SPASMOGENIC ACTIVITY OF THE AQUEOUS AND METHANOL EXTRACTS OF THE STEM BARK OF ANTHOCLEISTA VOGELII PLANCH. (LOGANIACEAE) IN RATS

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Summary

The effect of aqueous and methanol extracts of the stem bark of *Anthocleista vogelii* on the albinos rat smooth muscles was studied. The two extracts, produced a dose-dependent effect on the tone and force of the spontaneous contraction of the rat ileal and stomach smooth muscle fragments at concentrations ranging from 0.13 to 8.00 mg/ml. A concentration of 8.00 mg/ml on ileum and 4.00 mg/ml on stomach muscle produced maximal contractile effect in a single concentration. In cumulative concentrations, the aqueous extract produced a dose-dependent contractile effect while the methanol extract produced a biphasic response. Atropine, administered 5 minutes before the contractile effect of the methanol extract at concentration of 0.1 µg/ml and 0.2 µg/ml completely inhibited the contractile effect of the methanol extract on both ileal and stomach smooth muscles. Similarly, the same concentrations of atropine reduced the contractile effect of acetylcholine by 55.01 and 100% on ileum and stomach fragments respectively.nifedipine (1.60×10^{-1} µg/ml) and pyrilamine maleate (6.00×10^{-1} µg/ml) completely abolished the spasmogenic effect of the methanol extract on both ileal and stomach smooth muscles fragments.
These results point to a possible stimulation of these muscle fragments through muscarinic receptors which increase Ca^{2+} mobilisation from both extra and intramuscular medium.

Key words: Aqueous and methanol extracts – ileum – stomach – spasmogenic activity.

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Introduction

A report on the various used of *Anthocleista vogelii* has shown that it is a multipurpose plant of wide use for the rural community. In the African traditional medicine, the stem bark of this plant is used for the treatment of gastro-intestinal disorders, to cure fever, stomach ache and as purgative; while the combination of the stem bark and the leaves is used as anti-inflammatory and antidiabetic agents and also in the treatment of wounds (1).

In Cameroon, the stem bark is reported to be used to treat abdominal pains (2). This plant is a tree of 6 to 20 meters high, usually with buttrous roots, with branches having spikes, which also have sessile leaves and short petals. It is a tree of the Loganiaceae family which commonly grows around river edges and banks or in marshy areas of the tropical humid forest of the West Africa (3), with great concentration in Cameroon and Gabon (4). Considering the traditional importance of the plant to the rural community, in vitro experiments have been conducted in the present work. Thus, in this paper, the results of the stem bark aqueous and methanol extracts of *Anthocleista vogelii* on smooth muscle preparations are presented.

Materials and methods

Animals:

The experiments were carried out on wistar strain male adult rats of 12 to 16 weeks old and weighing between 170 to 200 g. The rats were raised in the animal house of the Department of Animal Biology, Faculty of Science, University of Dschang, Cameroon and fed on normal laboratory rat diet with food and water given ad libitum. Prior to the experiment, the animals were subjected to fasting for 24 hours but allowed free access to water.

Collection and preparation of plant material:

The stem bark of *Anthocleista vogelii* was collected in Bandjoun, in the West Province of Cameroon during the month of August 2008. Mr. Paul Mesili, now a retired Botanist of the Cameroon herbarium, Yaoundé, carried out the authentication of the plant material. A voucher specimen coded BUD 0636 was deposited at the Botany Department, University of Dschang for future reference. The collected fresh stem bark was air dried and ground into fine powder in a high speed grinding mill.

Extraction of the aqueous plant material:

Two hundred grams of powdered Anthocleista vogelii stem bark were boiled in two litters of distilled water for 15 minutes. The decoction was taken and allowed to cool for 30 minutes at room temperature (24 ± 2°C). The decoction was filtered twice and evaporated to dryness in an air oven at 45°C, to give 20.8 g of the aqueous extract corresponding to an extraction yield of 10.4%.

Extraction of the organic plant material:

Three thousand grams of the stem bark powder was soaked with 5 litters of hexane for 72 hours. The filtrate was concentrated to dryness in a rotary evaporator under reduced pressure at a temperature of 35°C to give 31.6 g of hexane extract (01.05% yield). The original material was then macerated in 5 litters acetone for 72 hours and the filtrate obtained was concentrated at a temperature of 30°C to give 112.3 g of acetone extract (03.74% yield). The original plant material was further macerated in 5 litters methanol for 72 hours and the filtrate concentrated at a temperature of 35°C to give 676 g of methanol extract (22.53% yield). The aqueous and methanol extracts were the main spasmogenic active extracts and were soluble in distilled water.

Experimental

The rats were killed by cervical dislocation. The abdomens were opened and 1 cm of ileal ileal segments were cut and cleansed of adhering connective tissue. The segments were rinsed with warm (37 °C) tyrode solution composed as follows (mM): NaCl 137.93; KCl 2.70; $CaCl_2$ 1.83; $MgSO_4$ 0.83; $NaHPO_4$ 0.42; $NaHCO_3$ 11.90 and glucose 5.56. Stomach fragments were prepared by cutting a portion of stomach (0.5 x 1 cm) along the greater curvature. One end of the segment was attached to a hook at the bottom of an organ bath containing tyrode solution and the other end attached to a Ugo Basile isotonic transducer with the resting tension of 0,5g. Spontaneous contractile activities of these fragments were then recorded at 37 °C on a Ugo Basile Unirecorder Gemini model 7050. The organ bath was bubbled with air and the set–up allowed 45 minutes to stabilize. After a resting period of 45 minutes, with 15 minutes washout interval, four kinds of experiments were carried out on the tissues.

1- A single concentration-effect curve of the aqueous and methanol on the spontaneous activity was established. This was done by adding, every 5 minutes, graded concentrations (0.13 – 8.00 mg/ml) of the aqueous or methanol extract to the tissue bath and observing their effects on the force of contraction. The effects of these concentrations on the baseline tone of the tissues were also observed.

2- Isolated rat ileums or stomach fragments were exposed to cumulative concentrations of aqueous and methanol extracts (0.13 – 8.00 mg/ml) in order to obtain concentration-response curves.

3- In order to determine the possible mechanism of action of the methanol extract, the following protocol was used: control curves were obtained at the beginning of the experiment when the tissues were contracted with 4.00 mg/ml of the methanol extract. This was followed by exposure to atropine (2.50×10^{-2} – 8.00×10^{-1} µg/ml), nifedipine (10^{-2} – 1.60×10^{-1} µg/ml) and pyrilamine maleate (7.50×10^{-2} – 6.00×10^{-1} µg/ml) for 5 minutes after the addition of the methanol extract.

Also, the determination of the possible mechanism of action of the aqueous extract used the following protocol: control curves were also obtained at the beginning of the experiment when the tissues were contracted with cumulative concentrations of aqueous extract ranging from 0.13 – 8.00 mg/ml. After the resting period of 45 minutes with 15 minutes washout interval, the exposure of little dose of atropine (2.50×10^{-2} or 5.00×10^{-2} µg/ml), nifedipine (10^{-2} or 2.00×10^{-2} µg/m and pyrilamine maleate (7.50×10^{-2}, 1.50×10^{-1} or 3.00×10^{-1} µg/ml) follow by the cumulative addition of the aqueous extract (0.13 – 8.00 mg/ml).

The responses of the aqueous and methanol extracts on the contractions elicited by the three antagonists were recorded for 5 minutes and the corresponding effect was determined by comparing the average amplitude of ileum or gastric contractions with the amplitude of contraction obtained when the antagonists alone were added to the bath.

4- In the last set of experiments, the effect of the methanol extract was studied in a free calcium medium. This Ca^{2+}-free medium was obtained by omitting $CaCl_2$ from the reference tyrode solution. The results were expressed as force of contraction of the internal standard Ca^{2+}-contraction.

Statistical analysis:

Statistical analysis was performed using ANOVA and Duncan's test and significance of difference between treatment was accepted at $p<0.05$. Data were expressed as mean ± standard error on the mean

Results

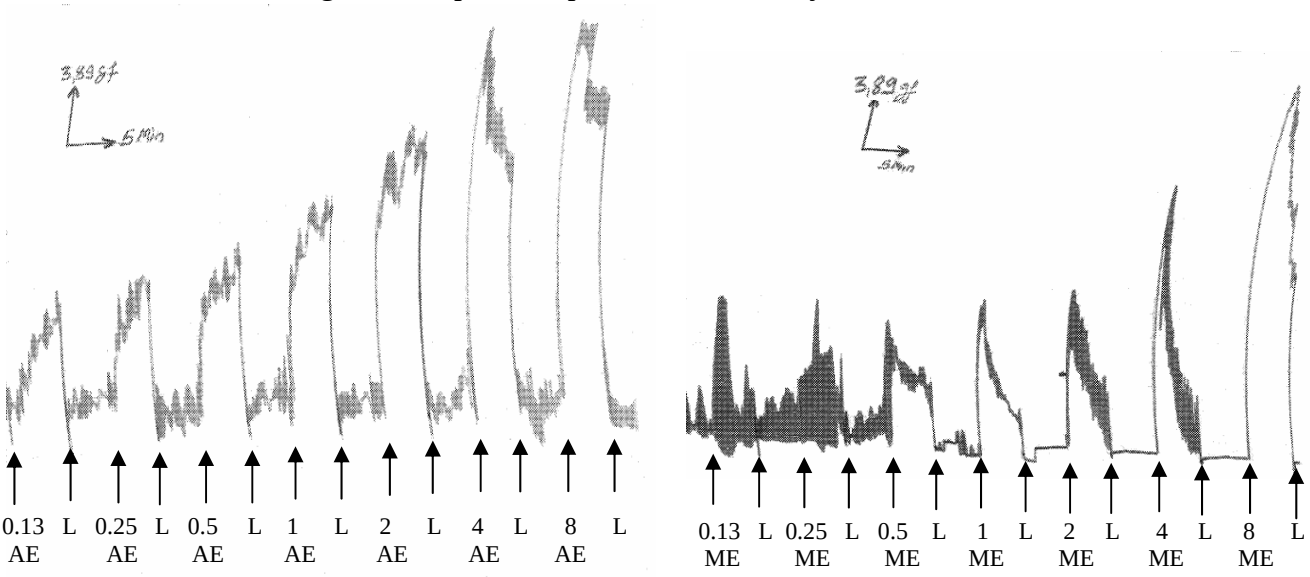
Effect of aqueous and methanol extracts on rat ileum

The aqueous and methanol extracts (0.13 – 8.00 mg/ml) produced a significant ($p<0.05$) concentration – dependent increase of spontaneous rat ileal contractions (figure 1). The force of contraction increased from 1.74 ± 0.86 mN at 0.13 mg/ml to 6.24 ± 1.52 mN at 8.00 mg/ml for the aqueous extract and from 1.33 ± 1.28 mN at 0.13 mg/ml to 5.52 ± 2.31 mN at 8.00 mg/ml for the methanol extract. Cumulative addition of the aqueous extract was observed which showed a significant ($p<0.05$) concentration – dependent increase with increases in the in the aqueous extract concentrations added (figure 3). Methanol extract at concentrations similar to those of aqueous extract produced a biphasic response with concentration – dependent decrease with increases in the methanol concentrations added (figure 3). Pre-treatment of ileal fragments with increased concentrations of atropine (2.50×10^{-2} - 8.00×10^{-1} µg/ml) in the case of methanol extract (figure 5) and in single concentration of atropine (2.50×10^{-2} or 5.00×10^{-2} µg/ml) in the aqueous extract (figure 8) completely prevented the expression of the previously observed spasmogenic activity of the methanol extract and prevent by over 80% the activity of the aqueous extract. Nifedipine (4.00×10^{-2} µg/ml) and pyrilamine maleate (6.00×10^{-1} µg/ml) completely abolished contractions induced by the methanol extract (4.00 mg/ml) (figures 6 and 7). Also, nifedipine (2.00×10^{-2} µg/ml) and pyrilamine maleate (3.00×10^{-1} µg/ml) completely inhibited contractions induced by the cumulative administration of the aqueous extract (0.13 – 8.00 mg/ml) (figures 9 and 10). The methanol extract in the free Ca2+ medium produced a low contraction of the ileal fragment as compared to a normal tyrode medium.

Effect of aqueous and methanol extracts on rat ileum

Figures 2 and 4 also show the the spasmogenic activity of single and cumulative concentrations of the aqueous and methanol extracts on rat stomach strips. Results similar to those for ileal fragments were obtained. Thus, concentrations of aqueous and methanol extracts (0.13 – 4.00 mg/ml) at single concentrations and (0.13 – 8.00 mg/ml) at cumulative concentrations for the aqueous extract only significantly ($p<0.05$) increased contraction forces while cumulative concentrations of the methanol extract (0.13 – 8.00 mg/ml) produced a biphasic response with concentrations forces decrease with increases in the methanol concentrations added. The spasmogenic activity of a single dose of methanol extract (4.00 mg/ml) was completely abolished by atropine at the concentration of (2.00×10^{-1} µg/ml) (figure 5) while cumulative concentration of the aqueous extract (0.13 – 8.00 mg/ml) was reduced by over 85% by atropine at concentration of 5.00×10^{-2} µg/ml (figure 8).

Pre-treatment with nifedipine (1.60 x 10^{-1} µg/ml) and pyrilamine maleate (6.00 x 10^{-1} µg/ml) completely inhibited the spasmogenic activity of a single concentration of the methanol extract (4.00 mg/ml) (figures 6 and 7). Spasmogenic activity of cumulative concentrations of the aqueous extract was significantly ($p<0.05$) reduced by the pre-treatment with a single dose of nifedipine (0.01 or .0.02 µg/ml) (figure 9) and pyrilamine maleate (0.075; 0.15 or 0.3 µg/ml) (figure 10). 'In a free Ca^{2+} medium, the methanol extract produced a low contraction of gastric strip as compared to a normal tyrode medium.



A B

Figure 1_a: Original concentration-response curves of the aqueous (A) and methanol (B) extracts of the stem bark of *Anthocleista vogelii* added to the intestinal smooth muscles fragments

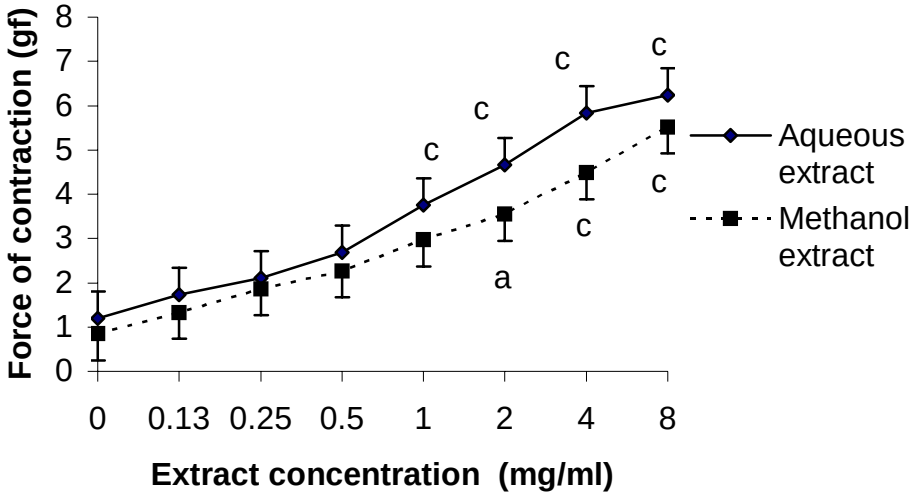
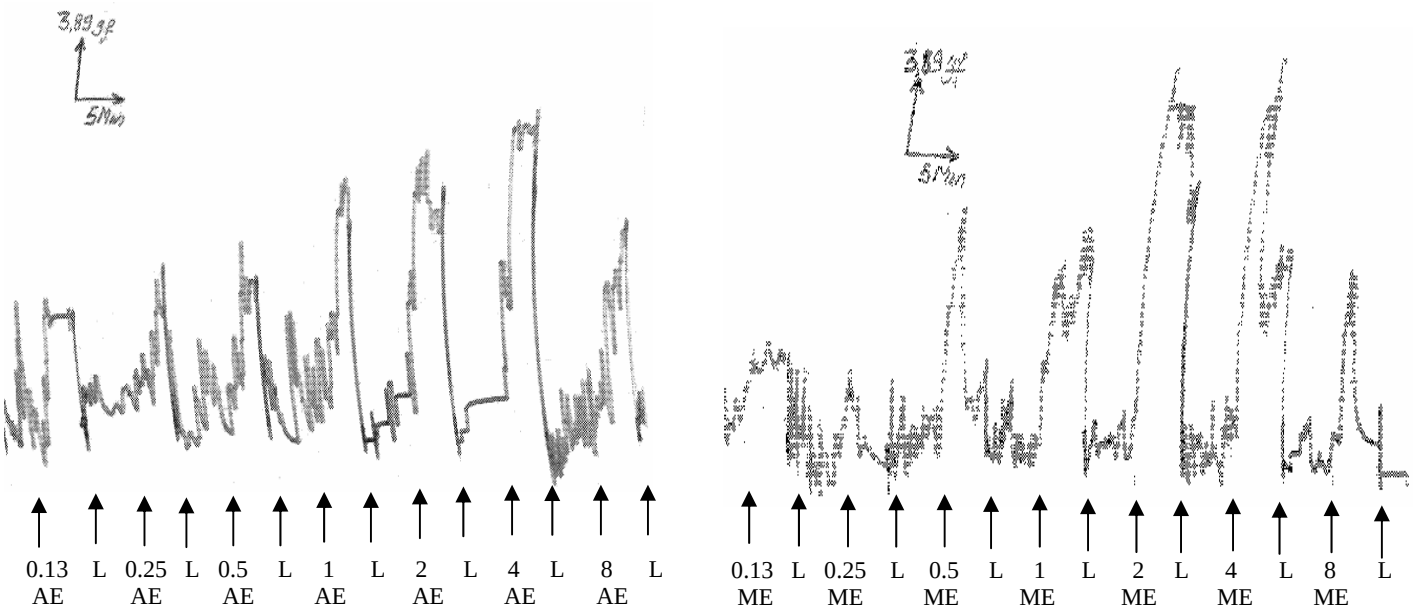


Figure 1_b concentration-response curves of the aqueous (A) and methanol (B) extracts of the stem bark of *Anthocleista vogelii* added to the intestinal smooth muscles fragments. Each point represents the means ± SE of 5 experiments. $^{a}p<0.05$ $^{c}p<0.001$ compared with initial concentration aqueous and methanol extracts (0 mg/ml).



A B

Figure 2_a: Original concentration-response curves of the aqueous (A) and methanol (B) extracts of the stem bark of *Anthocleista vogelii* added to the gastric smooth muscles fragments

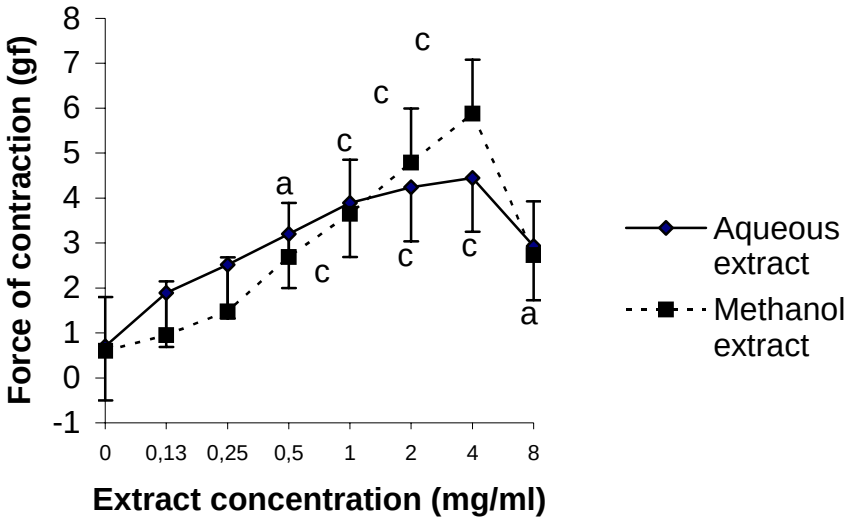


Figure 2_b: concentration-response curves of the aqueous (A) and methanol (B) extracts of the stem bark of *Anthocleista vogelii* added to the gastric smooth muscles fragments. Each point represents the means ± SE of 5 experiments. $^{a}p<0.05$ $^{c}p<0.001$ compared with initial concentration aqueous and methanol extracts (0 mg/ml).

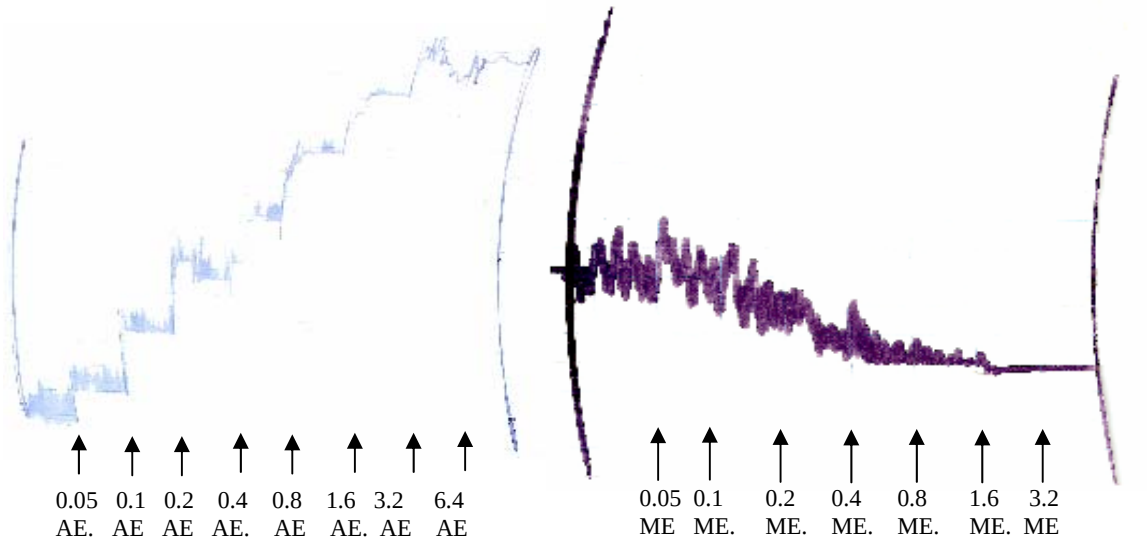


Figure 3_a: Original cumulative concentration-response curve of the aqueous (A) and methanol (B) extracts of the stem bark of *Anthocleista vogelii* added to the rat intestinal smooth muscle fragment.

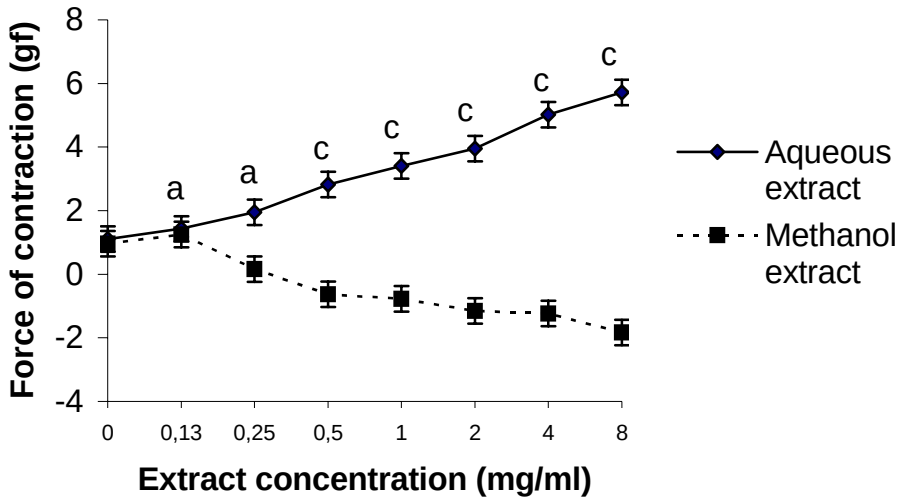


Figure 3_b: cumulative concentration-response curve of the aqueous (A) and methanol (B) extracts of the stem bark of *Anthocleista vogelii* added to the rat intestinal smooth muscle fragment. Each point represents the means ± SE of 5 experiments. $^{a}p<0.05$ $^{c}p<0.001$ compared with initial concentration aqueous and methanol extracts (0 mg/ml).

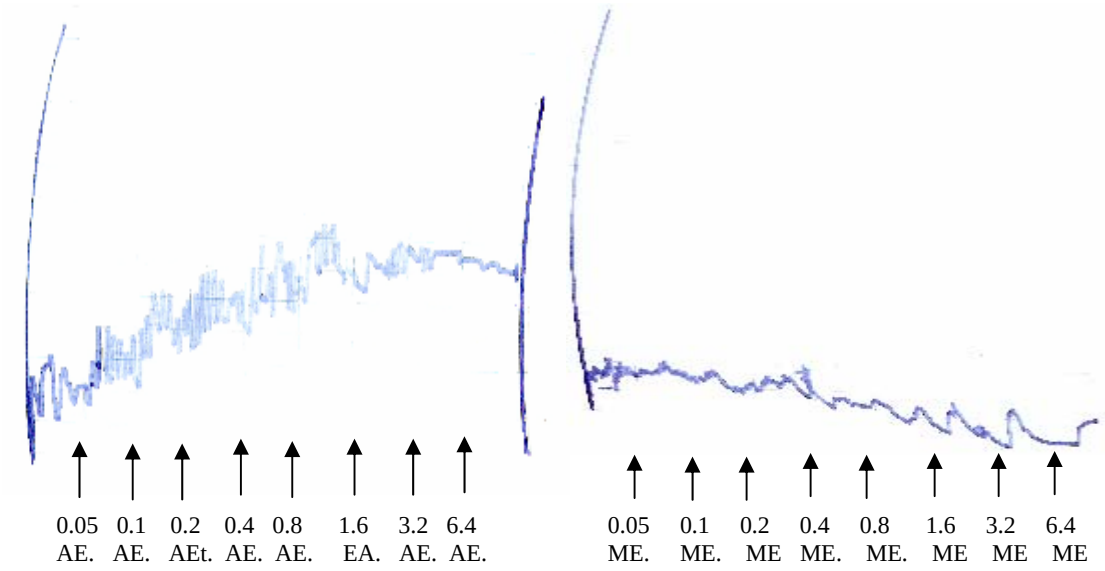


Figure 4_a: Original cumulative concentration-response curve of the aqueous (A) and methanol (B) extracts of the stem bark of *Anthocleista vogelii* added to the rat ileum smooth muscle fragment.

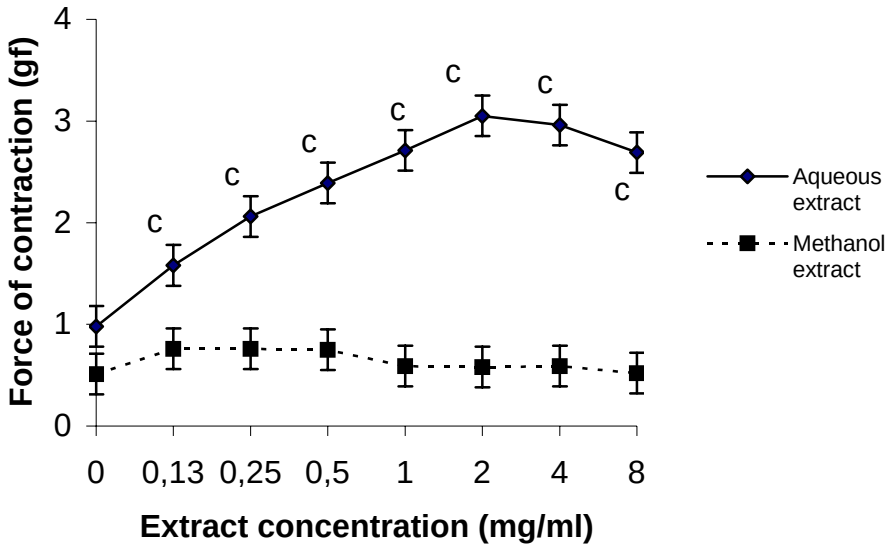
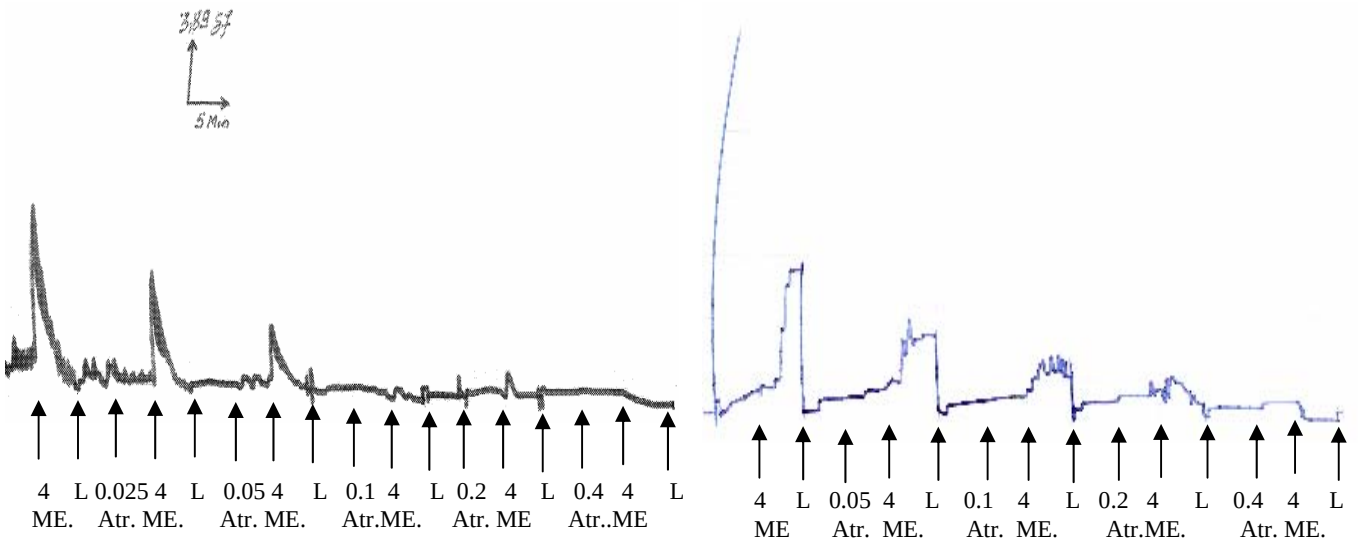


Figure 4_b: cumulative concentration-response curve of the aqueous (A) and methanol (B) extracts of the stem bark of *Anthocleista vogelii* added to the rat gastric smooth muscle fragment. Each point represents the means ± SE of 5 experiments. $^{c}p<[0.001$ compared with initial concentration aqueous and methanol extracts (0 mg/ml).



A B

Figure 5_a: Original curve representing the inhibitory effect of atropine on methanol induced contraction of rat intestinal (A) and gastric (B) smooth muscle fragments

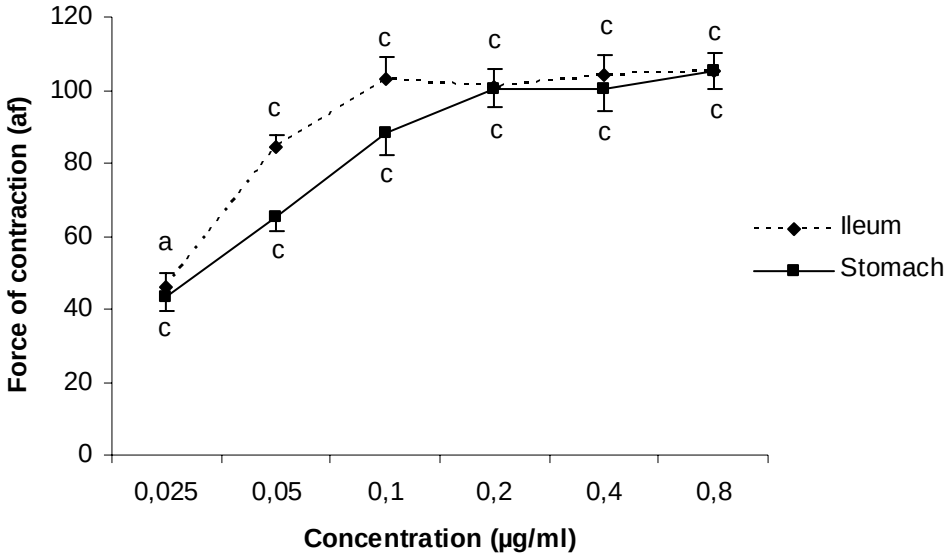
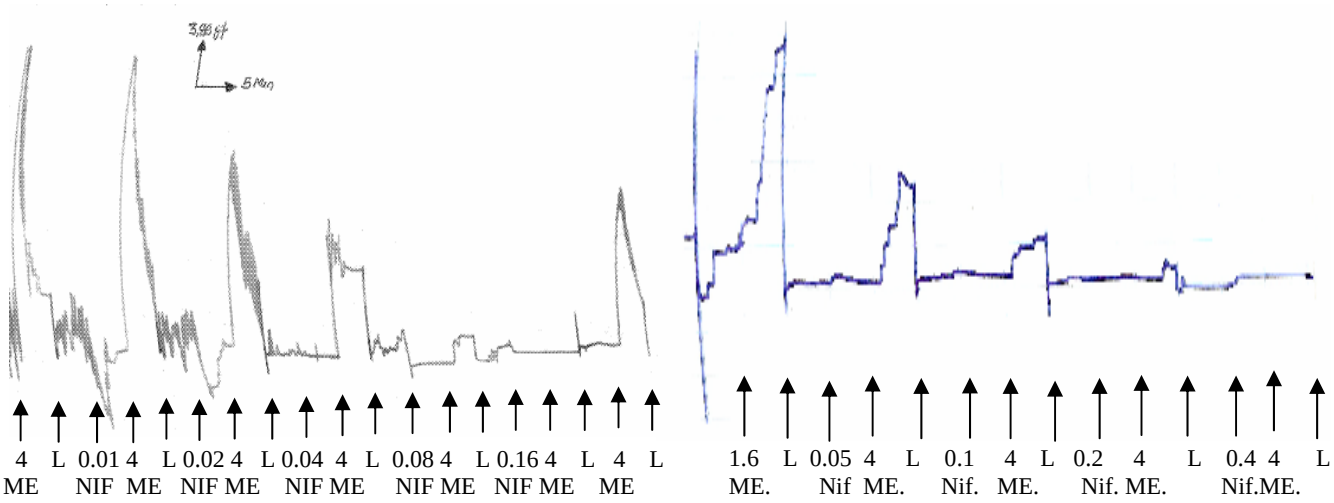


Figure 5_b: inhibitory effect of atropine on methanol induced contraction of rat intestinal (A) and gastric (B) smooth muscle fragments. Each point represents the means ± SE of 5 experiments. $^{a}p<0.05$ $^{c}p<0.001$ compared with initial concentration of atropine (0µg/ml).



A B

Figure 6$_a$: Original curve representing the inhibitory effect of nifedipine on methanol induced contraction of rat ileum (A) and stomach (B) smooth muscle fragments

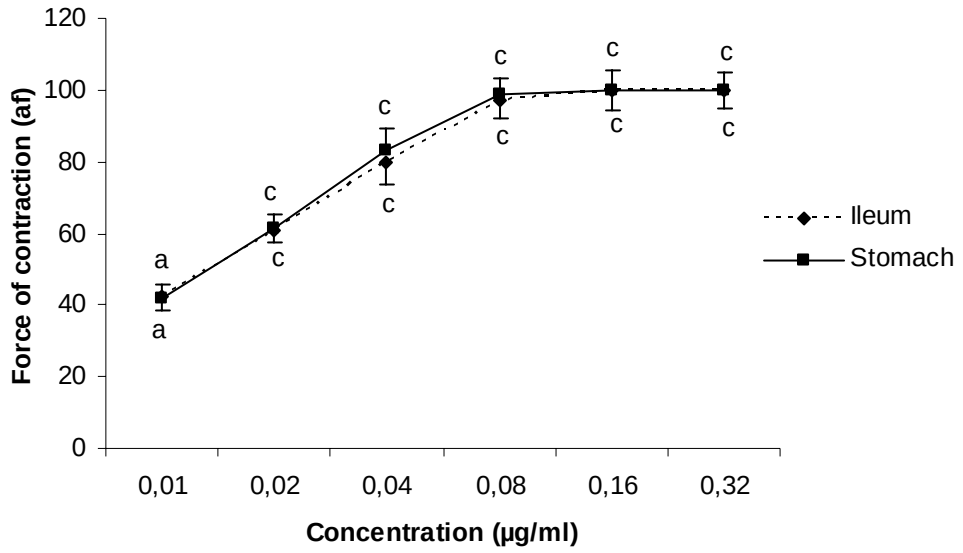


Figure 6$_b$: inhibitory effect of nifedipine on methanol induced contraction of rat intestinal (A) and gastric (B) smooth muscle fragments. Each point represents the means ± SE of 5 experiments. $^a p<0.05$ $^c p<0.001$ compared with initial concentration of atropine (0µg/ml).

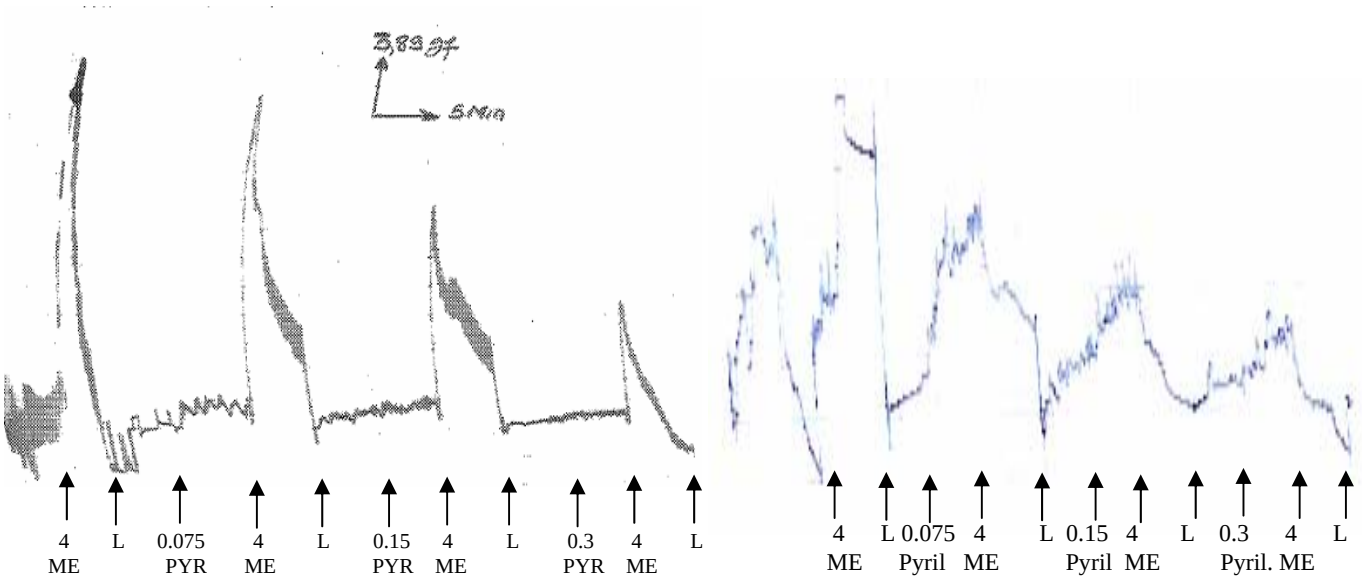


Figure 7_a: Original curve representing the inhibitory effect of pyrilamine maleate on methanol induced contraction of rat intestinal (A) and gastric (B) smooth muscle fragments

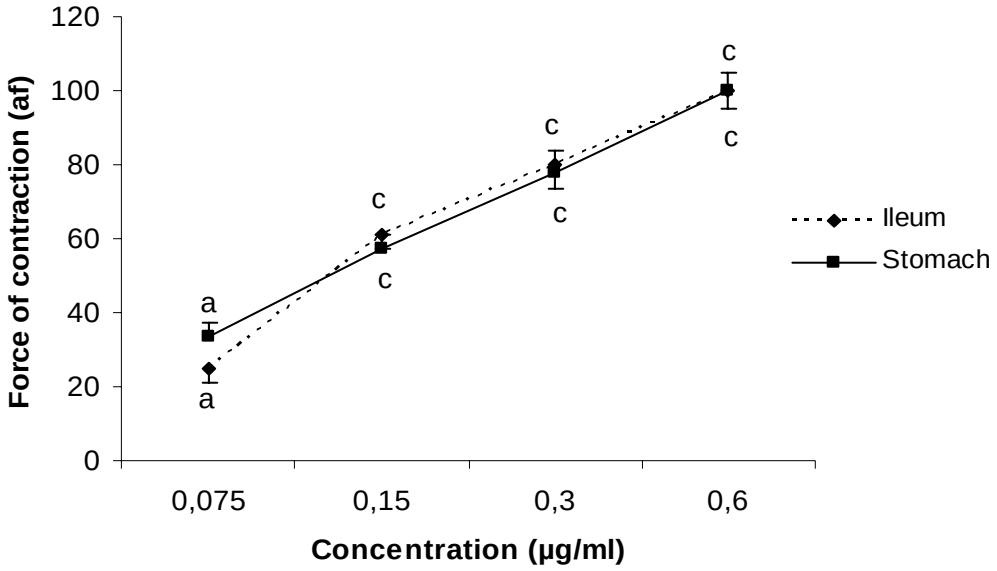
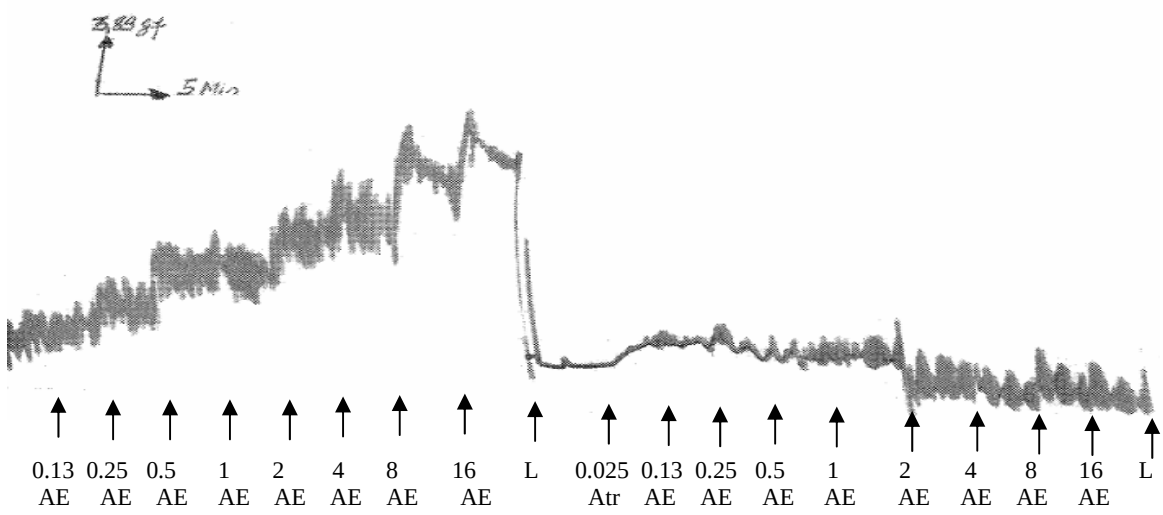


Figure 7_b: inhibitory effect of pyrilamine maleate on methanol induced contraction of rat intestinal (A) and gastric (B) smooth muscle fragments. Each point represents the means ± SE of 5 experiments. $^{a}p<0.05$ $^{c}p<0.001$ compared with initial concentration of atropine (0µg/ml).



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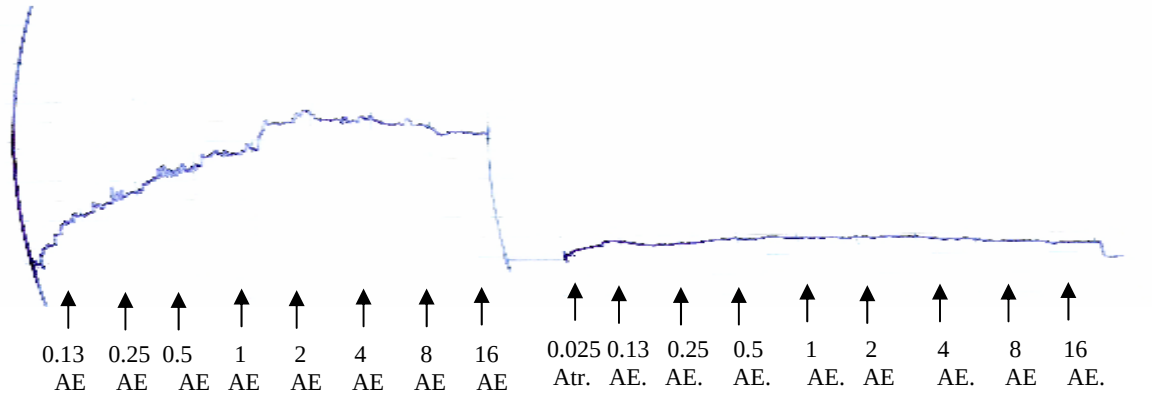

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Figure 8_a: Original curve representing the inhibitory effect of atropine on aqueous cumulative concentration-response on rat ileal (A) and gastric (B) smooth muscle fragments

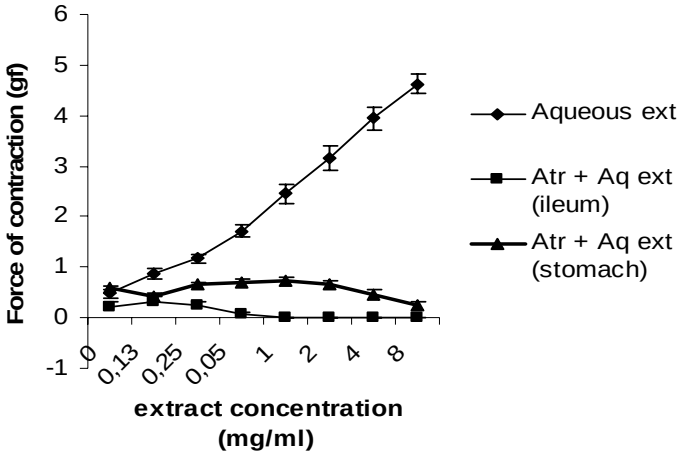
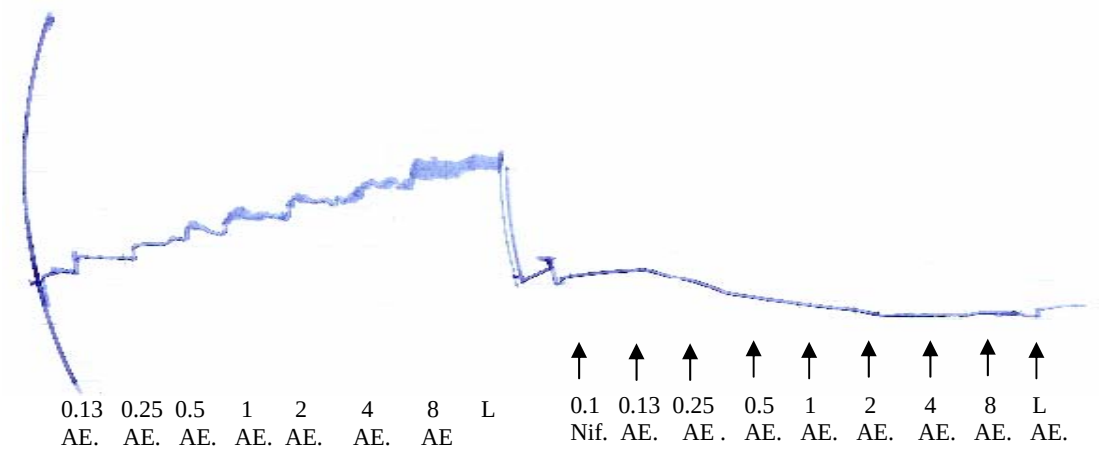


Figure 8_b: Inhibitory effect of atropine on aqueous cumulative concentration-response on rat ileal (A) and gastric (B) smooth muscle fragments



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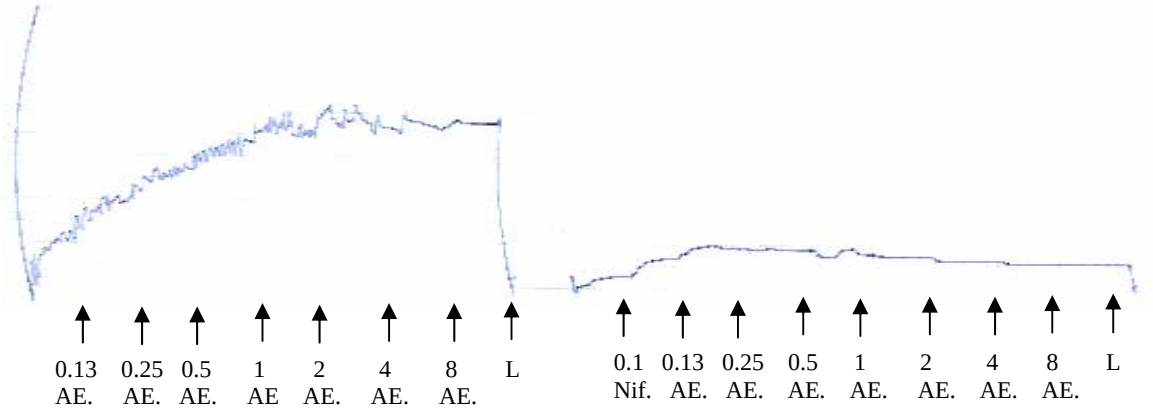


B

Figure 9_a: Original curve representing the inhibitory effect of nifedipine on aqueous cumulative concentration-response on rat ileum (A) and gastric (B) smooth muscle fragments

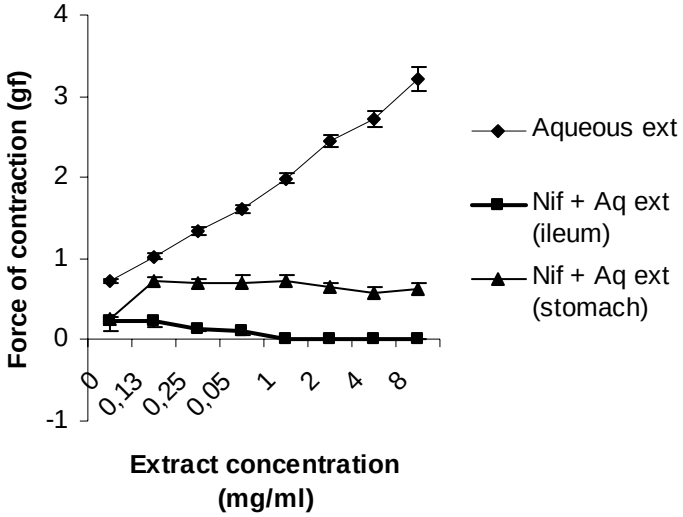
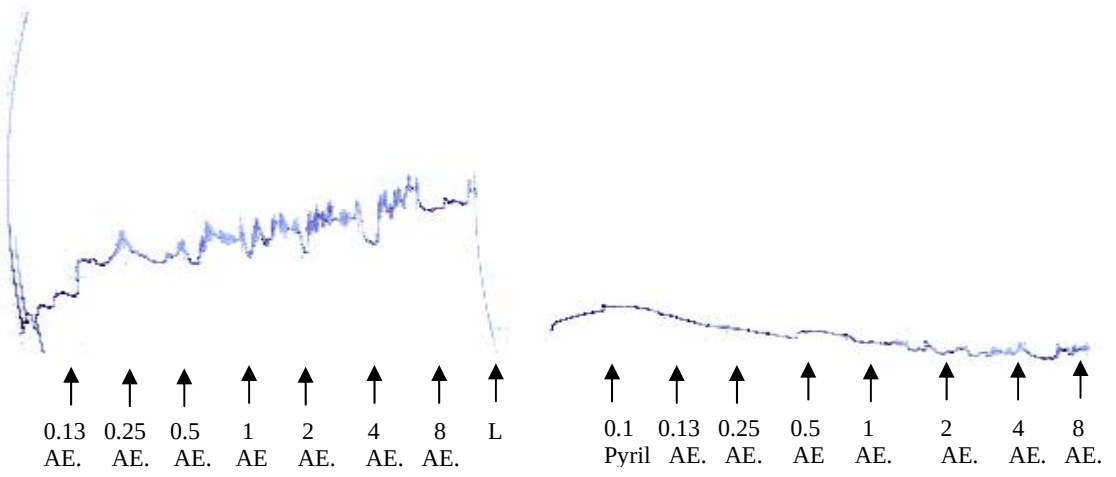


Figure 9_b: Inhibitory effect of nifedipine on aqueous cumulative concentration-response on rat ileum (A) and gastric (B) smooth muscle fragments



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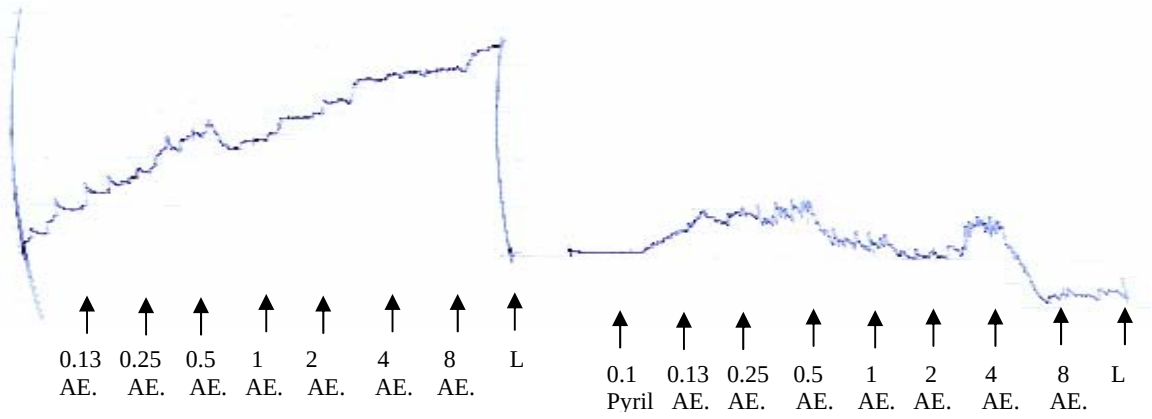

B

Figure 10_a: Original curve representing the inhibitory effect of pyrilamine maleate on aqueous cumulative concentration-response on rat intestinal (A) and gastric (B) smooth muscle fragments

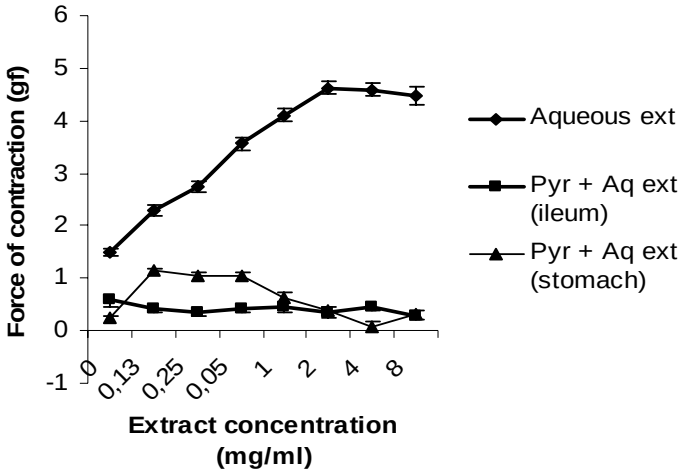

Figure 10_b: Inhibitory effect of pyrilamine maleate on aqueous cumulative concentration-response on rat intestinal (A) and gastric (B) smooth muscle fragments

Discussion

The results from the present study demonstrate that the aqueous and methanol extracts of the stem bark of Anthocleista vogelii possesses stimulant effect on rat intestinal and gastric smooth muscle preparations in vitro. The increase of ileum and stomach sensitivity to calcium strengthens the hypothesis of a postjunctional action of aqueous and methanol extracts, possibly activating the calcium entry via voltage dependent calcium channels in both intestinal and gastric smooth muscles (5, 6, 7). Contractions of smooth muscle are dependent on an increase in the concentration of the cytosolic free Ca^{++} which activates the contractile elements. The source of Ca^{2+} may be intracellular or extra cellular, depending on the contractile agent and the type of smooth muscle (8). Acetylcholine, a major excitatory transmitter, in both intestine and stomach by binding to muscarinic receptors, activates receptor-operated channels which become permeable to mono and bivalent cations such as Na^{+} and Ca^{2+} (9). Atropine, a known anti cholinergic agent, significantly inhibited the spasmogenic effect of the aqueous and methanol extracts on ileal and stomach smooth muscle, suggesting that the two extracts may be acting through muscarinic receptors (10). Nifedipine a calcium channel blocker and pyrilamine maleate a specific histamine channel inhibitor completely abolished the spasmogenic effect of the methanol extract and significantly reduced the contractile effect of the aqueous extract suggesting that the extracts may exerts their activity through calcium channel and histaminic receptors.

When the ileum and stomach fragments were exposed to a calcium free bath medium, force of contraction was low compared to what obtained in a normal medium due to the mobilisation of intracellular calcium only. This prompts us to think that the source of calcium is both intra and extra cellular. The methanol extract may thus be acting by mobilizing intracellular Ca^{++}.

Finally, the present study indicates that the aqueous and methanol extracts of the stem bark of *Anthocleista vogelli* possesses spasmogenic activity on both ileal and stomach smooth muscle fragments. Pharmacological characterization of the extracts suggests that the mechanism of action might be due to an interference with calcium metabolism in smooth muscle.

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