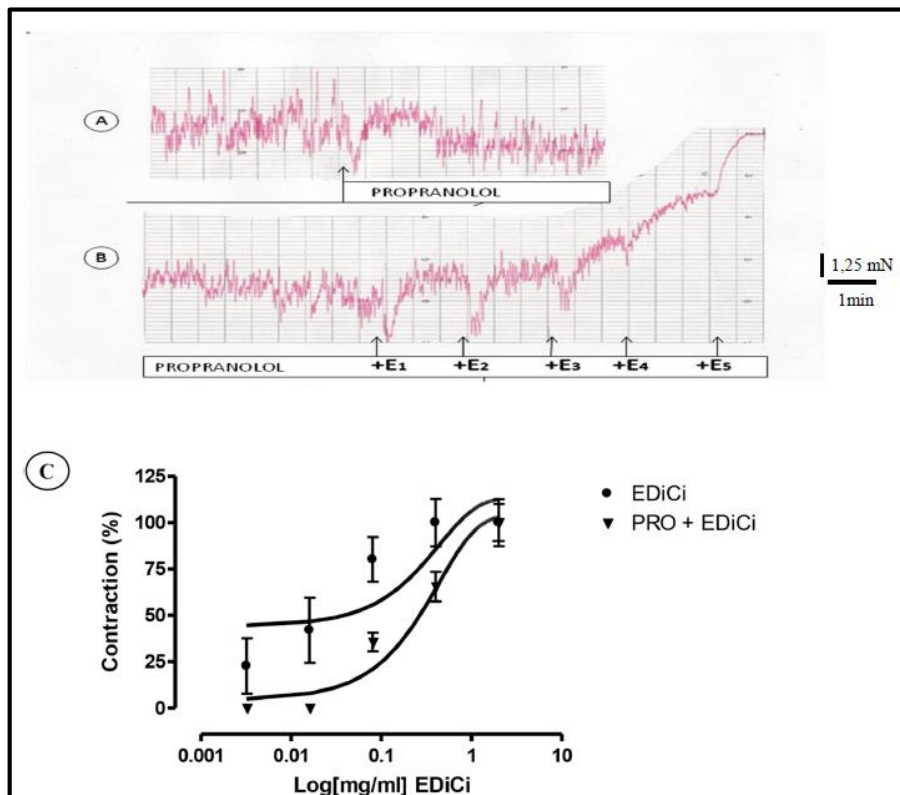


Figure 2: Effects of the hydroalcoholic extract of *Dichrostachys cinerea* on the contractile activity of *taenia coli* isolated from guinea pigs

The recording shows the concentration response effect of the hydroalcoholic extract:  $3.2 \times 10^{-3}$  mg / ml;  $1.6 \times 10^{-2}$  mg / ml;  $8 \cdot 10^{-2}$  mg / ml; 0.4 mg / ml. The arrows indicate the time of administration of the substance used.

### 3.1.3 Effects of hydro alcoholic extract of *Dichrostachys cinerea* on the contractile activity of intestinal smooth muscle isolated from guinea pigs in the presence of propranolol

The hydro alcoholic extract of *Dichrostachys cinerea* induces a concentration-response myostimulant effect on the smooth muscle of *tænia coli* isolated from guinea pigs. Pre-treatment of the organ with propranolol ( $10^{-5}$  M) leads to a decrease in the contractions induced by the hydro alcoholic extract in increasing cumulative concentrations (Figure 3).



**Figure 3: Effects of hydro alcoholic extract of *Dichrostachys cinerea* on the rhythmic contractile activity of *tænia coli* isolated from guinea pigs in the presence of propranolol**

A) Normal recording of the rhythmic activity of *tænia coli* isolated from guinea pigs followed by impregnation of the preparation of propranolol ( $10^{-5}$  M).

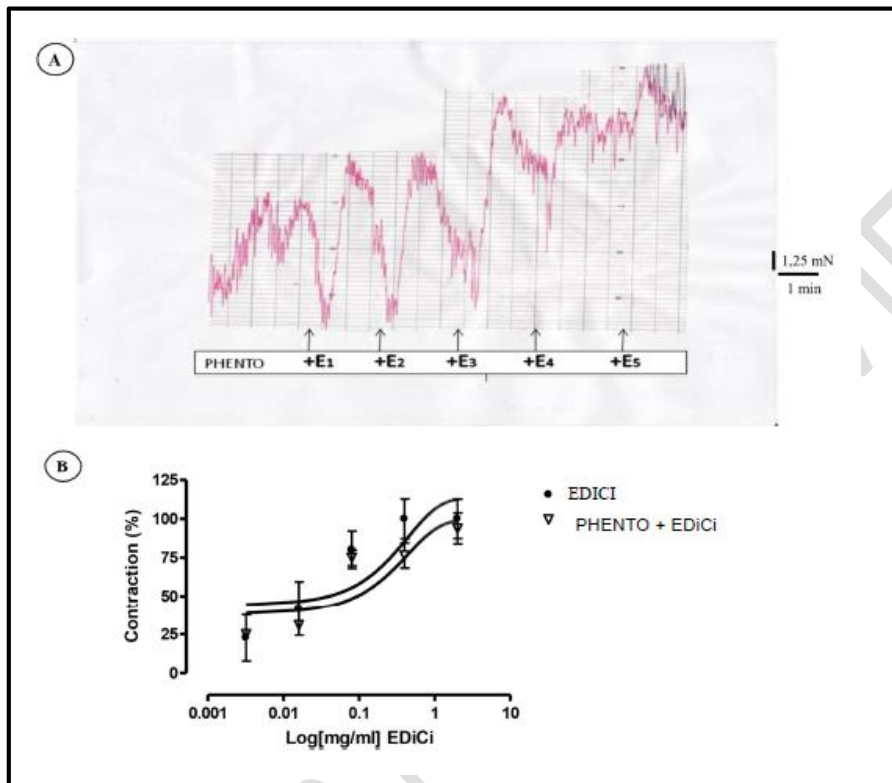
B) Continuation of the recording with the addition of increasing concentrations of hydro extract alcoholic ( $3.2 \times 10^{-3}$  mg / ml;  $1.6 \times 10^{-2}$  mg / ml;  $8 \cdot 10^{-2}$  mg / ml; 0.4 mg / ml; 2 mg / ml). The arrows indicate the time of administration of the substance used.

C) Evolution of contractions of isolated *tænia coli* as a function of increasing concentrations Hydro alcoholic extract with and without propranolol (n = 4, p < 0.05).

### 3.1.4 Effects of hydro alcoholic extract of *Dichrostachys cinerea* on the contractile activity of intestinal smooth muscle isolated from guinea pigs in the presence of phentolamine

Phentolamine, an  $\alpha$ -adrenergic receptor antagonist, is used at a concentration of  $6 \times 10^{-5}$  M has no effect on spontaneous contractions in guinea pigs. However, the pre-treatment with phentolamine has a weak effect on the hydro alcoholic extract of *Dichrostachys cinerea* used in increasing concentration (figure 4 A). A graphic representation indicating the

evolution of contractions of *tænia coli* isolated from *guinea pigs* in the presence of increasing concentrations of hydro alcoholic extract and with prior impregnation of phentolamine, is produced in figure 4B.



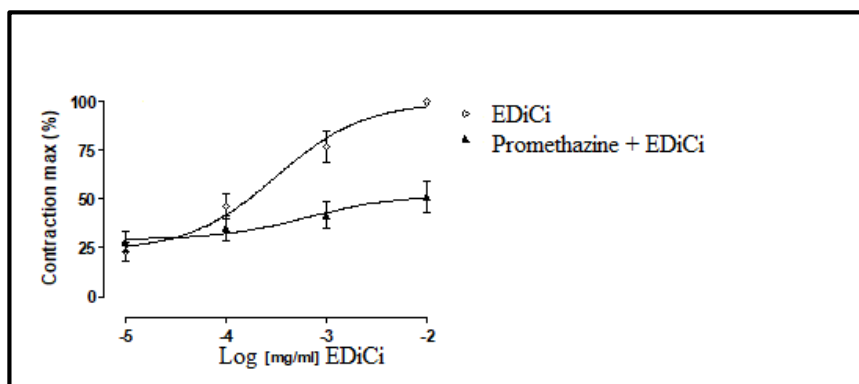
**Figure 4: Effects of hydro alcoholic extract of *Dichrostachys cinerea* on activity rhythmic contractile of *tænia coli* isolated from *guinea pig* in the presence of phentolamine**

A) The recording shows the effect of phentolamine ( $6 \cdot 10^{-5}$  M) on *tænia coli* isolated from *guinea pig* followed by the addition of increasing concentrations of the hydro alcoholic extract ( $3.2 \cdot 10^{-3}$  mg / ml;  $1.6 \cdot 10^{-2}$  mg / ml;  $8 \cdot 10^{-2}$  mg / ml; 0.4 mg / ml; 2 mg / ml).

B) Evolution of contractions of *tænia coli* isolated from *guinea pig* in the presence of concentrations increasing amounts of extract without and with prior impregnation of phentolamine ( $n = 4$ ,  $p < 0.05$ ).

### 3.1.5 Effect of promethazine on spontaneous contractions of *tænia coli* isolated from *guinea pig*

Promethazine is an antihistamine substance which inhibits  $H_1$  receptors of the phenothiazine family. This antihistamine has no effect on the smooth muscle of *tænia coli* from *isolated guinea pigs*. Following the addition of increasing doses of Edici, we observed a significant reduction in the contractions of *tænia coli* isolated from *guinea pigs* (figure 5).



**Figure 5: Evolution of contractions of *taenia coli* isolated from guinea pig in the presence of concentrations increasing amounts of extract without and with prior impregnation of Promethazine**  
(n = 4, p <0.05).

### 3.2 DISCUSSION

*Dichrostachys cinerea* is a small tree of the Leguminosae family, widely used for various conditions such as against rheumatism, for its anti-viral properties, for its laxative action and its effects against asthma. Many publications report its use as a laxative without this being demonstrated [4, 14]. However, there are articles reporting the antidiarrheal effect of *D. cinerea* on diarrhea induced by castor oil in rats [15, 16]. Vennapoosa et al. [14] showed that methanolic and ethyl acetate extracts of *Dichrostachys cinerea* have significant microbial activity (against *Shigella sonnei* and *Staphylococcus aureus*). This work shows that *Dichrostachys cinerea* has an anti-diarrheal effect because of its compounds which have an anti-microbial effect [17]. Phytochemical investigation performed on the plant species collected in Libreville (Gabon) forest revealed the presence of bioactive compounds such as alkaloids, flavenoids, tannins, sterols and triterpenes, phenolic compounds. The UV-VIS spectrum of this plant extract presents absorption peaks at 330, 350, 480, 530 and 580. The peaks 330 and 350 nm which have been identified are characteristic of flavonoids and its derivatives which generally absorb in the 300-350 zone [18]. The peak identified at 480 nm is characteristic of tannins which absorb between 350 and 500 nm. The 530 nm peak is characteristic of terpenoids which absorb from 400 to 550 nm [19]. Only the peak identified at 580 nm remains due to lack of reference. In view of the peaks, flavonoids and their derivatives are the majority in this extract, in fact, Aworet Samseny et al. [20], showed that the aqueous and organic extracts of *Dichrostachys cinerea* are very rich in phenolic compounds. Here we evaluate the effects of the hydroalcoholic extract on the preparation of tapeworm isolated from guinea pig (PTGP). The addition of increasing doses of hydroalcoholic extract of *Dichrostachys cinerea* (Edici) to the PTGP leads to an increase in the force of contraction, as well as an increase in their frequency. The addition of the first dose E<sub>1</sub> leads to an increase in contractions of 33.33%, and up to 313.33% for the last dose E<sub>4</sub>. Our extract thus leads to an increase in contractile force on GPTP. In Figure 1 we observe an increase in the amplitude of contraction; this is similar to that described by Moreto et al. [21] on the increase in colon motility following the administration of a compound called DAN-603. To attempt to analyze the effect of our extract, a specific  $\beta$  receptor antagonist, propranolol, is used. It is a non-cardiac selective antagonist because it blocks the cardiac  $\beta_1$  and non-cardiac  $\beta_2$  receptors (vessels, bronchi) [22]. We observe in Figure 3 that the addition of propranolol ( $10^{-5}$  M) to GPTP does not result in the suppression of contractions by Edici. We go from a contraction percentage of 13.33% when adding dose E<sub>3</sub> to 40% for dose E<sub>4</sub> and up to an increase of 160% for dose E<sub>5</sub>. Subsequently, the effect of phentolamine, an  $\alpha$ -adrenergic blocker [23], followed by the addition of increasing doses of Edici is observed on smooth muscle. The extract causes an increase in basic tone with an increase in the amplitude of contraction. We go from an increase of 29.5% for the concentration of E<sub>1</sub> to 75% when adding the E<sub>3</sub> dose. This percentage increase increases to 112.5% for the concentration of E<sub>5</sub> (fig. 4A, fig. 4B).  $\alpha$ -adrenergic blockers prevent the activation of  $\alpha$  receptors responsible for the contraction of smooth muscle fibers [24], while Edici always causes an increase in contractions on PTGP. The effect of our extract is comparable to that obtained by Nene Bi et al. [25] on the study of an aqueous extract of *Bridelia ferruginea* which increases the motility of *taenia coli* isolated from guinea pigs. In a final series of experiments, we evaluate the effect of our extract in relation to the histaminergic system and note that they are in agreement with those of Matsumoto et al. [26] who demonstrated an inhibition of contractions caused by histamine on guinea pig ileum by ketotifen, an H<sub>1</sub> receptor antagonist. We know that histamine causes contractions in the guinea pig ileum [27]. The effect of our extract is partially blocked by promethazine which is an antihistamine. On the intestinal smooth muscle of *taenia coli*, our extract induces a contractile activity whose mechanism seems to depend on the inhibition of antihistamine receptors. The laxative effect of our extract is due to the fact that certain compounds in our plant increase PTGP smooth muscle contractions. It has in fact been demonstrated that the contraction of smooth fibers, particularly bronchial and gastrointestinal, is achieved by G proteins which activate phospholipase C, leading to an increase in intracellular Ca<sup>2+</sup> [28]. Kongdang et al. [29] showed in

their literature review that among the preclinical models for studying laxative substances, the exploration method on isolated duodenum was part of the first investigation method (in vitro). This is followed by in vivo studies on rats or mice and when possible on animal models of constipation. Additional studies need to be conducted to determine the mechanism of action of our extract, as well as fractionation of the plant to evaluate the most active fractions.

#### 4. CONCLUSION

On the intestinal smooth muscle of *tænia coli*, our extract induces a major contractile activity which could explain its laxative effect. *Dichrostachys cinerea* extract increases intestinal peristalsis and accelerates transit. Which justifies its action as a purgative by populations.

#### CONSENT

Not applicable

#### ETHICAL APPROVAL

The Ethics Committee of the Institute of Pharmacopoeia and Traditional Medicine has approved this work (approval date: 08 11 2021 and number: 003/MESRSIT)

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UNDER PEER REVIEW

