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Research Article

**IN VITRO & IN VIVO ANTI-ASTHMATIC STUDIES ON THE
FLORAL EXTRACTS OF GOMPHRENA SERRATA L.**Mamillapalli Vani^{*1}, AbdulRahaman², Avula Prameela Rani³¹Department of Pharmacognosy & Phytochemistry, Vijaya Institute of Pharmaceutical Sciences for Women, Enikepadu, Viayawada, Pin: 521108, Krishna District, Andhra Pradesh, India.²Department of Medicinal Chemistry, Nirmala college of Pharmacy, Atmakur, Mangalagiri, Guntur (dt.), A.P., India.³Dept. of Pharmaceutics, Acharya Nagarjuna University, Nagarjuna nagar, Guntur (dt.). A.P., India.**Abstract:**

The present study has been conducted to evaluate the antiasthmatic potential from the inflorescence extracts of *Gomphrena serrata*. to validate its traditional use. The antiasthmatic activity of hydroalcoholic and acetone extracts was studied by two models histamine and acetyl choline induced bronchospasm in guinea pigs and histamine and acetyl choline induced contraction on isolated guinea pig ileum. The preconvulsive time at a dose of 400mg/Kg in guinea pigs and inhibition of contractions on guinea pig ileum at a concentration of 0.5mg were investigated and compared with the control groups. Phytochemical studies revealed the presence of flavonoids, phenolics, steroids, triterpenoids. The extracts have significantly reduced the bronchospasm induced by histamine and acetylcholine, as well as the contractions of ileum. Therefore the present study concludes that the hydroalcoholic and acetone extracts of *Gomphrena serrata* exhibited antiasthmatic activity which may be due to phytochemical substances probably causing suppression of antibody production or inhibition of antigen induced histamine and acetylcholine.

Key words: *Gomphrena serrata*, asthma, bronchospasm, histamine, acetylcholine**Corresponding author:****Mrs. Mamillapalli Vani,**

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INTRODUCTION:

Bronchial asthma is one of the most common respiratory syndrome affecting humans in millions[1]. It is a chronic inflammatory disorder of airways characterized by chest tightness, wheezing and breathlessness especially at night and on morning awakening[2]. It is caused by various factors like allergens, respiratory infections, dust, cold air, chemicals, histamine, acetyl choline, hereditary, emotions etc. These factors activate immunoglobulin-E mediated mast cells and release of inflammatory mediators like interleukins, eosinophils, neutrophils, histamines, bradykinins that cause inflammation in the throat and bronchial hyper secretions[3]. Histamine is one of the important mediators of broncho constriction and inflammation which is present in mast cells, basophils and various body fluids. It participates in various cell physiological processes like allergic reaction, inflammation, gastric acid secretion, central and peripheral neuro transmission[4]. Hence use of antihistamines is a part of anti-allergic therapy. Apart from it parasympathetic nerves which release acetyl choline as neuro transmitter also control the symptoms and inflammation of allergic diseases through peripheral muscarinic receptors which are present on air way smooth muscle and secretory glands. So, muscarinic antagonists are also used in the treatment of asthma[5]. Therefore the drugs which block the effects of inflammatory mediators like histamine, acetyl choline are used in the treatment of asthma. The drugs currently available show symptomatic and poor response with few side effects. Hence, natural drugs with antioxidant, immune modulatory and anti-inflammatory properties would be beneficial[6].

Gomphrena serrata Linn., *Amaranthaceae*, the genus *Gomphrena* (Family: *Amaranthaceae*) comprises approximately 120 species found in the America, Australia, and Indo-Malaysia; 46 species occur in Brazil, in savanna vegetation (cerrado), napeadic grassland (campo limpo), high altitude grassland (campo rupestre), and caatinga; only a few species are found in forest. A number of Brazilian *Gomphrena* species are employed in the treatment of bronchial asthma, diarrhea, and fever, and as an analgesic, tonic, or carminative[7]. This species show antimalarial and diuretic activities[8,9]. There is little phytochemical and pharmacological screening report on this genus[7,10]. Oleuropein, a phenolic glycoside, a novel anticancer agent was isolated[11]. On the basis of above facts present study has been undertaken to carry out antiasthmatic evaluation on the flower extracts of *Gomphrena serrata* L.

MATERIALS AND METHODS:

Chemicals and reagents:

Histamine hydrochloride, acetyl choline chloride, chlorpheniramine maleate, atropine sulphate were purchased from Sigma- Aldrich chemical Co. all other chemicals were of analytical grade.

Collection of plant material:

The plant material was collected from local grounds of Prasadampadu and Enikepadu of Viayawada rural region, Andhra Pradesh, India. The plant specimen was identified and authenticated by Dr. P. Satya Narayana Raju, Plant taxonomist, Dept. of Botany & Micro biology, Acharya Nagarjuna University, Guntur (dt), Andhra Pradesh, India.

Preparation of the extract:

The flowers were dried under shade, powdered coarsely using mechanical grinder. Then the powder was extracted with 50:50 methanol, water and with acetone alone using soxhlet apparatus. The extracts obtained were dried under vacuum preserved in refrigerator till use.

Experimental animals:

Guinea pigs (400–600 g) of either sex were housed in standard conditions of temperature ($22 \pm 2^\circ\text{C}$), relative humidity ($55 \pm 5\%$), and light (12 h light/dark cycles) were used. They were fed with standard pellet diet and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee as per the guidelines of CPCSEA, Ministry of Social Justice and Empowerment, Government of India.

Phytochemical screening:

Preliminary phytochemical screening was carried out on the hydroalcoholic and acetone extracts to reveal the presence of phytochemicals present in the extracts [12].

Acute toxicity testing:

The animals were overnight fasted prior to the experiment. Different doses (50–2000 mg/kg, orally) of the hydro alcoholic and acetone extracts were administered to groups of guinea pigs and they were observed continuously for 1 hr, next half-hourly intervals for 4 hrs for any gross changes in their behavior and then up to 24 hrs for any mortality as per the OECD Guidelines 425[13].

Histamine and acetylcholine-induced bronchospasm in guinea pigs:

Guinea pigs of either sex were divided into four groups. Each group comprised four animals, which were exposed to 0.1% w/v of histamine dihydrochloride aerosol in a histamine chamber. Progressive dyspnea was observed in animals when exposed to histamine aerosol. The end point, preconvulsion dyspnea (PCD), was determined from the time of aerosol exposure to the onset of dyspnea leading to the appearance of convulsion. As soon as PCD commenced, the animals were removed from

the chamber and placed in fresh air. The PCT (Pre convulsion time) at this time point was taken as the value on day 0. Both groups of guinea pigs were given hydro alcoholic and acetone extracts at a dose of 200 and 400 mg/kg orally, respectively, once a day for 7 days. On the seventh day, 2 h after the last dose, the time of onset of PCD was recorded as on day 0. The same procedure was followed in another set of animals (n = 4) for control group which received distilled water, the standard group (n=4) received chlorpheniramine maleate, the test group (n = 4) for acetylcholine-induced bronchospasm study, except that 0.5% acetylcholine chloride was used in place of histamine dihydrochloride, the std group (n = 4) received atropine sulphate [14]. The percentage increase in time of onset of PCD was calculated using the following formula:

Percentage increased in time of PCD = $(1 - T_1 / T_2) \times 100$, where T_1 is time for PCD onset on day 0, T_2 is time for PCD onset on day 7.

Histamine and acetylcholine-induced guinea pig ileum contraction:

Guinea pigs of body weight 200–500 g were selected and allowed to starve overnight with free access to water. The animals were killed by a blow on the head and exsanguinated. The ileum was isolated, cut into individual sections of 1cm, and then divided into four groups; each group consisted of four ileums. Group I was the control group (histamine 0.5 µg/ml only) and group II were treated as standard group received chlorpheniramine, group III received hydro alcoholic extract and group IV received acetone extract were treated as test groups and were administered histamine with extracts at 0.5 µg/ml, respectively. The isolated ileum was mounted in a 30 ml organ bath containing tyrode solution, maintained at $37 \pm 1^\circ\text{C}$, and gassed with air. The tissue was equilibrated for 45 min during which the bath solution was replaced every 10 min. One end of ileum was attached to an S-shaped aerator tube and other attached to isotonic frontal writing lever to smoked drum. At the end of the equilibration period, histamine (0.5 µg/ml)- induced contraction as well as the effect of extracts (0.5 µg/ml) was recorded. A drug tissue contact time of 1 min was maintained and 15 min time cycle was followed for recording the response of Histamine. After obtaining a dose response curve of histamine on ileum, the extracts (0.5 µg/ml) were added to the reservoir and same doses of histamine were repeated in presences of extracts. The extracts were dissolved in PEG 400 and water (PEG 400 used alone was without any contractile effect). Graph of percentage of maximum contractile response on ordinate and negative logarithm of molar concentration of histamine on

abscissa was plotted to record dose response curve of histamine, in absence and presence of extracts. The percentage response of each tested group was calculated from the height of the peaks obtained and compared with histamine controls¹⁵. The same procedure was followed for acetylcholine induced guinea pig ileum contraction using acetylcholine in place of histamine (0.1 µg/ml) and atropine sulphate was taken as standard drug.

Statistical analysis

The results of various studies were expressed as mean \pm SEM and analyzed statistically using one-way analysis of variance, followed by Tukey test to find out the level of significance. Data were considered statistically significant at $P < 0.05$ and $P < 0.001$ respectively.

RESULTS:

Phytochemical screening:

Preliminary phytochemical screening of hydroalcoholic and acetone extracts of *Gomphrena serrata* L. showed the presence of alkaloids, glycosides, tannins, flavonoids, steroids amino acids and proteins.

Acute toxicity testing:

The hydroalcoholic and acetone extracts were found to be safe up to 2000 mg/kg body weight when administered orally in guinea pigs. After 24 h, the animals were found to be well tolerated; there was no mortality and no signs of toxicity. The extracts were found to be safe and hence to study the qualitative evaluation of antiasthmatic activity for the flower extracts of *Gomphrena serrata* L. the dose of 400 mg/kg of body weight was selected which was found to be safe and in therapeutically effective range for the present study.

Effect of hydroalcoholic and acetone extracts of *Gomphrena serrata* L. flowers on histamine and acetylcholine aerosol-induced bronchospasm in guinea pigs

The hydroalcoholic and acetone extracts significantly increased the time to onset of PCD following exposure to histamine ($P < 0.05$) and acetylcholine ($P < 0.001$) aerosol-induced bronchospasm in guinea pigs (Table 1,2). The increase in time to onset of PCD in the histamine-induced bronchospasm group at a dose of 400 mg/kg body weight was found to be 10.88 and 5.89 for the hydroalcoholic and acetone extracts respectively, whereas in case of acetylcholine-induced bronchospasm at the same dose the increase in time was 10.71 and 8.72 respectively when compared to histamine control groups (Figure 1,2).

Effect of hydroalcoholic and acetone extracts of *Gomphrena serrata* L. flowers on

Histamine and acetylcholine induced contractions on guinea pig ileum

In isolated guinea pig ileum studies, the hydroalcoholic and acetone extracts significantly inhibited the contraction of ileum induced by histamine as well as acetylcholine compared with

controls (Table 3,4). In the histamine-treated groups, the percentage of inhibition was found to be 56 and 28.6% and in the acetylcholine treated group it was 47 and 41% respectively. The percentage of inhibition was found to be higher in histamine treated groups when compared with acetylcholine treated group.

Table 1: Effect of *Gomphrena serrata* floral extracts on histamine induced guinea pig bronchial contraction

S.no	Groups	Drug and Dose	PCT Mean \pm SEM
1	Control	Distilled water p.o.	2.22 \pm 0.24
2	Standard	Chlorpheniramine 2mg/kg p.o.	8.77 \pm 0.43*
3	Test-1	GSHA 400mg/kg p.o.	10.88 \pm 2.08**
4	Test-2	GSAE 400mg/kg p.o.	5.89 \pm 0.93

Note: Each value was expressed as mean \pm SEM, where n=4 in each group; GSHA-hydroalcoholic extract of *Gomphrena serrata*; GSAE-acetone extract of *Gomphrena serrata*; p.o.-orally; PCT-Pre convulsion time; *p<0.01, **p<0.05 as compared with control by one-way ANOVA, Tukeys test.

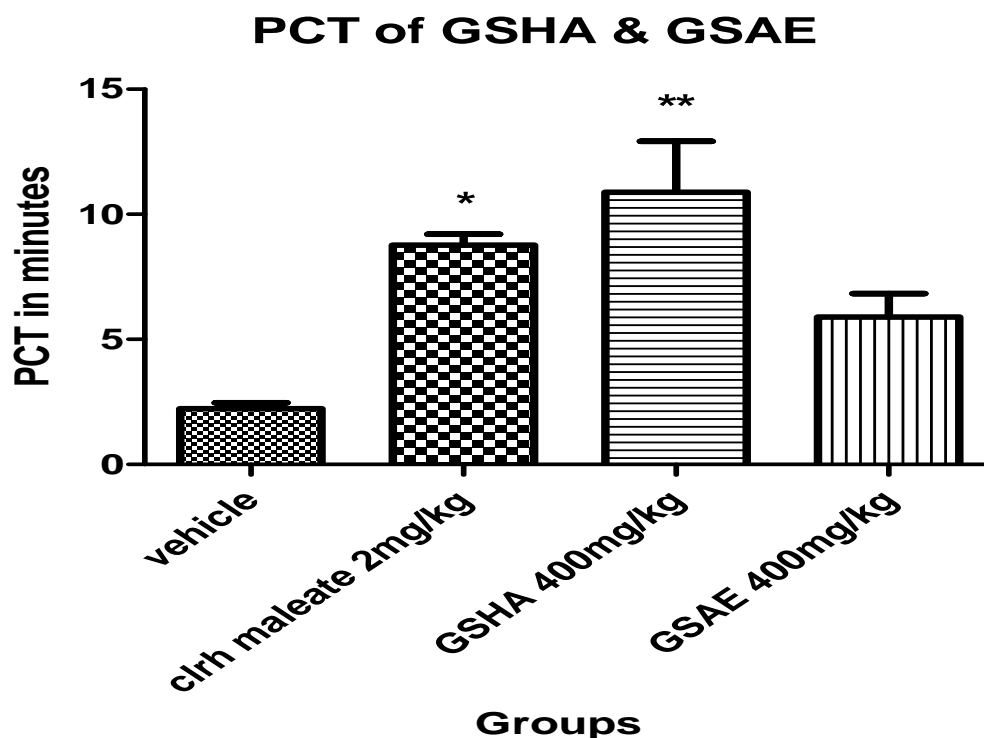


Fig 1: Effect of *Gomphrena serrata* floral extracts on histamine induced guinea pig bronchial contraction

Table 2: Effect of *Gomphrena serrata* floral extracts on acetylcholine induced guinea pig bronchial contraction

S.no	Groups	Drug and Dose	PCT Mean \pm SEM
1	Control	Distilled water p.o.	3.22 \pm 0.60
2	Standard	Atropine sulphate 2mg/kg p.o.	11.60 \pm 1.24
3	Test-1	GSHA 400mg/kg p.o.	10.71 \pm 1.29
4	Test-2	GSAE 400mg/kg p.o.	8.72 \pm 1.64

Note: Each value was expressed as mean \pm SEM, where n=4 in each group; GSHA-hydroalcoholic extract of *Gomphrena serrata*; GSAE-acetone extract of *Gomphrena serrata*; p.o.-orally; PCT-Pre convulsion time; *p<0.01, **p<0.05 as compared with control by one-way ANOVA, Tukeys test.

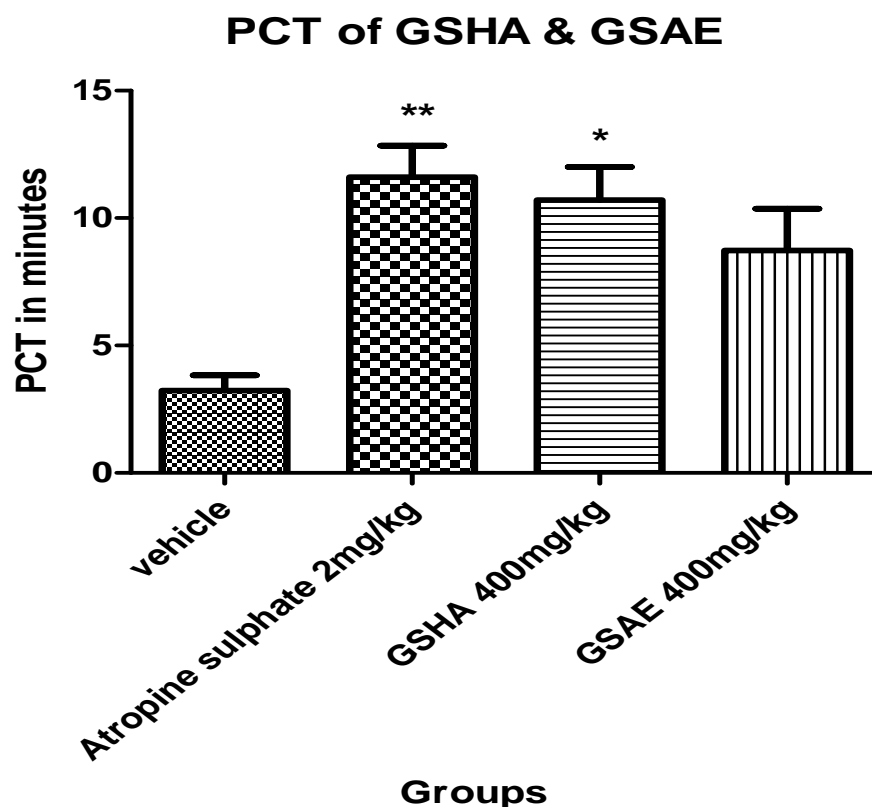


Fig 2: Effect of *Gomphrena serrata* floral extracts on acetylcholine induced guinea pig bronchial contraction

Table 3: Effect of *Gomphrena serrata* floral extracts on histamine induced guinea pig contractions on ileum

S.no	Groups	Drug and Dose	Response Mean ± SEM	% inhibition
1	Control	Histamine 0.5mg	4.9±0.08	0%
2	Standard	Chlorpheniramine 0.5mg	1.8±0.91	63.3%
3	Test-1	GSHA 0.5mg	2.2±0.32	56%
4	Test-2	GSAE 0.5mg	3.5±0.22	28.6%

Note: Each value was expressed as mean±SEM, where n=4 in each group; GSHA-hydroalcoholic extract of *Gomphrena serrata*; GSAE-acetone extract of *Gomphrena serrata*

Table 4: Effect of *Gomphrena serrata* floral extracts on acetyl choline induced guinea pig contractions on ileum

S.no	Groups	Drug and Dose	Response Mean ± SEM	% inhibition
1	Control	Acetylcholine 0.1mg	5.5±0.27	0%
2	Standard	Atropine sulphate 0.5mg	2.2±0.81	60%
3	Test-1	GSHA 0.5mg	2.9±0.05	47.3%
4	Test-2	GSAE 0.5mg	3.2±0.64	41.9%

Note: Each value was expressed as mean±SEM, where n=4 in each group; GSHA-hydroalcoholic extract of *Gomphrena serrata*; GSAE-acetone extract of *Gomphrena serrata*

DISCUSSION:

Bronchial asthma is characterized by airway reactivity to exposure to various spasmogens. The airway stimulation leads to the release of numerous mediators like histamine, acetylcholine, leukotrienes, and prostaglandins, which cause an acute attack of bronchoconstriction³. Inhalation

of histamine and acetylcholine is a classic method of inducing bronchoconstriction, which results in intense smooth muscle contractions, increased vascular permeability, mucus formation, hypoxia, and convulsions in guinea pigs. Bronchodilators can delay the occurrence of these symptoms [15]. In the present study hydroalcoholic extract showed an inhibitory effect on preconvulsive breathing and prolonged latent period of convulsion in the guinea pigs exposed to aerosolized histamine and acetylcholine when compared to acetone extract. The results of the study suggest that the extract significantly increased the time of occurrence of PCD through dilatation of the bronchial smooth muscles.

Antiasthmatic drugs act on the contraction of the Ileum muscle through several mechanisms, including stimulation of b-adrenergic receptors, inhibition of histamine (H1) receptors, or through an anticholinergic property [16]. In the present study, in the isolated guinea pig ileum preparation, percentage of inhibition was more in hydroalcoholic extract when compared to acetone extract indicating antiasthmatic activity, which may be due to inhibition of H1 receptor or by anticholinergic property.

Phytochemical screening showed the presence of flavonoids, steroids, saponins, terpenoids, lignins, etc. Flavonoids are reported to possess smooth muscle relaxant and bronchodilator and spasmolytic property [17,18,19], whereas saponins are reported to have mast cell-stabilizing property and lignins are responsible for antibacterial, antioxidant, spasmolytic, and anti-inflammatory effects [20]. Steroids and terpenoids were also responsible for spasmolytic action by relaxing the tracheobronchial tree of lungs [21,22]. The antiasthmatic activity of HMPF may be due to the presence of the above-mentioned phytoconstituents.

CONCLUSION:

Histamine when inhaled has been shown to induce bronchoconstriction by direct H1-receptor activation and also by a naturally mediated bronchoconstrictor effect via vagal reflexes. In the present study, the result of hydroalcoholic and acetone extracts of flowers of *Gomphrena serrata* L. 200 mg/kg significantly protected the guinea pigs against histamine and acetylcholine induced bronchospasm. Therefore, the result of present study indicates the

plant extracts possess direct in-vivo antihistaminic and anticholinergic activity and therefore utility of the extracts in antiasthmatic activity. These pharmacological activities collectively constitute significant preventive action against asthma. However, further studies should be carried out to evaluate its mechanism of action and for identification of the compound responsible for antiasthmatic activity.

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Conflicts of interest

There are no conflicts of interest.

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