

## Effects of aqueous leaf extract of *Bryophyllum pinnatum* on guinea pig tracheal ring contractility

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**Summary:** Aqueous leaf extract of *Bryophyllum pinnatum* Lam (Crassulaceae) is used as a cough remedy and for the prophylaxis of asthma. Since drugs used for the prophylaxis of asthma may be acting on airway smooth muscles, we investigated the effects of aqueous leaf extract of the plant on the contractile responses of isolated tracheal rings. Guinea pigs were grouped into non-sensitized, ovalbumin (OA)-sensitized, OA-sensitized but 200 mg/kg/day x 21 extract-treated, and OA-sensitized but 400 mg/kg/day x 21 extract-treated. The extract was administered orally. Tracheal rings obtained from the four groups were mounted in organ baths and used to test spasmolytic and antispasmodic effects of the extract on histamine or carbachol-induced contractions. Concentrations of 0.125 – 1.0 mg/ml of the extract did not relax histamine or carbachol-induced precontractions. The presence of 0.25 – 1.0 mg/ml of the extract in organ baths significantly ( $p < 0.0001$ ) reduced the maximal contractile responses ( $E_{max}$ ) to cumulative concentrations of histamine or carbachol irrespective of the experimental group.  $pD_2$  values were significantly reduced for histamine ( $p < 0.05$ ) and carbachol ( $p < 0.002$ ) in rings obtained from 400 mg/kg/day x 21 extract-treated group. It is concluded that aqueous leaf extract of *B. pinnatum* possesses antispasmodic effects on the guinea pig tracheal rings. The results lend credence to the use of the extract for the prophylaxis of asthma in ethnomedicine.

**Keywords:** *Bryophyllum pinnatum*; Tracheal rings; Anti-asthmatic; Antispasmodic; Herbal medicine

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### INTRODUCTION

The use of herbal products for the treatment of chronic obstructive airway diseases such as asthma is becoming increasingly popular (Ng et al., 2003; Rivera et al., 2004; Argüder et al., 2009). Although orthodox medicines are available for asthma, their costs often put them out of the reach of many people in resource poor countries (Osujih, 1993; Cohen-Kohler, 2008). These herbal products are also assumed to have lower adverse effects compared to the orthodox drugs (Larrey, 1994).

*Bryophyllum pinnatum* (Lam.) Oken (= *Kalanchoe pinnata* Pers) of the family Crassulaceae

is one plant with extensive ethnomedicinal use. It is also called air plant, Canterbury bells, cathedral bells, life plant, Mexican love plant, miracle plant and resurrection plant. It is found in many parts of the world largely because of its ease of cultivation which may also be the reason for its extensive promotion in herbal medicine. Phytoconstituents of the leaves include flavonoids, saponins, tannins and alkaloids (Okwu and Josiah, 2006; Yamagishi, 1989). Vitamins including ascorbic acid, riboflavin, thiamine, niacin and minerals such as calcium, zinc and phosphorus are also present in the leaves (Okwu and Josiah, 2006).

The leaves have been reported to possess a variety of medicinal properties including, antimicrobial (Akinpelu, 2000), antifungal (Misra and Dixit, 1979), antiulcer (Pal and Nag Chaudhuri, 1991), antihypertensive (Ojewole, 2002), tocolytic (Gwehenberger et al., 2004; Plangger et al., 2006), antidiabetic (Ojewole, 2005), anti-inflammatory and analgesic (Ojewole, 2005), and wound healing (Khan, 2004). Other reported properties include anti-tumour (Yamagishi, 1989; Obaseiki-Ebor et al., 1993), sedative and muscle relaxant effects (Yemitan and Salahdeen, 2005). It may also be effective in the treatment of leishmaniasis (Torres-Santos et al., 2003).

Herbal practitioners in Nigeria and other parts of Africa use the aqueous extract for the treatment of coughs and as a prophylactic medicine for asthma. In Benin City, Nigeria, the leaves are boiled, filtered through a clean white cloth and the yield reconstituted for daily oral use by asthmatic patients. Since change in diet type has been implicated as a major reason for the rising incidence of asthma worldwide (Romieu and Trenga, 2001; Devereux, 2010), diet recommendation now includes antioxidants which are present in plants to varying degrees (Missoand Thompson, 2005; Kawai et al., 2007). Since many conventional anti-asthmatic drugs act on smooth muscles of the airways (Hall, 2000), we hypothesized that the extract of *B. pinnatum* may possess similar properties. We therefore designed the present study to investigate its effects on the isolated guinea pig tracheal ring responses to spasmogens.

## MATERIALS AND METHODS

### *Plant material*

Fresh leaves of *B. pinnatum* were collected from the wild in a suburb of Benin City, Nigeria, in June 2009. Although the plant is commonly known, it was confirmed by Dr B. A. Ayinde of the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Nigeria. A herbarium specimen with voucher number FHI 107762 of the same plant has been deposited with the Forest Research Institute of Nigeria by Miss Joy Odimegwu. Adulterants were carefully picked out and the leaves were thoroughly rinsed in tap water. The leaves (2 kg) were cut into small pieces with a knife and boiled in 4 L of distilled

water for 1 h, allowed to cool and then filtered two times with a clean white cloth. The final filtrate which was free from particles was concentrated in a rotary evaporator and dried in an oven at 40°C over 24 h (yield = 3.6 % w/w) and then stored in amber-coloured bottles in a fridge (4°C). The aqueous extract was reconstituted in distilled water and administered according to the experimental protocol.

### *Animals*

All experiments were performed using adult guinea pigs of either sex weighing 350 - 400 g. The animals were obtained from the animal house, Department of Physiology, Ambrose Alli University, Ekpoma, Nigeria. They were housed in standard plastic cages and allowed access to pellets (Bendel Feeds and Flour Mill Ltd, Ewu, Nigeria) and tap water *ad libitum*. Animals were exposed to natural lighting conditions and room temperature. They were handled according to standard protocols for the use of laboratory animals (National Institute of Health USA: Public Health Service Policy on Humane Care and Use of Laboratory Animals, 2002). The study was approved by institutional ethical committee on the use of animals for experiments. The animals were randomly allotted into four groups comprising of:

1. Non-sensitized (Control). The guinea pigs were given 5 ml/kg/day of saline for 21 days
2. Ovalbumin sensitized + saline treated. The guinea pigs were sensitized but administered 5 ml/kg/day of saline for 21 days. The animals were sensitized by intraperitoneal injection of 100 mg ovalbumin (OA), another intramuscular 100 mg OA, and a booster dose of 50 mg OA (i.m.) 24 h later (Bramley et al., 1995).
3. OA-sensitized + 200 mg/kg/day aqueous extract-treated. The animals in this group were sensitized as described but given the extract for 21 consecutive days.
4. OA-sensitized + 400 mg/kg/day aqueous extract treated. The animals were sensitized as described but administered the extract for 21 consecutive days.

The administration of extract and saline was done by use of an orogastric tube (CU.FNC-16-3).

### *Isolated tracheal ring experiments*

The tracheae were quickly dissected from sacrificed guinea pigs and placed in a Petri dish containing physiological salt solution (PSS). The tracheae were

cleaned of adherent connective tissues as much as possible and cut into rings of 2 mm length. The rings were suspended in L-shaped wire loops in 10 ml organ baths containing the PSS. The composition of the PSS was (g/l): NaCl 7.52, KCl 0.44, MgSO<sub>4</sub> 0.29, KH<sub>2</sub>PO<sub>4</sub> 0.16, NaHCO<sub>3</sub> 2.10, glucose 2.18, CaCl<sub>2</sub> 0.37. The PSS was bubbled throughout with 95% O<sub>2</sub> and 5% CO<sub>2</sub> gas mixture (BOC Gases, Nig. Plc) with the temperature maintained at 37°C. The upper loop was attached to a Grass model FT302 force transducer connected to a Grass Model 7D polygraph (Grass Instrument Co; Quincy, MA, USA). The rings were given a resting force of 1g (McCaig et al., 1992; Ozolua et al., 2009). An equilibration period of 45 min was allowed at the end of which the tissues were stimulated twice at 15 min intervals with 1 μM histamine in order to establish viability.

After equilibration the tracheal rings were exposed to drugs and extract according the following experimental protocol:

1. Effects of cumulative concentrations of 0.125, 0.25, 0.5, and 1 mg/ml of extract on histamine or carbachol-precontracted rings. Precontraction was done with 1 x 10<sup>-5</sup> M histamine or 2 x 10<sup>-6</sup> M carbachol. These were the concentrations that produced approximately 75% of their respective maximum contraction in tracheal rings obtained from non-sensitized guinea pigs. The appropriateness of these concentrations was confirmed by obtaining 100% relaxation with 10<sup>-6</sup> M isoprenaline.
2. Effects of different concentrations of 0.25, 0.5, and 1 mg/ml of extract on cumulative concentrations (1 x 10<sup>-10</sup> to 4 x 10<sup>-4</sup> M) of histamine or carbachol.

In order to ascertain tissue viability, fresh rings were mounted for each concentration of the extract tested in the organ bath.

#### Chemicals

Histamine, carbachol, isoprenaline and ovalbumin were all obtained from Sigma (UK) and prepared fresh by dissolving in distilled water. Other chemicals and reagents were of analytical grade and were obtained from Sigma, May & Baker, BDH or Scharlau Chemie S.A. The extract was dissolved in PSS before addition to the organ bath such that the volume of bath was not altered by more than 200 μl.

#### Statistics

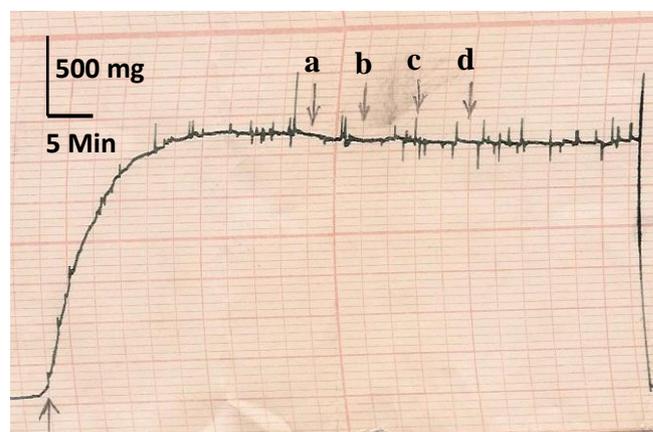
##### Tracheal antispasmodic effects of *B. pinnatum*

Data are presented as mean ± SEM (standard error of the mean) and *n* represents the number of rats used for a particular experiment. All contractions were related to the maximum responses by rings obtained from non-sensitized guinea pigs. EC<sub>50</sub> (molar concentration producing 50% of maximum contractile response) values were computed using a programme for logit transformation of concentration-response and converted to pD<sub>2</sub> (negative log of EC<sub>50</sub>) values. The maximal response (E<sub>max</sub>) was determined graphically. Comparisons were made where appropriate, by use of one-way ANOVA followed by Tukey post hoc. All data were analyzed using GraphPad Prism software (UK). Statistical significance was set at *p* < 0.05.

## RESULTS

### Effect of extract on precontracted tracheal rings

Addition of cumulative concentrations (0.125 – 1.0 mg/ml) of the extract to histamine (10<sup>-5</sup> M) or carbachol (2 x 10<sup>-6</sup> M) precontracted tracheal rings did not produce relaxant effect. The pattern of response is shown in Figure 1 and is the same regardless of the animal group from which the ring was sourced.



**Figure 1.**

Representative tracing showing absence of relaxation by tracheal rings exposed to cumulative organ bath concentrations of aqueous extract of *B. pinnatum*. The tracheal rings were precontracted with 10<sup>-5</sup> M histamine or 2 x 10<sup>-6</sup> M carbachol. a, b, c, d represent organ bath concentrations of 0.125, 0.25, 0.5, and 1.0 mg/ml respectively.

Effect of extract pretreatment on tracheal ring responses to cumulative concentrations of spasmogens

Comparative effects of cumulative concentrations of histamine and carbachol on tracheal rings obtained from guinea pigs in all the four groups are shown in

Figure 2. Maximum response ( $E_{max}$ ) was highest in the sensitized group. Rings obtained from extract-pretreated (200 or 400 mg/kg/day x 21) guinea pigs showed significantly ( $p < 0.0001$ ) attenuated responses to either histamine or carbachol compared to those from sensitized and non-sensitized groups.

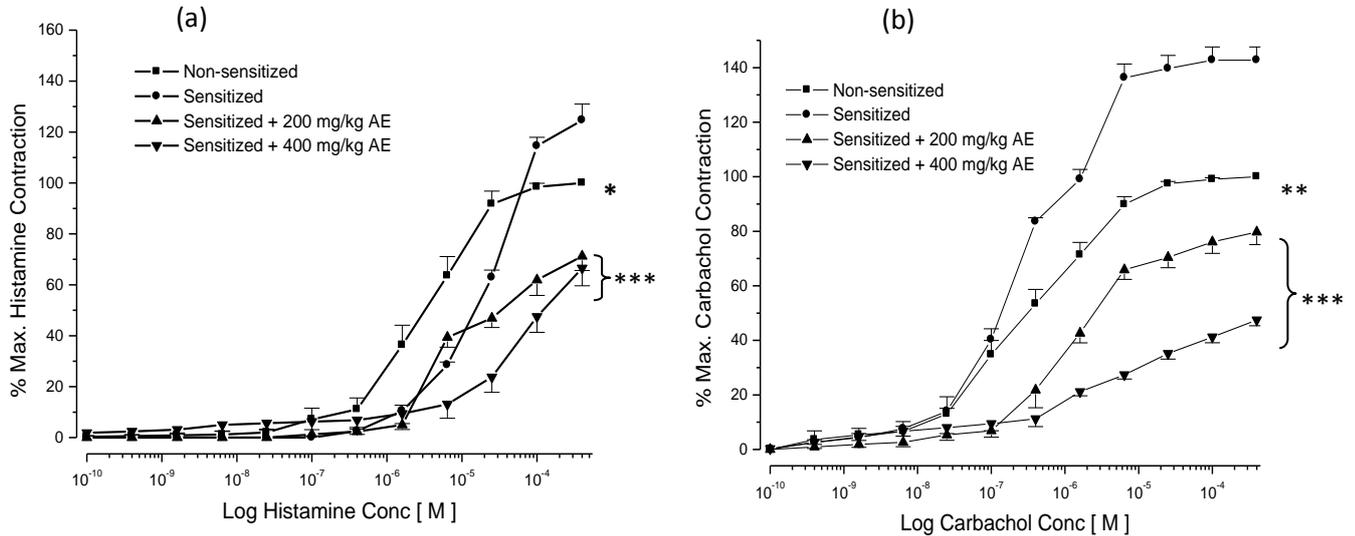


Figure 2.

Effects of cumulative concentrations of histamine (a) and carbachol (b), on responses by tracheal rings obtained from control, ovalbumin-sensitized, 200 mg/kg/day (x21) and 400 mg/kg/day (x21) aqueous extract (AE) of *B. pinnatum*-treated guinea pigs. \* $p < 0.05$  compared to sensitized; \*\*\* $p < 0.0001$  compared to sensitized and non-sensitized; \*\* $p < 0.001$  compared to sensitized.  $n = 5$  per group.

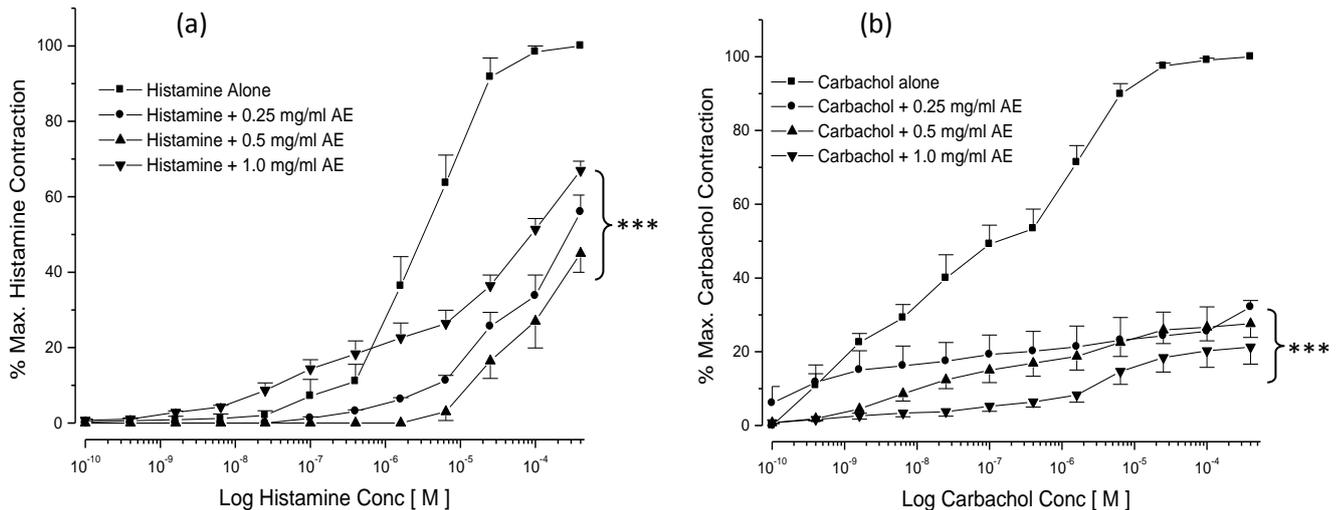


Figure 3.

Effects of cumulative concentrations of histamine (a) and carbachol (b) on isolated tracheal rings obtained from control guinea pigs. The spasmogens were applied in the presence or absence of different concentrations of the aqueous extract (AE) of *B. pinnatum*. All concentrations of the extract significantly attenuated the maximum responses by tracheal rings. \*\*\* $p < 0.0001$  compared to histamine or carbachol alone.  $n = 5$  per group

There was no statistically significant difference between maximum histamine-induced responses in rings from 200 and 400 mg/kg/day x 21 groups (Figure 2a). In carbachol-induced responses (Figure 2b), rings from 400 mg/kg/day x 21 extract-pre-treated group showed the lowest response which were significantly different ( $p < 0.05$ ) from responses produce by the 200 mg/kg/day x 21 group. Table 1 shows that in histamine-induced contractions,  $pD_2$  values were significantly lower in the 400 mg/kg/day x 21 group compared to the non-sensitized group. In carbachol-induced contractions, 200 and 400 mg/kg/day x 21 extract-treated groups had significantly ( $p < 0.05$ ) lowered  $pD_2$  values compared to the other two groups (Table 1).

*Effect of different concentrations of extract on tracheal ring responses to cumulative concentrations of spasmogens*

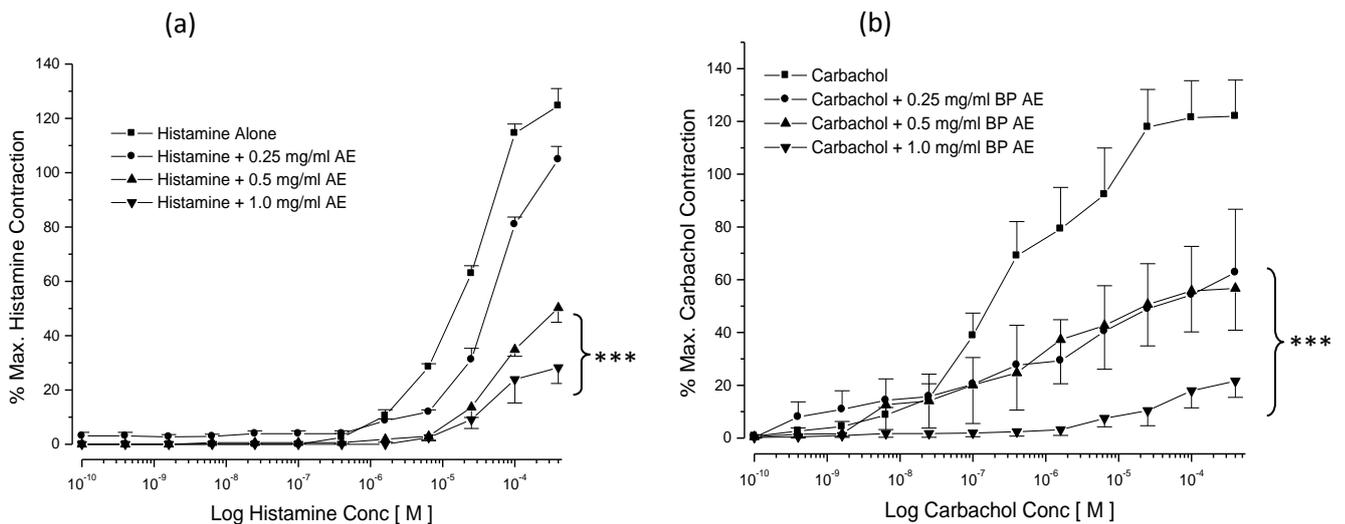
The pattern of response by tracheal rings obtained from non-sensitized guinea pigs to cumulative concentrations of histamine and carbachol in the presence of different organ bath concentrations of the extract is shown in Figure 3. Although lower concentrations of 0.25 and 0.5 mg/ml of the extract appeared to be more effective, the three concentrations significantly ( $p < 0.0001$ ) attenuated tracheal rings responses to histamine (Figure 3a). The attenuating effect of the extract on cumulative concentrations of carbachol (Figure 3b) was significant ( $p < 0.0001$ ) and independent of its concentration in the organ bath.

Table 1.

Maximum contractile responses ( $E_{max}$ ) and the  $-\log$  of the concentrations producing 50% of the  $E_{max}$  ( $pD_2$ ) of isolated guinea pig tracheal rings exposed to cumulative concentrations of histamine or carbachol.

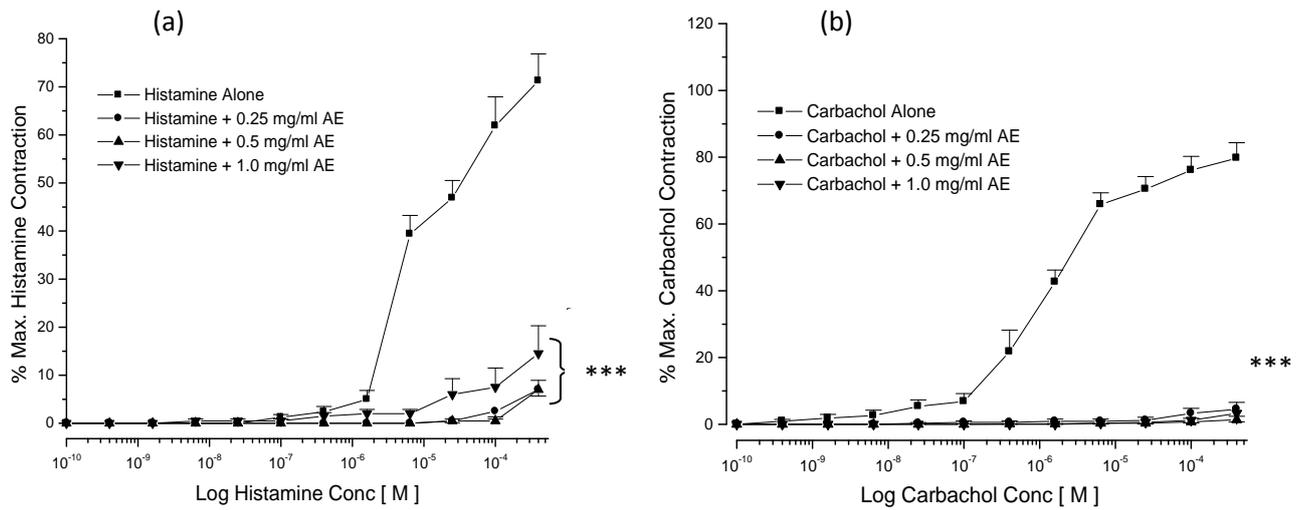
	Histamine		Carbachol	
	$E_{max}$	$pD_2$	$E_{max}$	$pD_2$
<b>Non-sensitized</b>	100.00 ± 0.00	5.50 ± 0.40	100.00 ± 0.00	6.84 ± 0.35
<b>OA-sensitized</b>	124.63 ± 6.35	4.67 ± 0.14	142.73 ± 4.88	6.50 ± 0.02
<b>OA-sensitized + 200 mg/kg/day x21</b>	71.25 ± 5.61***	5.20 ± 0.03	79.74 ± 4.61***	5.98 ± 0.07*
<b>OA-sensitized + 400 mg/kg/day x21</b>	66.50 ± 6.88***	4.44 ± 0.24*	47.46 ± 2.13***	5.39 ± 0.06**

For  $E_{max}$ , \*\*\* $p < 0.0001$  compared to non-sensitized and ovalbumin (OA)-sensitized. For  $pD_2$ , \* $p < 0.05$  compared to non-sensitized; \*\* $p < 0.002$  compared non-sensitized and OA-sensitized. n = 5 per group.



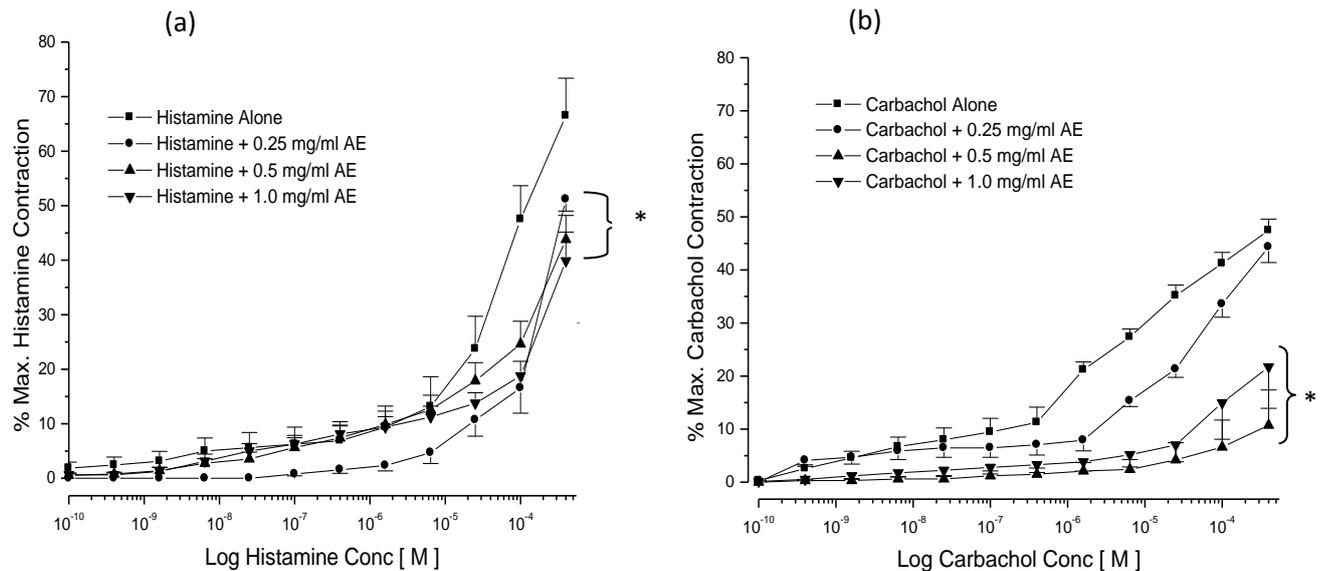
**Figure 4.**

Effects of cumulative concentrations of histamine (a) and carbachol (b) on isolated tracheal rings obtained from ovalbumin-sensitized guinea pigs. The drugs were applied in the presence or absence of various concentrations of the aqueous extract of *B. pinnatum*. Concentrations of the extract significantly attenuated the maximum tracheal ring responses. \*\*\* $p < 0.0001$  compared to histamine or carbachol alone. n = 5 per group.



**Figure 5.**

Effects of cumulative concentrations of histamine (a) and carbachol (b) on responses by tracheal rings obtained from ovalbumin-sensitized by 200 mg/kg/day (x21) aqueous extract (AE) of *B. pinnatum*-treated guinea pigs. The contractile agents were applied in the presence or absence of different concentrations of AE. Concentrations of the extract significantly attenuated the maximum tracheal ring responses. \*\*\* $p < 0.0001$  compared to histamine or carbachol alone.  $n = 5$  per group.



**Figure 6.**

Effects of cumulative concentrations of histamine (a) and carbachol (b) on responses by tracheal rings obtained from ovalbumin-sensitized but 400 mg/kg/day (x21) aqueous extract (AE) of *B. pinnatum*-treated guinea pigs. The drugs were applied in the presence or absence of different concentrations of the extract. Concentrations of the extract significantly attenuated the maximum tracheal ring responses. \*\*\* $p < 0.01$  compared to histamine alone or compared to carbachol alone and carbachol + 0.25 mg/ml extract.  $n = 5$  per group

Figure 4 shows that the presence of the extract significantly attenuated responses of tracheal rings obtained from ovalbumin-sensitized guinea pigs.

Reduction in histamine-induced responses was significant ( $p < 0.0001$ ) in the presence of 0.5 and 1.0 mg/ml of the extract (Figure 4a). All three

concentrations were not significantly different in their attenuating effect on carbachol-induced responses (Figure 4b).

Tracheal rings obtained from sensitized guinea pigs which were also given 200 mg/kg/day x 21 and then exposed to organ bath concentrations of the extract showed significantly ( $p < 0.0001$ ) lesser contractile responses compared to responses in the absence of the extract. Results obtained with histamine-induced contractions (Figure 5a) were similar to those of carbachol (Figure 5b).

The different organ bath concentrations of the extract also significantly ( $p < 0.05$ ) attenuated histamine-induced responses by rings obtained from 400 mg/kg/day x 21 extract-treated guinea pigs (Figure 6a). In carbachol-induced contractions, organ bath concentrations of 0.5 and 1.0 mg/ml significantly reduced tracheal ring responses.

## DISCUSSION

Agents that are used in the management of asthma may act by inducing bronchodilatation, inhibiting responses to spasmogens and preventing inflammation and secretions (Gordon and Lazarus, 2009; Lai and Rogers, 2010). The present study has shown that the aqueous leaf extract of *B. pinnatum* possesses effect on the isolated tracheal rings. While the extract was able to attenuate contractile responses to cumulative organ bath concentrations of histamine and carbachol, it was unable to induce relaxation in tracheal rings precontracted by the spasmogens. Attenuation of responses occurred independently of the animal group from which the tracheal rings were obtained, although it was more pronounced in rings obtained from extract-pretreated animals. Also, the inhibitory effect of the extract on tracheal ring contractility almost always depended on its organ bath concentrations. Sensitization of guinea pig is a model of asthma in which the animals become hyper-responsive to spasmogens (Bramley et al., 1995; Kucharewicz et al., 2008). Sensitized animals have higher levels of IgE which are activated when the animals are exposed to provocative agents (Kucharewicz et al., 2008; Choi et al., 2009). This explains the higher responses by rings obtained from ovalbumin-sensitized animals to cumulative organ bath concentrations of the spasmogens. However, attenuating effect of the extract on tracheal ring

responsiveness to the spasmogens was irrespective of whether the animal had been sensitized or non-sensitized. The results obtained from non-sensitized guinea pigs, suggest that the extract could prevent bronchospasms if used by non-asthmatic humans.

Drugs used in the management of asthma are often given daily as prophylactics against acute attacks (Udem, 2006). This informed the treatment of the guinea pigs with doses of 200 mg/kg/day x 21 or 400 mg/kg/day x 21 of the extract within the period of sensitization. Pretreatment of animals is a protocol often used for studying the prophylactic properties of herbal extracts (Ladenius and Nijkamp, 1993; Ozolua et al., 2009). Results from this study suggest that prophylactic treatment with the extract can reduce airway narrowing which follows exposure to spasmogens.

The mechanism responsible for the antispasmodic effect of extract in the tracheal rings is not clear. The extract has been shown to possess anti-inflammatory properties (Ojewole, 2005). Although inflammation is a component of chronic asthma the ability of the extract to equally attenuate responses by rings obtained from non-sensitized guinea pigs suggests that mechanisms other than inhibition of inflammation may be involved. The extract is known to be rich in flavonoids (Okwu and Josiah, 2006; Yamagishi, 1989). These secondary plant metabolites have been associated with various smooth muscle relaxant and antioxidant effects (Cishek et al., 1997; Ajay et al., 2003). They are known to inhibit  $Ca^{2+}$  release and utilization mechanisms in smooth muscles (Gordon and Lazarus, 1988; Ghayur and Gilani, 2006). The inflammatory process in asthma and airway hyper-responsiveness are often associated with the generation of oxygen radicals. Flavonoids are very potent antioxidants (Romieu and Trenga, 2001; Tapiero et al., 2002; Raviv and Smith, 2010). Flavonoids present in the leaf extract (Okwu and Josiah, 2006; Yamagishi, 1989) may also play a role in mopping up these oxygen radicals.

Histamine and carbachol stimulate  $H_1$  and  $M_3$  receptors respectively on the tracheal smooth muscle cells culminating in a common pathway for the release of intracellular  $Ca^{2+}$  which is the forerunner of contraction. Although the use of  $H_1$  receptor blockers has not been popular in the management or prophylaxis of asthma (Bousquet et al., 1992; Lordan

and Holgate, 2002 ), drugs such as ipratropium which block muscarinic receptors are used in the management of the disease (Proskocil and Fryer, 2005).

In conclusion, the present study has shown that the aqueous leaf extract of *B. pinnatum* possesses antispasmodic effect on tracheal smooth muscle cells. This action lends credence to the use of the extract for the prophylaxis of asthma in ethnomedicine.

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