

# PHYTOCHEMICAL COMPOSITION AND EFFECT OF AQUEOUS EXTRACT OF *STRUCHIUM SPARGANOPHORA* (L) ON COCKROACH CRUDE EXTRACT – INDUCED AIRWAY INFLAMMATORY RESPONSES IN WISTAR RATS

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## ABSTRACT

Asthma, a chronic immune inflammatory disorder of the airways has been responsible for high mortality in both infants and adults in Africa and appropriate therapy has long been sought. Phytochemical composition and effect of aqueous extract of *Struchium sparganophora* on pulmonary inflammation induced in Wistar rat asthma model were studied. Pulmonary inflammation was induced in Wistar rats against appropriate controls with cockroach (*Periplaneta americana*) crude extract. Twenty-four hours after asthma induction treatment, *S. sparganophora* extract was administered by oral gavage to induced rats. The inflammatory responses and effect of the extract were assessed by analysis of leukocytes infiltration in bronchoalveolar lavage fluid at 12 hourly intervals. The aqueous extract of *S. sparganophora* contained alkaloids, flavonoids, steroids, glycosides, polyphenols, saponins, tannins etc. The aqueous extract significantly ( $p < 0.001$ ) reduced inflammation indices so that mean total leukocytes ( $\times 10^6$ ) of cockroach crude extract-induced rats administered with *S. sparganophora* aqueous extract were  $5.7 \pm 0.18$ ,  $4.4 \pm 0.24$ ,  $3.4 \pm 0.19$  and  $3.0 \pm 0.31$  respectively at the different times post aqueous extract administration. These values were significantly ( $P < 0.001$ ) lower compared with those in the placebo rats viz:  $16.9 \pm 0.31$ ,  $15.0 \pm 0.37$ ,  $15.1 \pm 0.87$  and  $14.4 \pm 0.31$ . Similarly, mean percentages of neutrophils, lymphocytes, and eosinophils in cockroach crude extract-induced rats administered with *S. sparganophora* crude extract were significantly ( $p < 0.01$ ) lower compared with means in cockroach crude extract-induced rats administered with distilled water (placebo). The reduction of inflammation in rats by *S. sparganophora* extract administration may be due to their phytochemical components. These are discussed in relation to their significance in asthma management.

**KEYWORDS:** Phytochemical, *S. sparganophora* (L), inflammation, rats, asthma.

## INTRODUCTION

*Struchium sparganophora* (L) is a tropical leaf vegetable of the family asteraceae (Abbiw, 1990). This herb has long been one of the neglected plants in the traditional practices of the tropics although earlier reports show that the plant has been used in Ghana as fodder for animals, as spinach in human diet and in the treatment of migraine when applied as poultice, pressed or compressed to the head or temple (Abbiw, 1990). It is also used in soup preparation in some parts of South-eastern Nigeria and it contains some levels of ascorbic acid (Falade *et al.*, 2004). In Nigeria, the leaves are effective in the alleviation of symptoms of asthma, although this has not been scientifically proven.

Asthma is a chronic inflammatory disorder of the airways in which many cells play a role, causing recurrent episodes of wheezing, breathlessness, chest tightness, and cough particularly at night or in early morning (Global Strategy for Asthma Management and Prevention, 1995). Chronic airways inflammation is a characteristic feature of asthma disease and is usually marked by prominent infiltration of eosinophils, neutrophils, lymphocytes and monocytes in the bronchial airways of patients (Ohashi *et al.*, 1992; Campbell *et al.*, 1998; Willskarp, 1999; Chen *et al.*, 2001; Kim *et al.*, 2001). Cockroach allergen-induced pulmonary airway inflammation has been demonstrated in animal models of asthma including mice (Campbell *et al.*, 1998; Kim *et al.*, 2001), guinea pigs (Zhou *et al.*, 1998; Chen *et al.*, 2001) and humans (Howarth, 1998).

Herbal – based therapies have been increasingly reported in the management of asthma and other allergic diseases in many parts of the world. These therapies include treatment with flavonoids especially quercetin (Middleton and

Kandaswami, 1992), extracts of the plant species *Tylophora indica* (Gupta *et al.*, 1979), *Coleus forskohlii* (Kreutner *et al.*, 1985), *Boswellia sp.* (Gupta *et al.*, 1998) and *Boerhaavia diffusa* (Taylor, 2004). Other reports show that in China, herbal therapies commonly used in management of asthma and other allergies include 'Ma huang', Ginseng, Cinnamon, Bromelin, *Gingko biloba*, Lacorice, Bu pleurum and nettle root extract. Most of these are known to reduce or inhibit airway inflammatory response (Simon, 2004). For instance, quercetin inhibited histamine release in human basophil cell and rat mast cells (Fewtrell and Gomperts, 1977; Middleton *et al.*, 1981) and inhibited neutrophil lysosomal enzyme secretion (Showell *et al.*, 1981; Bennett *et al.*, 1981). Airway inflammation in human asthmatic patients has been found to be inhibited by extracts of *Boswellia serrata* (Gupta *et al.*, 1998) and *Tylophora indica* (Gupta *et al.*, 1979). Similarly, extract of *Boerhaavia diffusa* inhibited inflammatory response in rats (Taylor, 2004).

There is need to study the effect of *S. sparganophora* extract in the inflammatory response in asthma, this may add to the list of herbal therapies effective in inhibiting airways inflammation and hence in the management of asthma. This study set out to examine the phytochemical composition of *S. sparganophora* (L), and in addition, to assess the effect of administration of its aqueous extract in bronchial airways inflammation induced in Wistar rat model by cockroach crude extract.

## MATERIALS AND METHODS

The experiments were performed in the Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar, Nigeria

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### Preparation of aqueous extract of *Struchium sparganophora*

Fresh plants of *S. sparganophora* (L) herb were collected from the swamp behind the staff quarters of Cross River University of Technology, Calabar, Nigeria. They were brought in a polythene bag to the Department of Botany, University of Calabar, Calabar and identified. Leaves were washed with distilled water and allowed to drain of water, after which 100g were crushed into a paste in a mortar using pestle for 25 minutes. The paste was put in a cheesecloth and squeezed to extract all the liquid from it. The liquid obtained weighed 25g and was preserved in a clean sterile sample bottle of 100ml in a refrigerator at -4°C until used.

### Analysis of Phytochemical composition of aqueous leaf extract of *S. sparganophora*

*S. sparganophora* aqueous extract was subjected to phytochemical analysis using standard laboratory methods (Harbone, 1998). The following tests were performed on aliquots of the aqueous extract: Test for alkaloids (Safowora, 1982, modified by Harbone, 1998), test for flavonoids, test for tannins, test for glycosides, test for saponins, test for polyphenol, test for phlobatins, test for anthraquinones and test for hydroxymethyl anthraquinone. Each test was repeated thrice.

### Experimental animals

Healthy female Wistar rats aged 12wk and weighing 180- 200g were obtained from the Animal Resource Centre of the Department of Zoology, University of Calabar, Calabar, Nigeria. They were housed in plastic cages (500cm x 250cm) with screen tops, in the Animal Resource Facility of the Department of Biochemistry, College of Medical Sciences, University of Calabar, Calabar and allowed to acclimatize for 2wk. The room was maintained under standard laboratory conditions, at room temperature of 26°C ± 2°C, relative humidity of 46%, with adequate ventilation and a 12hr light and dark cycle. Peletized growers mash for their feeding and water were provided the rats *ad libitum*. Permission for animal studies was obtained from the Animal Ethics Committee of the College of Medical Sciences, University of Calabar, Calabar.

### Preparation of cockroach crude extract

Cockroach crude extract was prepared following our method reported previously (Eko *et al.*, 2008).

### Induction of pulmonary airway inflammation in Wistar rats with cockroach crude extract

Induction of pulmonary airway inflammation in Wistar rats was done by sensitizing healthy female rats with

intraperitoneal (ip) injection of American cockroach (*Periplaneta americana*) crude extract (diluted 1: 10v/v with sterile phosphate buffered saline, PBS of pH 7.2) together with sterile PBS vehicle once, followed by two separate intratracheal challenges with the diluted cockroach crude extract.

Thirty-six healthy female Wistar rats were randomly assigned to three groups of 12 each. Rats in two groups were sensitized on day 0, with 0.5ml diluted cockroach crude extract together with 0.5ml PBS vehicle to make a total volume of 1ml. This was followed on days 14 and 21, by separate intratracheal challenges with 0.5ml cockroach crude extract each. Rats in the third group were sensitized on day 0, with 1ml of sterile PBS, followed on day 14 and 21 by intratracheal challenges with 0.5ml sterile PBS each to serve as control. The procedure used for the intratracheal challenge was as described in our previous paper (Eko *et al.*, 2008).

### Administration of aqueous extract of *S. sparganophora* on Wistar rats induced with cockroach crude extract

Administration of aqueous extract of *S. sparganophora* on induced rats was done by oral gavage using sterile orogastric tube (Bard, Georgia, 30229, USA, size 11) at 24 hours after induction, on day 22. Each rat in one of the cockroach crude extract-induced groups was administered with 0.5ml aqueous extract of *S. sparganophora*, while the second group of induced rats was administered with 0.5ml distilled water each, and used as placebo. The rats in the PBS-treated control group were administered with 0.5ml distilled water each.

### Collection and analysis of bronchoalveolar lavage (BAL) fluid at different times post *S. sparganophora* or water administration

At 12 hourly intervals up to 48 hours post administration of aqueous extract of *S. sparganophora* or distilled water, BAL fluid was collected from 3 rats separately and analysed for total leukocyte counts, and differential counts of percentage neutrophils, lymphocytes and eosinophils using the method described in our previous paper (Eko *et al.*, 2008). All the experiments were repeated twice. The experimental groupings and schedule of treatments were as shown on table 1.

### Statistical Analysis

All values were expressed as means or percentages ± SEM. Differences between treatment means were compared by analysis of variance (Chen *et al.*, 2001). When the overall F value was significant, pairwise comparison was performed between groups using students' 't' test (Onwioduokit, 2000).

**Table 1:** Experimental animal groupings and schedule of treatments

Treatment	Total No. of rats	NO. of rats analysed at different times post aqueous extract administration (hrs)			
		12hr.	24hr	36hr	48hr
Induced + administered with <i>S. sparganophora</i>	12	3	3	3	3
Induced + administered with water (placebo)	12	3	3	3	3
PBS-induced control	12	3	3	3	3

All the experiments were repeated twice

## RESULTS

### Phytochemical composition of aqueous extract of *S. sparganophora*

The phytochemical composition of aqueous extract of *S. sparganophora* is presented on table 2. The aqueous extract of the leaves contained various phytochemical components in varying relative abundance, including alkaloids,

flavonoids, steroids, tannins, phlobatins, anthraquinone and reducing compounds, which were present in medium amounts.

### Airway inflammation in bronchoalveolar lavage (BAL) fluid of Wistar rats

Assessment of airway inflammation at different times was done by estimation of total leukocyte cell counts ( $\times 10^6$ ) and percentage differential cells in the BAL fluid of the rats.

**Total leukocyte cell counts in BAL fluid at 12 hourly intervals post *S. sparganophora* aqueous extract administration**

Table 3 showed that means of total leukocyte counts ( $\times 10^6$ ) in BAL fluid of induced Wistar rats administered with aqueous extract of *S. sparganophora* were significantly ( $p < 0.01$ ) lower compared with values in induced Wistar rats administered with distilled water (placebo) at all the times. On the other hand, these values were not significantly ( $p < 0.05$ ) different compared with the values in PBS-treated control rats at all the times. Induced rats administered with distilled water (placebo) had mean values significantly ( $p < 0.001$ ) higher compared with values in PBS-treated control rats at all the times.

**Percentage neutrophil cells in BAL fluid of Wistar rats at 12 hourly intervals post *S. sparganophora* aqueous extract administration**

Table 4 showed that the mean percentage neutrophil cells in BAL fluid of induced rats administered with aqueous extract of *S. sparganophora* were significantly ( $p < 0.01$ ) lower compared with the values in induced rats administered with distilled water (placebo) at all the times. Induced rats administered with aqueous extracts of *S. sparganophora* had mean values that were not significantly ( $p < 0.05$ ) different compared with the values in PBS-treated control rats. The values in induced rats administered with distilled water (placebo) were significantly ( $p < 0.01$ ) higher compared with values in PBS-treated control rats at all the times.

**Percentage lymphocyte cells in BAL fluid of Wistar rats at 12 hourly intervals post *S. sparganophora* aqueous extract administration**

Table 4 showed that the mean percentage lymphocyte cells in BAL fluid of induced rats administered with aqueous extract of *S. sparganophora* were significantly ( $P < 0.01$ ) lower compared with the values in induced rats administered with distilled water (placebo) at all the times. Induced rats administered with aqueous extract of *S. sparganophora* had mean values that were not significantly ( $P > 0.05$ ) different compared with the values in PBS-treated control rats. Induced rats administered with distilled water (placebo) had means significantly ( $P < 0.01$ ) higher compared with means in PBS-treated control rats at all the times.

**Percentage eosinophil cells in BAL fluid of Wistar rats at 12 hourly intervals post *S. sparganophora* aqueous extract administration**

The data on table 4 showed that the mean percentage eosinophil cells in BAL fluid of induced rats administered with aqueous extract of *S. sparganophora* were significantly ( $P < 0.001$ ) lower compared with the means in induced rats administered with distilled water (placebo) at all the times. On the other hand, these means were not significantly ( $P > 0.05$ ) different compared with the mean in PBS-treated control rats at all the times. Induced rats administered with distilled water (placebo) had means significantly ( $P < 0.001$ ) higher compared with means in PBS-treated control rats at all the times.

**Table 2: Phytochemical composition of Aqueous Extract of *Struchium Sparganphora* (L)**

Phytochemical Component	Relative Presence
Alkaloids	+
Flavonoids	+
Steroids	+
Tannins	+
Phlobatinnins	+
Anthraquinone	+
Hydroxymethylantraquinone	+
Saponins	+
Glycosides	+
Polyphenols	+
Reducing compounds	+

Key:

- + Present in low amounts
- ++ Present in medium amounts
- +++ Present in large amounts

**Table 3: Total leukocyte cell counts ( $\times 10^6$ ) in BAL Fluid at different times in Wistar rats induced with cockroach crude extract (CCE) and administered with aqueous extract of *Struchium sparganophora* (L)**

Treatment	Duration (hr. post aqueous extract administration)			
	12	24	36	48
Induced + administered with <i>S. sparganophora</i> aqueous extract	5.7 ± 0.18 <sup>a,b</sup>	4.4 ± 0.24 <sup>a,b</sup>	3.4 ± 0.19 <sup>a,b</sup>	3.0 ± 0.31 <sup>a,b</sup>
Induced + administered with distilled water (placebo)	16.4 ± 0.31 <sup>c</sup>	15.8 ± 0.37 <sup>c</sup>	15.1 ± 0.87 <sup>c</sup>	14.4 ± 0.31 <sup>c</sup>
PBS-treated (control)	3.8 ± 0.14	3.4 ± 0.33	3.7 ± 0.33	3.4 ± 0.37

Values are means of 6 determinations ± SEM

- 'a' = significantly different means of induced rats administered with *S. sparganophora* aqueous extract compared with means of Placebo rats ( $p < 0.001$ )
- 'b' = no significant difference in means of induced rats administered with means of PBS-treated control rats ( $P > 0.05$ ).
- 'c' = significantly different means of placebo rats compared with means of PBS-treated control rats ( $p < 0.001$ ).

**Table 4:** Percentage differential cells in BAL fluid at different times in Wistar rats induced with cockroach crude extract and administered with aqueous extract of *S. sparganophora*

Treatment	Duration (hr post aqueous extract administration)	Differential cells		
		% Neutrophils	% lymphocytes	% Eosinophils
Induced + administered with <i>S. sparganophora</i> aqueous extract	12	10.2±0.47 <sup>b,c</sup>	4.8±0.43 <sup>b,c</sup>	4.9±0.17 <sup>a,c</sup>
	24	6.4±0.18 <sup>b,c</sup>	4.1±0.14 <sup>b,c</sup>	4.6±0.21 <sup>a,c</sup>
	36	5.6±0.27 <sup>b,c</sup>	4.0±0.17 <sup>b,c</sup>	3.1±0.11 <sup>a,c</sup>
	48	5.2±0.21 <sup>b,c</sup>	3.7±0.47 <sup>b,c</sup>	2.3±0.14 <sup>a,c</sup>
Induced + administered With distilled water (placebo)	12	21.9±0.41 <sup>e</sup>	19.2±0.62 <sup>e</sup>	20.4±0.97 <sup>d</sup>
	24	19.1±0.25 <sup>e</sup>	20.1±0.47 <sup>e</sup>	24.4±0.80 <sup>d</sup>
	36	18.7±0.47 <sup>e</sup>	19.8±0.41 <sup>e</sup>	22.4±0.64 <sup>d</sup>
	48	18.4±0.54 <sup>e</sup>	19.4±0.58 <sup>e</sup>	21.1±0.24 <sup>d</sup>
PBS-treated control	12	6.1±0.19	4.4±0.22	2.2±0.15
	24	5.9±0.31	4.7±0.33	2.1±0.15
	36	5.6±0.37	4.3±0.47	2.7±0.42
	48	5.9±0.31	4.6±0.39	2.4±0.37

Values are means of 6 determinations ± SEM

- 'a' = significantly different means of induced rats administered with *S. sparganophora* extract compared with means of placebo rats ( $p < 0.001$ ).  
 'b' = significantly different means of induced rats administered with *S. sparganophora* extract compared with means of placebo rats ( $p < 0.01$ ).  
 'c' = no significant differences in means of induced rats administered with *S. sparganophora* extract compared with means of PBS treated control rats ( $P > 0.05$ ).  
 'd' = significantly different means of placebo rats compared with means of PBS-treated control ( $P < 0.001$ ).  
 'e' = significantly different means of placebo rats compared with means of PBS-treated control ( $P < 0.01$ ).

## DISCUSSION

Administration of aqueous extract of *Struchim sparganophora* (L) to cockroach crude extract-induced Wistar rats reversed the pulmonary inflammation shown in BAL fluid of induced rats administered with distilled water (placebo). The marked elevated mean total leukocyte cell counts, mean percentages of neutrophils, lymphocytes and eosinophils in BAL fluid was reversed to levels similar to those in PBS-treated control rats. There was a progressive time-related reduction in the inflammatory cells in BAL fluid of induced rats administered with aqueous extract of *S. sparganophora* beginning from 12 up to 48 hours post administration of the aqueous extract. Previous reports have shown that administration of extracts of some plant species exerted anti-inflammatory effects in asthmatic subjects. For instance, extracts of *Tylophora indica* (Gupta *et al.*, 1979), *Coleus forskohlii* (Kreutner *et al.*, 1985), *Boswellia sp* (Gupta *et al.*, 1998) and *Boerhaavia diffusa* (Taylor, 2004) were reported to exert anti-inflammatory effects in asthmatic patients and in rats. However, these reports have not shown the anti-inflammatory effects of these plant extracts on a time-related basis.

The current most potent anti-inflammatory drugs, corticosteroids, have been reported to effect a marked reduction in the mast cells, T-lymphocytes and eosinophils in bronchial epithelium and submucosa (Barnes, 1995), and further block the activation of inflammatory cells, suppress mediator generation from lymphocytes and reduce the expression of vascular adhesion molecules (Djukanovic *et al.*, 1992; Laitinen *et al.*, 1992). However, it was reported that inflammation still persisted at a low level in the airways of patients with chronic asthma despite regular and prolonged treatment with inhaled steroids (Booth *et al.*, 1995; Wenzel *et al.*, 1997). These findings led to the conclusion that corticosteroids only affect certain inflammatory mediators in asthma, substantially reducing the eosinophil/lymphocyte-driven processes, while leaving behind or even augmenting a neutrophil mediated

process (Wenzel *et al.*, 1997). These reports made the search for a more effective anti-inflammatory therapy more crucial.

In the present study, oral administration of aqueous extract of *S. sparganophora* (L) on cockroach crude extract-induced Wistar rats reversed all leukocyte cell infiltration including neutrophils, lymphocytes and eosinophils in bronchoalveolar lavage fluid and therefore achieved the role of a very effective anti-inflammatory herbal therapy. The phytochemical constituents detected in *S. sparganophora* included alkaloids, flavonoids, steroids, glycosides, polyphenol, phlobatinins, tannins, anthraquinone and hydroxymethyl anthraquinone. The phytochemical constituents are known to be responsible for pharmacological effects shown by medicinal plants. For instance, studies have shown ample evidence of the anti-inflammatory effects of flavonoids (Gabor, 1972; Baumann *et al.*, 1980; Middleton *et al.*, 1981; Middleton and Kandeswami, 1992).

Quercetin and a number of other flavonoids such as fisetin, rutin, murin, kaempferol and myricetin have been shown to inhibit secretions in human mast cells, basophils and neutrophils (Schneider *et al.*, 1979), in rat mast cells (Fewartell and Gamperts, 1977) and in cytotoxic T lymphocytes (Schwart *et al.*, 1982). Busse *et al.* (1984) reported that the flavonoids, quercetin, fisetin, rutin, chalcone, hesperetin, catechin, apigenin, taxifolin and tangeretin, inhibited three aspects of human neutrophil function that were commonly associated with inflammation viz., the release of lysosomal enzymes, the chemiluminescence (CL) response, and the production of superoxide anion. It is therefore likely that the presence of these phytochemicals in aqueous extract of *S. sparganophora* was responsible for the anti-inflammatory effects on neutrophils, lymphocytes and eosinophils resulting in the reduction of cockroach crude extract-induced inflammation in Wistar rats.

We conclude that the aqueous extract of *S. sparganophora* reversed the asthmatic inflammation induced in Wistar rats with cockroach crude extract, and that phytochemical constituents in the aqueous extract have

relationship with the reversal of airway inflammation. The data of anti-inflammatory effect of aqueous extract of *S. sparganophora* (L) is of significance in herbal asthma therapy particularly in Africa, especially as this extract unlike many others reported had effect on all leukocyte cells including neutrophils, lymphocytes and eosinophils.

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