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(RESEARCH ARTICLE)

The antispasmodic activity of ethanol extract of the stem bark of *Piliostigma reticulatum* Horscht D.C (Ceasalpiniaceae), and its dichloromethane fraction isolated

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Abstract

Piliostigma reticulatum (Caesalpiniaceae) has been used in traditional medicine for the treatment of gastrointestinal disorders like diarrhea. The aim of the present study was to examine the relaxant activity of an extract of *P. reticulatum* stem bark and five of its fractions on isolated rabbit duodenum. Segments of duodenum of rabbits were suspended in an organ bath. The ethanol extract had been fractioned to obtain some fractions heptane, dichloromethane, ethyl acetate, butanol and aqueous extract. The ethanol extract and the dichloromethane fraction were tested on the contraction of rabbit duodenum at the increasing concentrations. Increasing concentrations of *P. reticulatum* ethanol extract and its fraction induced a relaxant effect on spontaneous rabbit duodenum. The ethanol extract reduced duodenum contraction totally at 1.32 mg/mL at IC_{50} = 0.62 mg/mL. At the test concentration of 0.52mg/mL in organ bath, the dichloromethane fraction showed a relaxation of 100% (IC_{50} = 0.24 mg/mL). *Piliostigma reticulatum* stem bark possesses antispasmodic properties that can at least explain and support its traditional use against diarrhea.

Keywords: Antispasmodic activity; *Piliostigma reticulatum*; Gastrointestinal disorders.

1. Introduction

Treatment of spastic motility disorders continues to be challenging. Therapeutic options remain limited due in part to our lack of understanding of the pathophysiology and significance of these disorders [1]. Antispasmodics are drugs used to relieve or prevent smooth muscle spasms. By reducing the intestinal hyper-contractility of smooth muscles, these drugs allow the gastrointestinal muscle to return to their proper tone, therefore reducing many of abdominal pains and symptoms [2]. Hence, antispasmodics are frequently prescribed for a number of gastrointestinal diseases, including irritable bowel syndrome, a condition which affects 10–25% of the general population [3]. Main antispasmodic drugs include anti-muscarinic compounds (e.g. the alkaloids derived from the plant belladonna and their synthetic derivatives) and calcium channel blockers (e.g. otilonium and pinaverium) [3, 4]. Since, the use of these drugs may be associated with the appearance of unwanted side effects like dry mouth and urinary retention for anti-muscarinic drugs, and headache, nausea, vomiting and constipation for calcium blockers. Thus, the search of safe, plant-derived antispasmodics becomes a challenging option.

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Some medicinal plant species are used in traditional medicine for the treatment of gastrointestinal disorders including diarrhea, indigestion and constipation [5, 6, 7, 8]. Scientific investigations using different pharmacological tests are performed to prove their potential efficiency. Evaluation of antispasmodic activity on some isolated organs has demonstrated that some of these medicinal plant species possess significant and interesting spasmolytic activity [9, 10, 11] or spasmogenic activity [12]. In some cases, mechanisms concerning the antispasmodic activity of tested extracts are also reported [12, 13]. Extensive investigations of active antispasmodic extracts have led to the isolation of active compounds belonging to different phytochemical groups [14, 15, 16, 17, 18]**.**

Piliostigma reticulatum which is found in tropical forests of West African countries like Côte d'Ivoire, Mali and Burkina Faso is traditionally used against many disorders like ulcers [19], boils, wounds, syphilitic cancer [20] and diarrhea [21]. The antidiarrheal activity of this specie has been demonstrated by [22] and [23]. Others studies have been realized like the anti-secretory activity [24], the anti-bacterial activity [25] and the toxicity [26].

The antispasmodic property of this plant has not yet been investigated veritably to our knowledge to show that this plant inhibits diarrhea by reduction of intestinal contraction. However, chemical compounds isolated from *P. reticulatum* like flavonols (6-C-methylquercetin-3-methyl ether-5; 6,8-di-C-methylkaempferol-3-methyl ether-6 and 6-C-methylquercetin-3,3',7-trimethyl ether-7) and oxychromonol (6-C-methyl-2-p-hydroxyphenyloxychromonol, called piliostigmol) have been shown to exhibit antimicrobial activities against bacteria (*Escherichia coli* and *Bacillus subtilis*) and fungi (*Aspergillus niger* and *candida albicans*) which cause infectious diarrheas [25]. We have chosen dichloromethane fraction because this fraction showed an important antidiarrheal activity [23] and antibacterial activity [25] in our previous studies. The aim of this research was to separate different fractions of total hydro-alcoholic extract of *P. reticulatum* and, screen for their spasmolytic activity of the total extract and its dichloromethane fraction and, as well as to screen its phytochemical constituents.

2. Material and methods

2.1. Plant material

Stem barks of *Piliostigma reticulatum* (DC.) Horscht (Ceasalpiniaceae) were collected in Abidjan (South region of Côte d'Ivoire) in October 2018. The plant was identified and authenticated by the National Centre of Floristic of University of Cocody-Abidjan of Pr AKE Assi Laurent. A voucher specimen (N° 18033) of the plant has been deposited in the herbarium of this Centre.

2.2. Preparation of the ethanol extract

Stem barks of *Piliostigma reticulatum* were washed with distilled water, cleaned, cut into smaller pieces and kept at room temperature for two weeks. Then they were ground into a fine powder. The powder (100 g) was extracted with 2 liters of a solution of ethanol (96 %) / water (80:20) for 24 hours under constant stirring (this operation was repeated twice). The extract was filtered twice through cotton wool, then through Whattman filter paper (N° 1). The filtrate was evaporated to dryness using a rotavapor (Buchi R110/NKE6540/2) at 45 ° C, and dried under reduced pressure. Percentage yield was found to be 14.4 %.

Five liquid fractions (heptane, dichloromethane, ethyl acetate, butanol and aqueous) were extracted from the total extract by using successive liquid–liquid extraction [27].

2.3. Animals

Healthy rabbits (weighing 1.5-1.8 kg) of both sexes provided from UFR Biosciences (University of Cocody-Abidjan, Côte d'Ivoire) were used. They were kept and maintained under standard laboratory conditions of temperature one week before the experiments. The animals were fed with commercial pelleted diet (Ivograin®, Abidjan, Côte d'Ivoire) and were given water *ad libitum*. They were deprived of food and for at least 24h prior to experiments. The equipment, handing and sacrificing of the animals were in accordance with the European Council legislation 87/609/EEC for the protection of experimental animals [28].

2.4. Smooth muscle preparation

The experiments were carried out according to the Magnus general technique [29]. The animals were killed by decapitation and the duodenum was removed. A two cm long segment of the duodenum was suspended in 150 mL organ bath containing Tyrode's solution of the following composition (mM): NaCl 136.89, KCl 2.68, $CaCl_2$ 1.80, $MgCl_2$ 1.05, $NaHCO_3$ 11.90, $NaHPO_4$ 0.42 and glucose 5.55, maintained at 37 °C. The solution was aerated with a mixture of 95 % O_2

and 5 % CO_2.under a resting tension of 1g. The preparations were connected to transducer coupled to the graph paper. The suspended duodenum was allowed to equilibrate for 30 min. The smooth muscles relaxant action of test materials was observed by administration of extracts [30]. The bath was washed after testing each concentration of extracts.

The inhibition of duodenum contraction by test sample was expressed as percentage of mean ± SEM from six experiments in the presence of extracts and was calculated using the following formula:

$$\% \, Inhibition = \frac{A - B}{A} \times 100$$

Where A is the amplitude (cm) of the normal duodenum contraction and *B* the amplitude (cm) of the duodenum contraction induced by the extracts in the presence of the test sample [31].

To determine IC_{50} values, crude extracts and fractions were tested at different concentrations from 0.13 to 1.32 mg/ml (twofold dilution) in organ bath respectively. The IC_{50} value of each sample was derived from the sigmoid dose–response curves.

2.5. Phytochemical screening

The extract was screening for the presence of tannins, flavonoids, alkaloids, sterols, saponins, polyphenols, polyterpenes and quinons. Detection of these constituents was carried out as described by [32].

2.6. Statistical analysis

The results were expressed as mean ± SEM. Data were analyzed for statistical significance by one-way ANOVA followed by Tukey test using the GraphPad Demo 5 (San Diego). At 95 % confidence interval $p < 0.05$ was considered as statistically significant.

3. Results

3.1. Extraction of plant material

The amount of total ethanol extract was 14.4 %. From dried ethanol extract (starting with 10 g), heptane (90 mg; 3.6 %), dichloromethane (200 mg; 8 %), ethyl acetate (500 mg; 20 %), butanol (700 mg; 28 %) and aqueous (900 mg; 36 %).

3.2. Antispasmodic activity of ethanol extract

Rabbit duodenum suspended in tyrode's solution under 1 g tension after 30 min had a stable tension. The doses of 0.13 and 0.26 mg/mL product any effect on duodenum contraction. At doses of 0.52; 0.79 and 1.04 mg/mL (significantly reduced), the contraction was reduced respectively to 21; 84 and 94% (Table 1). The contraction of duodenum was totally and significantly inhibited at 1.32 mg/mL (n=6; Figure 1). The IC_{50} was 0.66 mg/mL (Figure 2).

Table 1 Effects of ethanol extract of *P. reticulatum* on duodenum contractions in rabbits**.**

Concentrations	Inhibition of contraction
0.26 mg/mL	0 ± 0
0.52 mg/mL	21 ± 0.66
0.79 mg/mL	84 ± 0.94
1.04 mg/mL	94 ± 1.84*
1.32 mg/mL	100 ± 0**

Values are the mean ± SEM (n=6); * $p < 0.05$; ** $p < 0.001$.

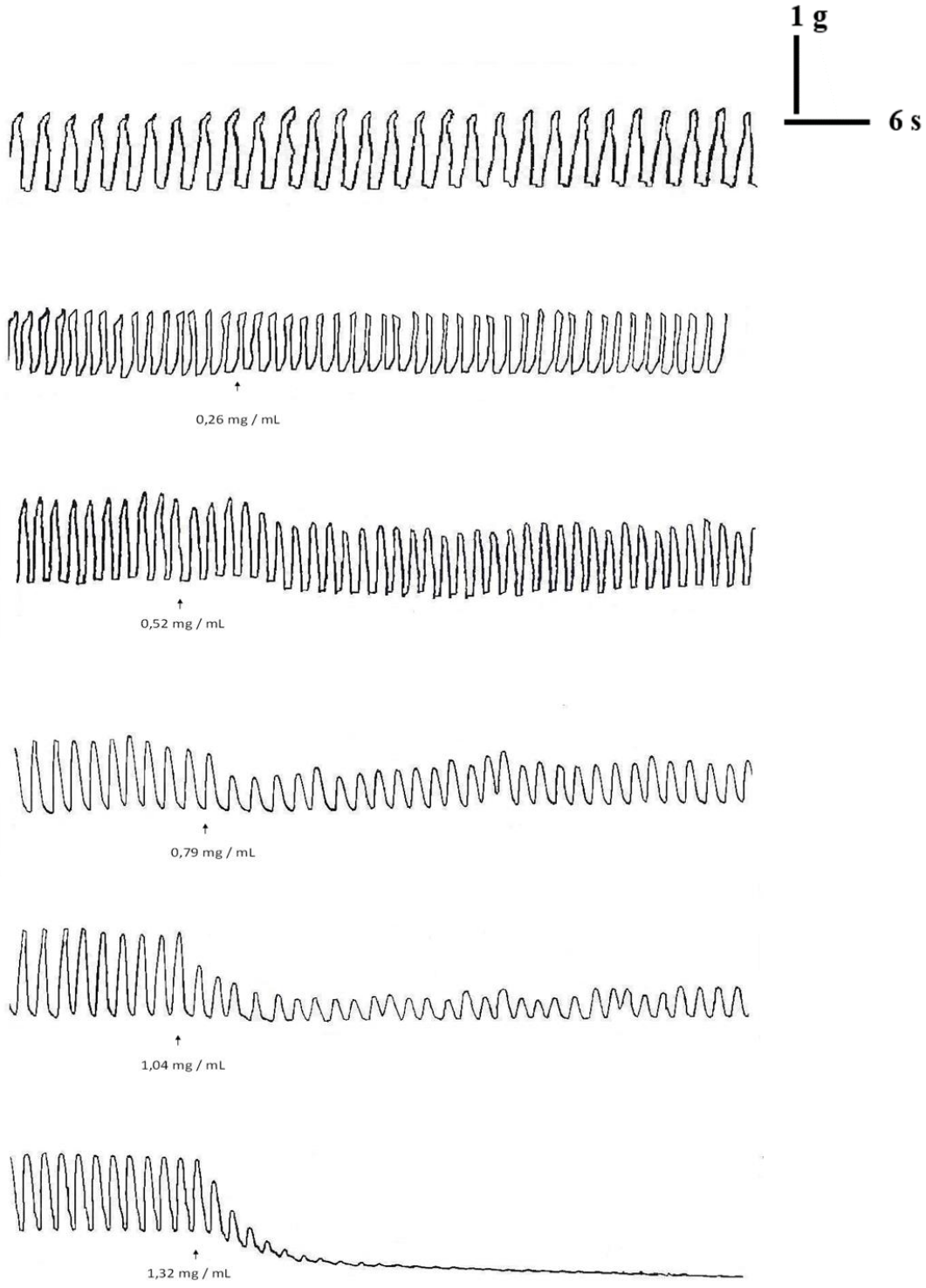

Figure 1 Effect dose-response of crude ethanol extract (A = normal contraction).

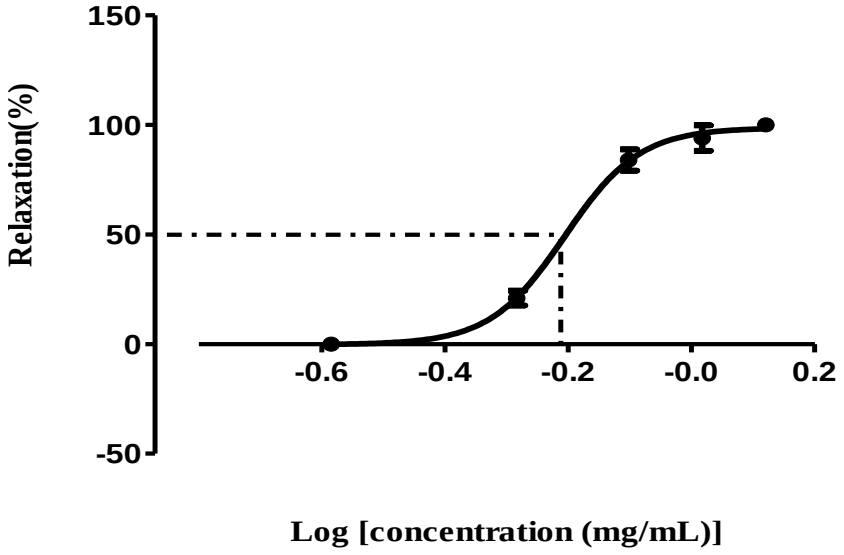

Figure 2 Sigmoid curve of ethanol extract effect.

3.3. Antispasmodic activity of dichloromethane fraction

At 0.13 mg/mL the extract reduced lightly duodenum contraction at 15% (Table 2). But at 0.26; 0.39 and 0.52 mg/mL, the relaxation was significantly and strongly observed (n=6; Figure 3). The IC_{50} was 0.24 mg/mL (Figure 4). The percentages of inhibition were 65% for 0.26 mg/mL, 96% for 0.39 mg/mL, and 100% for 0.52 mg/mL (Table 2).

Table 2 Effects of dichloromethane fraction on duodenum contractions in rabbits.

Concentrations	Inhibition of contraction
0.13 mg/mL	15 ± 1.30
0.26 mg/mL	65 ± 2.12*
0.39 mg/mL	96 ± 0.54**
0.52 mg/mL	100 ± 0**

Values are the mean ± SEM (n=6); * $p < 0.05$; ** $p < 0.001$.

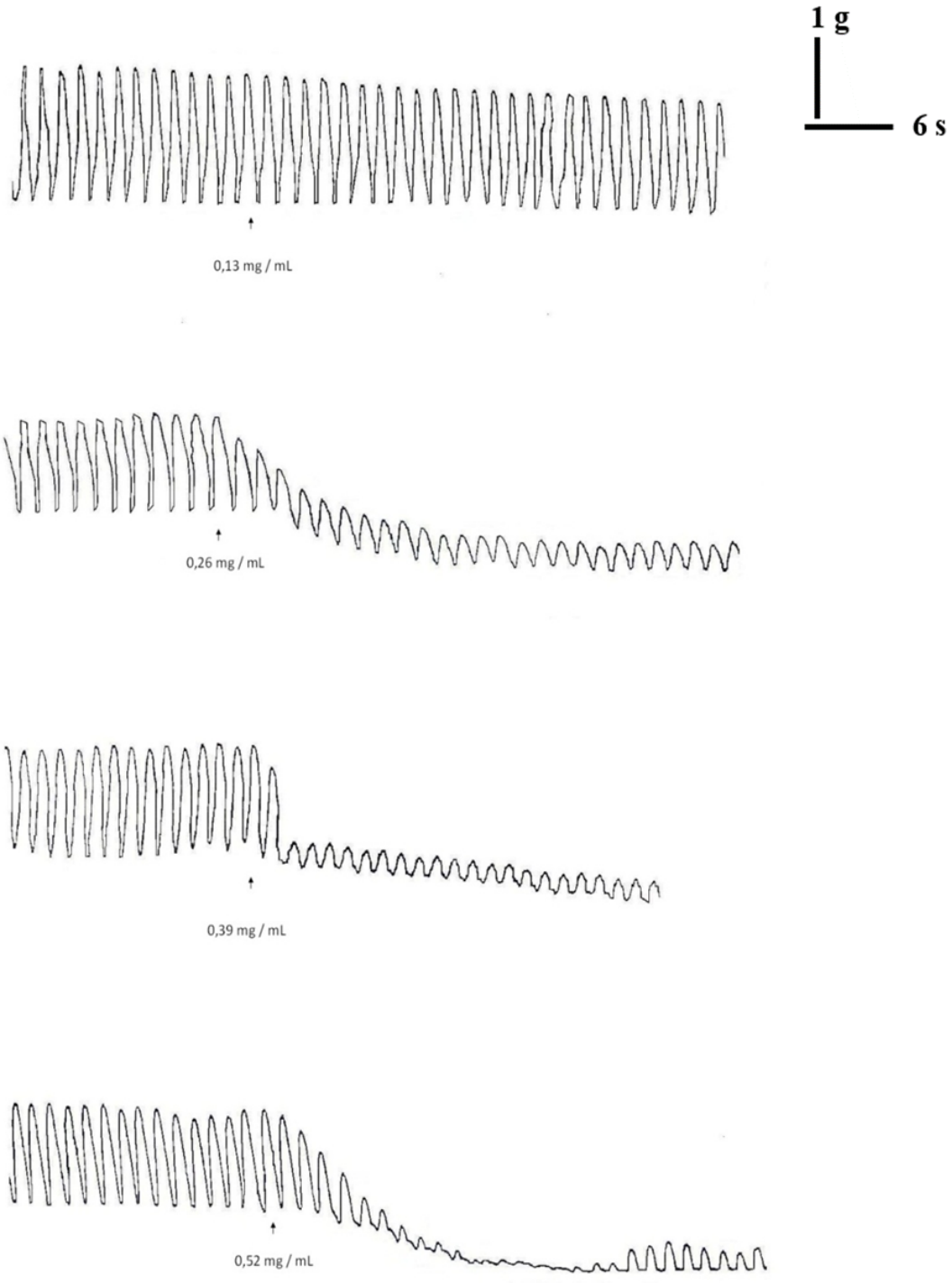

Figure 3 Effect dose-response of dichloromethane extract (A=normal contraction).

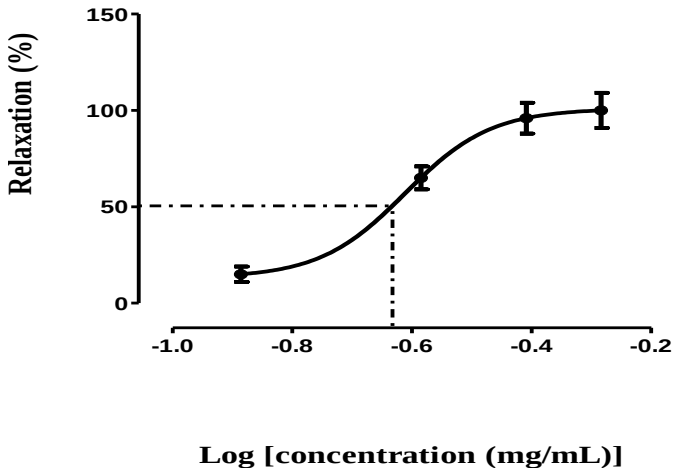

Figure 4 Sigmoid curve of dichloromethane extract effect**.**

3.4. Phytochemical screening of dichloromethane fraction

Phytochemical screening tests of dichloromethane fraction for various constituents revealed the presence of major components such as tannins and flavonoids. Polyphenols and reducing sugars were present, and anthraquinones, alkaloids, coumarins, polyterpenes and sterols were absent (table 3).

Table 3 Phytochemical screening of dichloromethane fraction and ethanol total extract of stem bark of *P. reticulatum.*

chemical constituents	*Piliostigma reticulatutum*
Polyphenols	+
Sterols et polyterpernes	-
Flavonoids	++
Saponins	-
Tannins	++
Alkaloids	-
Quinons	-
Reducing sugars	+
Coumarins	-

(-) absence, (+) presence, (++) major chemical constituents.

4. Discussion

The present study sought to assess the antispasmodic activity of *Piliostigma reticulatutum*. This plant was ethno-botanically selected during a survey ruled in 2018 in the City of Abidjan (Côte d'Ivoire) and realized through the actors of Ivorian traditional medicine including 8 herbalists and 1 healer. The results of the survey (data not shown) have allowed us to collect 17 antidiarrheal plant species among which *Piliostigma reticulatutum* was much advised for gastrointestinal disorders.

The objective of this work was to further investigate the antispasmodic effect of dichloromethane fraction of *P.reticulatum* and compare to the total extract effect to find out which is effective.

Our study showed that ethanol extract stem bark of *P. reticulatum* significantly inhibited the duodenum contraction, as shown by the figures. Also the dichloromethane fraction reduced significantly duodenum contraction. The relative potential action of the dichloromethane, indicate it contains constituents which are responsible for the inhibitory action of *P. reticulatum* on duodenum. The comparison of dose ratios (x=0.24 mg/mL) at IC_{50} points shows that dichloromethane-rich fraction is more potent than the ethanol extract (x=0.66 mg/mL). The dichloromethane extract have a better potency action on duodenum contraction in rabbits.

Antispasmodic agents are considered useful for the treatment of pains resulting from spasms of the gut and diarrhea due to the hypermobility of the gastrointestinal tract [12, 33]. Diarrhea mechanisms are various and involve often gastrointestinal disorders. Therefore we can assume that the antidiarrheal action of *P. reticulatum* could be mediated in by a mechanism involving the decrease of gastrointestinal mobility which results of inhibition of duodenum contraction.

The phytochemical screening of stem bark of *P. reticulatm* showed that tannins and flavonoids are the major components while polyphenols and reducing sugars were minors. The result also showed the absence of sterols, polyterpenes, saponins, alkaloids, quinons, and coumarins. These components observed could be responsible of antispasmodic activity of dichloromethane fraction and ethanol total extract of stem bark of *P. reticulatum*. Our results are in consonance with many studies in the literature. It was reported that flavonoids, [9, 34]; tannins, reducing sugars [35], were responsible of antispasmodic properties of certain plants like *Pynocycla spinosa* and *Morinda morindoides.*

The antispasmodic activity of flavonoids has been demonstrated and attributed to their ability to inhibit gastrointestinal mobility [36, 37]. *In vitro* experiments on animals have shown that flavonoids are able to inhibit the contractions induced by spasmogens [38, 39].

The antispasmodic activity of *P. reticulatum* could therefore due be to the presence of flavonoids, tannins, polyphenols and reducing sugars containing in dichloromethane fraction.

5. Conclusion

From this study, it can be concluded that the dichloromethane fraction which has been isolated in the total extract of *P. reticulatum*, contains an important antispasmodic activity. The mechanism action could be study by antagonism method with acetylcholine, propranolol and prazosin. It is suggested that the antispasmodic constituents must be isolated in order to identify the most active chemical substance, which could then be used possibly as an antispasmodic in gastrointestinal disorders such as diarrhea.

However, further bioassay-guided fractionation studies are required to identify the active principle (s) and their mechanism of action. Moreover, the use of aerial parts of the plant could be a solution to the utilization of its roots, therefore limiting the biodiversity degradation.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

If studies involve use of animal/human subject, authors must give appropriate statement of ethical approval. If not applicable then mention ‘The present research work does not contain any studies performed on animals/humans subjects by any of the authors’.

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