

## Original Article

# Bronchospasmolytic activity of the extract and fractions of *Asystasia gangetica* leaves

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**Summary:** The bronchospasmolytic constituent of leaves of *Asystasia gangetica* (L.) T. Anderson, sub-specie micrantha (Nees) Ensermu (Acanthaceae) was isolated by bio-activity-guided technique. The bronchospasmolytic effect of the fractions of the leaf extract as well as the isolate AG-1 was evaluated using histamine-induced contraction of the guinea pig trachea and pre-contracted trachea (pathological tissue). The results showed that the fractions and AG-1 inhibited contractions of the guinea pig trachea induced by histamine in a dose dependent manner. The isolated constituent, (AG-1) caused 82% inhibition of maximal contraction produced by histamine at a concentration of 400µg/ml. On histamine (8µg/ml) pre-contracted trachea, cumulative doses of the fractions evoked a dose dependent relaxation. Phytochemical analysis showed that the isolated compound (AG-1) tested positive to terpenoids while the fractions contained typical constituents such as carbohydrates, alkaloids, saponins, steroids, flavonoids and glycosides. These findings suggest that the usefulness of leaves of *A. gangetica* in the treatment of asthma may derive from bronchospasmolytic effect of terpenoid compounds in the leaves.

**Industrial relevance:** Asthma is currently a worldwide problem, with increasing prevalence in both children and adults; a prevalence rate of 5 – 10% has been reported for Nigeria. Drugs used in the management include bronchodilators which are short-term relievers and anti-inflammatory drugs which are long-term controllers. Despite the availability of oral and inhaled medications, the prevalence of asthma is on the rise (NHLBI/WHO 1995). The challenge of developing new effective, safe and long lasting antiasthmatic drugs from natural products appears inevitable. The leaves of *Asystasia gangetica* L. (T). (Acanthaceae), a traditional anti-asthma remedy, offer great potential for the development of a novel anti-asthmatic agent. The leaves have been shown to possess antihistaminic, bronchospasmolytic and anti-inflammatory properties. The aim of this research was to isolate and pharmacologically characterize the anti-asthmatic constituent of the leaves of this plant using bioactivity-guided fractionation of the leaf extract.

*Keywords:* *A. gangetica*, leaf extracts, bronchospasmolytic, anti-asthma, terpenoids.

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## Introduction

*Asystasia gangetica* (L.) T. Anderson, sub-specie micrantha (Nees) Ensermu (Acanthaceae) is an attractive, fast-growing, spreading, herbaceous groundcover that grows from 300 – 600 mm in height. It has green, oval-shaped leaves with rounded base occurring in opposite pairs. The flower is white – cream coloured with purple markings and the fruit is a club shaped capsule, splitting from tip to base (Saunders 1958). It is widely distributed from tropical Asia to Africa including Nigeria (Elliot 2004; GRIN 2007).

In the traditional medicine of East Africa, *A. gangetica* is used as an anthelmintic (Kokwaro 1976); while in Nigeria, the leaves are popularly used in the management of asthma.

Pharmacological studies have shown that the leaves of this plant possess bronchospasmolytic and anti-inflammatory properties (Akah et al., 2003). The leaf extracts inhibited histamine- and serotonin-induced contractions of the guinea pig trachea (Akah et al., 2003). Although the isolation of the phytochemical constituents of this plant is yet to be documented, the leaves have been shown to contain large amounts of proteins; as well as amino acids, minerals, carbohydrate, lipids and fibre (Yeoh & Wong 1993).

In our earlier study, we demonstrated the bronchospasmolytic effect of the leaf extracts as the probable mechanism underlying the anti-asthmatic effect of the plant's leaves (Akah et al., 2003). In asthma, bronchoconstriction or intermittent airway constriction is a major hallmark of the disease, giving rise to the characteristic symptoms of wheezing, coughing, chest tightness and shortness of breath (NHLBI/WHO 1995). Bronchoconstriction is the main component of the immediate phase of asthmatic response on exposure to allergen (Rang et al., 2007; Barnes 1996). Spasmolytic effect or relaxation of bronchial smooth muscles may provide relief in diseases of the airways (such as asthma) where spasm of bronchial smooth muscles is the major cause of increased airway resistance. Currently available therapeutic options employed in the treatment of bronchoconstriction include bronchodilators such as  $\beta_2$  agonists,

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muscarinic receptor antagonists and methylxanthines (Udem 2006). Besides their role in the management of bronchoconstriction in asthma, these bronchodilators are also the cornerstone of therapy in chronic obstructive pulmonary disease (Greene & Harris 2000).

Though some bronchodilators are known to have additional beneficial effects like increased mucociliary clearance, anti-inflammatory effect (Greene & Harris 2000; Barnes 2003), the effects of these bronchodilators are limited in clinical practice, because of their adverse effect profile (Udem 2006). Also, the prevalence of asthma is rapidly increasing around the world, especially in young children, and it has become a significant cause of morbidity and mortality in developed countries (Braman 2006). Therefore, there is a need for new or alternative approaches to the control of asthma and the development of better and safer drugs. On the basis that many useful anti-asthmatic drugs such as cromoglycate and theophylline, are of plant origin, herbal medicine should be a promising direction for the current search of improved anti-asthmatic drugs.

The acclaimed effectiveness of the leaves of this plant in traditional treatment of asthma and our earlier demonstration of its bronchospasmolytic property (Akah et al., 2003) stimulated our interest in further studies on this plant. The present study was therefore undertaken to isolate and characterize the potential bronchospasmolytic constituent(s) of the leaves using bioactivity - guided fractionation technique.

### Materials and Methods

**Collection and preparation of plant material:** Fresh leaves of *Asystasia gangetica* were collected in November at Orba, Enugu State, Nigeria and authenticated by Mr. A. Ozioko of Bioresources Development and Conservation Programme (BDPC) Centre, Nsukka. The leaves were sun-dried for 2 days and pulverized to coarse powder using a hand blender.

**Extraction of Plant Material:** About 15 kg of the powdered leaves was extracted by cold maceration with methanol for 48 h. The extract obtained was concentrated in a rotavapor under reduced pressure and completely dried over a regulated water bath maintained at 60°C to afford 726.93 g of the methanol extract (ME).

**Bioactivity-guided fractionation of ME:** The ME (540 g) was fractionated in a silica gel (70-230 mesh) column successively eluted with hexane: ethyl acetate (7:3) and methanol (100%). The fractions were collected in aliquots of 10 ml and pooled to obtain four broad fractions- A, B (16.41 g; 3.04% w/w), C and D. Screening of the four fractions for antihistaminic and bronchospasmolytic activities, using inhibition of histamine-induced contraction of the guinea pig trachea and relaxation of guinea pig trachea pre-contracted with histamine respectively as bioactivity guides, showed that fraction B exhibited the greatest inhibition of contractions of the trachea induced by histamine and relaxation of the pre-contracted trachea. Based on this result, fraction B (5.4 g) was further separated using a gradient solvent mixtures of hexane : ethyl acetate (9.5 : 0.5; 9 : 1; 8 : 2; 7 : 3, 6 : 4, 5 : 5, 0 : 1) to obtain eight fractions (I, II, III, IV, V, VI, VII, VIII). Further activity-guided tests on these fractions revealed greatest potency in fraction VIII (400 mg; 7.41% w/w), which on further chromatographic separation (69 mg) using petroleum ether: ethyl acetate (7:3) yielded a brown amorphous powder (AG-1; 30 mg; 43.48% w/w).

**Phytochemical analysis:** The extracts were subjected to phytochemical analysis for identification of constituents using standard procedures (Trease & Evans 1983; Harborne 1998).

**Studies on the guinea pig trachea:** Guinea pigs housed in the laboratory animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, for at least two weeks prior to the study were used. All animal experiments were in compliance with National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

**Effect of fractions and AG-1 on histamine-induced contractions of guinea pig trachea:** Guinea pigs of either sex (250-500 g), starved overnight, but allowed free access to water were used. The animals were killed by a blow on the head and exsanguinated. The trachea was dissected and cut along its length on the dorsal surface. Incomplete transverse cuts were made between the segments of cartilage to produce a zig-zag strip (Akah et al., 2003). The isolated trachea was mounted in a 10 ml organ bath containing Tyrode solution maintained at 37°C and gassed with air (Akah et al., 1997). The tissue was left to equilibrate for 60 min during which the bath solution was replaced every 10 min. At the end of the equilibration period, histamine (8 µg/ml) induced contractions as well as the effect of the extracts and fractions (0.4 – 6.4 mg/ml) on the contractions produced by histamine were recorded on an Ugo Basile Microdynamometer Recorder 7004. The tissues were bathed in the test substances for 5 min before the addition of histamine. A drug-tissue contact time of 75 s was observed.

**Effect of fractions and AG-1 on guinea pig trachea pre-contracted with histamine (pathological tissue):** The guinea pig trachea was prepared and mounted as described above. The trachea was contracted with 8 µg/ml histamine. After 2 min and without washing off, cumulative doses (2 – 64mg) of fraction B, fraction VIII or Control (3% v/v Tween 85) were added at 2 min intervals and the effects recorded on an Ugo Basile Microdynamometer Recorder 7004.

**Statistical analysis:** The results were analysed using One Way Analysis of Variance (SPSS version 13).

### Results and Discussion

The pathophysiology of asthma is marked by two phases, namely; an immediate phase on exposure to allergen consisting mainly of bronchoconstriction and a late phase consisting of a special type of inflammation, comprising vasodilation, airway wall edema, mucus secretion and airway hyper-responsiveness caused by inflammatory mediators (Chung 1993; Barnes 1996; Rang et al., 2007). Histamine, the first mediator implicated in the pathophysiological changes in asthma, mimics several features of the disease (Barnes et al., 1998) including bronchoconstriction, airway mucus secretion, plasma exudation and activation of cholinergic nerves. Evaluation of the effect of the fractions and AG-

1 on histamine-induced contractions of the guinea pig trachea showed that they exhibited a dose-dependent inhibition of contractions of the guinea pig trachea induced by histamine. The isolated constituent, (AG-1) caused 82% inhibition of maximal contraction produced by histamine at a concentration of 400 µg/ml. The inhibitory potency of the extract and fractions increased in the order of magnitude B< VIII<AG-1 as indicated by their IC<sub>50</sub> values representing the concentration of the fractions which inhibited 50% of the maximal contraction produced by histamine (Table 2 and Fig. 1). On the pre-contracted trachea, cumulative doses of the fractions also exhibited dose-dependent relaxation of the guinea pig trachea pre-contracted with histamine (8 µg/ml) (Table 3 and Fig.2). The bronchospasmolytic activity exhibited by the ME is consistent with our earlier finding (Akah et al., 2003).

Bronchodilation is strictly mediated by a variety of mechanisms such as, stimulation of β<sub>2</sub> adrenergic receptors, antagonism of muscarinic receptors, and direct relaxation of the bronchial smooth muscle (Udem 2006). Constituents of leaf extracts of this plant including the isolate, AG-1, may not possess agonistic action at β adrenergic receptors since they did not elicit direct relaxation of normal smooth muscles of the airway (Akah et al., 2003). Also the fact that they may lack β agonist effect implies that the use of the leaves in the management of acute symptoms of asthma may be bereft of the adverse effects associated with β agonists such as increased heart rate, cardiac arrhythmias, tremor and anxiety (Udem 2006).

**Table 1.** Phytochemical constituents of *A. gangetica* extractives

Phytochemical constituent	B (3.04%)	VIII (7.41%)	AG-1 (43.48%)
Carbohydrates	-	-	-
Alkaloids	-	-	-
Tannins	-	-	-
Steroids	-	+	-
Saponins	-	-	-
Flavonoids	+	+	-
Reducing sugars	Trace	Trace	-
Terpenes	+	+	+

Values in parenthesis represent extractive yield calculated relative to the weight of the starting material; + = present; - = absent.

**Table 2.** IC<sub>50</sub> values of fractions and AG-1 inhibiting histamine-induced contraction of the guinea pig trachea

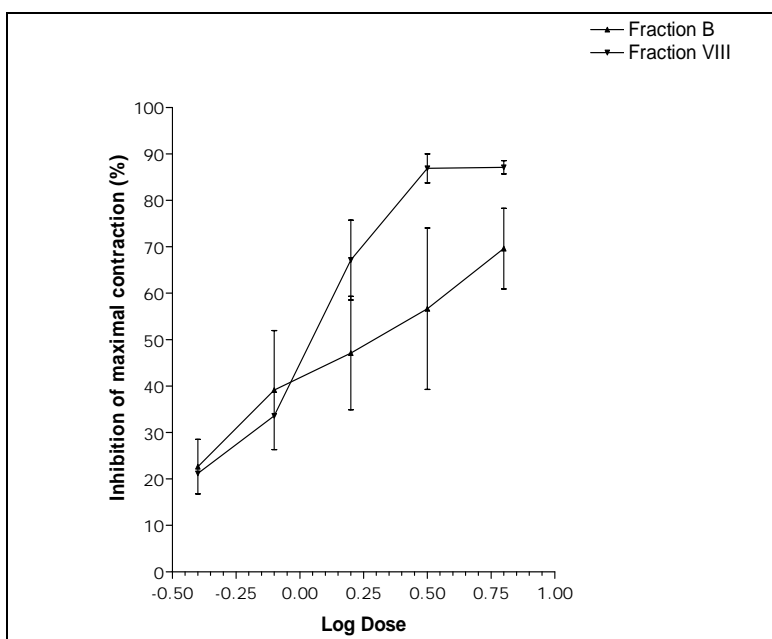
Extract / fraction	IC <sub>50</sub> (mg/ml)
B	1.91
VIII	1.10
AG-1	<sup>a</sup>

<sup>a</sup> A concentration of 0.4 mg/ml produced 82% inhibition of maximal contraction induced by histamine; IC<sub>50</sub> = concentration of extract and fraction that caused 50% inhibition of contraction

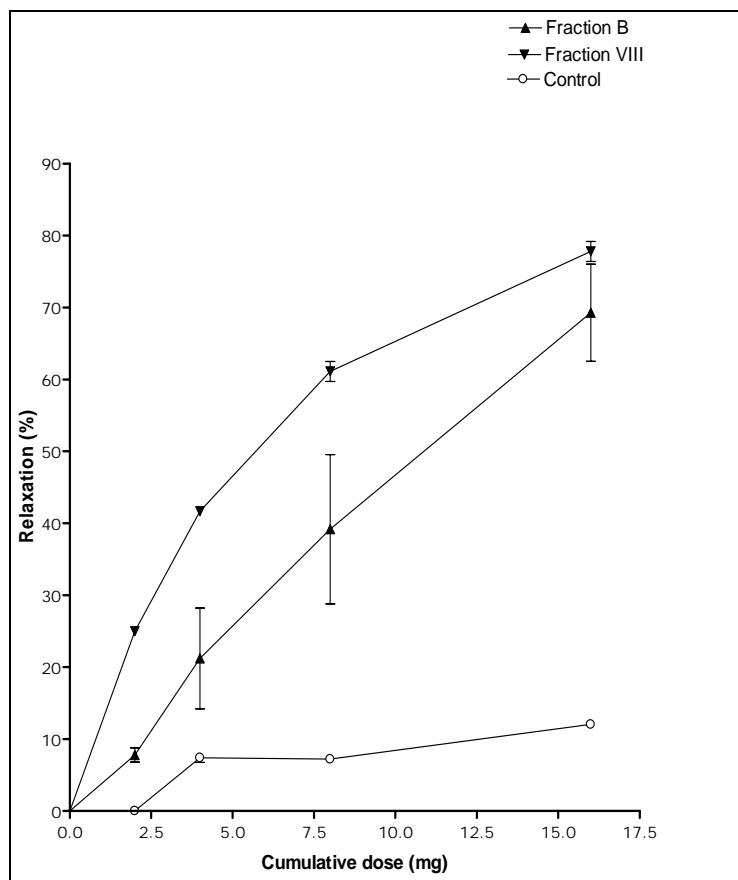
**Table 3.** Relaxant effect of cumulative doses of fractions on guinea pig trachea pre-contracted with histamine

Fraction	Relaxation (%) of Pre-contracted trachea					
	2 mg	4 mg	8 mg	16 mg	32 mg	64 mg
B	7.79 ± 0.10*	21.21 ± 7.00*	39.18 ± 10.30*	69.20 ± 6.75*	93.94 ± 3.50*	115.63 ± 0.77*
VIII	25.04 ± 0.00*	41.60 ± 0.00*	61.10 ± 1.39*	77.70 ± 1.39*	86.11 ± 1.39*	100 ± 0.00*
Control	0.00	7.39 ± 0.64	7.22 ± 0.55	12.04 ± 0.46	13.77 ± 1.61	30.03 ± 3.74

\*P< 0.05 compared to negative control (One Way ANOVA; LSD post hoc)



**Figure 1.** Effect of fractions B and VIII on contractions of guinea pig trachea induced by histamine



**Figure 2.** Relaxant Effect of fractions B and VIII on contractions of guinea pig trachea pre-contracted with histamine

Although histamine-induced contraction of the trachea is known to be mediated by H<sub>1</sub> receptors, results of our earlier study also showed that the leaf extracts inhibited serotonin- and acetylcholine- induced contractions of the trachea (Akah et al., 2003) thus suggesting that constituents of the extracts may not be specific antagonists of histamine. Histamine and serotonin are among the mediators released during the acute inflammatory response associated with asthma. Hence, extractives of this plant may as well provide relief in symptoms of asthma where these mediators are implicated.

The relaxant effect of the extract and fractions on pre-contracted trachea (pathological tissue) further reinforces the bronchospasmolytic action and implies the potential usefulness of the constituents of these extracts in relaxing an already constricted tracheobronchial tree.

Phytochemical analysis of the extractives showed the presence of typical plant constituents like carbohydrates, alkaloids, steroids, saponins, flavonoids, reducing sugars and terpenes (Table 1). However, the presence of terpenoids in the extractives suggests that this class of constituents may be responsible for the pharmacological activities. Phytochemical analysis also showed that the isolate, AG-1 tested positive to terpenoids.

### Conclusion

In conclusion, the terpenoid compounds in the leaves of *A. gangetica* are likely responsible for the usefulness of the plant's leaves in the management of asthma in traditional medicine practice. The anti-asthmatic effect of the plant's leaves may derive from the bronchospasmolytic action of these terpenoids.

Studies to elucidate the mechanism of the bronchospasmolytic effect, as well as the structure and precise identity of AG-1 are ongoing

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