



Antiallergic activity of ethanol extract from *Petiveria alliacea*

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Abstract

Objective: To screen the antiallergic effects of different extracts of the leaves of *Petiveria alliacea* in two experimental models *in vitro* assay of antiallergic activity in the RBL cell line and antagonism study of guinea pig ileum to LTD₄. **Materials and methods:** Crude extract of the leaves of *P. alliacea* obtained by successive extraction with hexane, chloroform and ethanol, were screened for antiallergic properties in the RBL cell line by measuring the inhibitory effects on release of β-hexosaminidase from RBL-2H3 (rat basophil leukemia) cells and also were evaluated by measuring the inhibitory effects on LTD₄-induced contraction of guinea-pig ileum. **Results:** β-hexosaminidase release induced by antigen (DNP-HAS) was strongly inhibited by ethanol extract; its IC₅₀ was 0.032mg/mL. Chloroform extract was moderately active. The hexane extract had no activity in the test. The ethanol extract showed a significant antihistamine action (91.1%) at a dose of 50μg/mL compared to control. The chloroform extract was found to have little activity (9.1%) and hexane extract did not show a significant antihistamine activity. The disodium cromoglycate (DSCG) one of the commercial antiallergic drugs was used as positive control. All the extracts were less active than DSCG at doses test. At 50μg/mL the ethanol extract of *P. alliacea* was the most potent antagonist of LTD₄-induced contractions of guinea pig ileum (91.1%). Chloroform and hexane extracts were less active in the test. **Conclusion:** From the results obtained, it can be observed that ethanol extract of leaves of *Petiveria alliacea* have significant antiallergic property. It also provides a rationale for their use in the Mexican traditional system of medicine.

Key words: *Petiveria alliacea*, antiallergic effects.

1. Introduction

Petiveria alliacea L. (Phytolaccaceae) is commonly know as “hierba del zorrillo”. It is a common herb that grows wild and abundantly in the fields of Mexico in the states

of Durango, Jalisco, Nayarit, Michoacan, Sinaloa, and Oaxaca. A water extract of the fresh leaves is used in folk medicine for the cure of different illnesses. Aqueous leaves

extract has long been used for treating allergies, inflammatory afflictions, and bacterial infection it is generally believed to produce beneficial effects. The hydroalcoholic extract of *P. alliacea* roots orally administered to rats showed anti-inflammatory activity. [1]

In a previous report on *P. alliacea* the presence of the sulfur compounds: trithio-laniacin, benzyl polysulfides (are useful for prophylactic and therapeutic treatment of liver disorders), dibenzyl trisulfides and trans-N-methyl-4-methoxyproline, were indicated [2-4]. 6-C-formyl and 6-C-hydroxymethyl flavonones and coumarins from *P. alliacea* have been reported. [5, 6]

In the present study was to used two methods were used which is widely employed for the evaluation of antiallergic activity of *P. alliacea* namely assay of antiallergic activity in the RBL cell line and antagonism study of guinea pig ileum to LTD₄.

2. Experimental

2.1 Materials

All the reagents were purchased from Sigma Chemical Co. (USA).

2.2 Plant material

The plant material, collected in the state of Colima (November 2002), was taxonomically identified at the Departamento of Botanica de la Universidad Autonoma Metropolitana, Xochimilco, and a voucher specimen (# 5643) of the plant is deposited at the herbarium for reference.

2.3 Preparation of extract

Fresh leaves of *P. alliacea*, were dried at room temperature and ground into a fine powder. 300 g of powder was heated to reflux temperature (Soxhlet) with 1 L of hexane, chloroform, and methanol for 5 h. The solvents were removed under reduced pressure using a rotatory evaporator to constant weight. The

yields obtained for the hexane, chloroform, and methanol extracts were of 3.05, 5.1, and 6.6% respectively. The extracts of *P. alliacea* were sonicated before addition to the organ bath. Histamine was prepared by adding the substance directly to Tyrode solution.

2.4 Animals

Male Hartley guinea pigs, of 8-10 week of age, weighing 250-400 g were used for the study. They were maintained on a standard diet and water was given *ad libitum*. The procedures involving animals and their care conformed to the international guidelines Principles of Laboratory Animals Care.

2.5 Assay of antiallergic activity in the RBL cell line

The inhibitory activity of the extracts against the release of β -hexosaminidase from RBL-2H3 (rat basophil leukemia) cells was evaluated according to Choi. [7] The cells were grown in DMEM supplemented with 10% fetal bovine serum and l-glutamine. Before the experiment, cells were dispensed into 24-well plates at a concentration of 5×10^5 cells per well, and using a medium containing 0.5 μ g/mL of mouse monoclonal IgE.

The cells were sensitized by incubation overnight at 37°C in 5% CO₂. They were then washed with 500 μ L of siraganian buffer (pH 7.2, 119mM NaCl, 5mM KCl, 0.4mM MgCl₂, 25mM PIPES, 40mM NaOH). And incubated in 160 μ L of siraganian buffer containing 5.6mM glucose, 1mM CaCl₂ and 0.1% BSA for additional 10 min at 37°C. Then cells were exposed to 40 μ L of test material for 20 min treated with 20 μ L of antigen (dinitrophenol-human serum albumin, DNP-HSA, 1 μ g/mL) for 10 min at 37°C to activate cells and to evoke allergic reactions (degranulations).

The reaction mixture was centrifuged at 2000 rpm for 10 min and 25 μ L aliquots of the supernatant were transferred to 96-well plates

and incubated with 25µL of substrate (1mM p-nitrophenyl-N-acetyl-β-D-glucosamide) for 1 h at 37°C. The reaction was stopped by adding 200 µL of 0.1 M Na₂CO₃/NaHCO₃. Absorbance was measured at 405 nm. [8,9] The disodium cromoglycate (DSCG) one of the commercial antiallergic drugs was used as positive control.

2.6 Antagonism study of guinea pig ileum to LTD₄

LTD₄ (leukotriene D₄) was performed on the isolated ileum of male guinea pigs, which was mounted at a load of 1.0 g in 20 mL organ bath filled with Tyrode's solution and bubbled with 5% CO₂ in O₂. The contractile activity of 10⁻⁸ M LTD₄ was assayed in the presence or absence of compounds dissolved in 10µL(DMSO). Extract was given 10 min before the addition of LTD₄.

To know the responsiveness of the tissue, histamine or acetylcholine (10⁻⁵ M) were added to the bath. Following the histamine challenge, the tissues were washed and allowed 20 min to stabilize to baseline tension before LTD₄ was added.

The inhibitory activity of the extract was expressed as percent inhibition, calculated by a comparison of the contraction with height (100%) induced by 10⁻⁸ M LTD₄. The DSCG was used as positive control. [10,11]

3. Result and discussion

The inhibitory effects of the extracts from *P. alliacea* on β-hexosaminidase release from RBL-2H3 cells were examined. β-hexosaminidase release induced by antigen (DNP-HAS) was strongest inhibited by ethanol extract; its IC₅₀ was 0.032 mg/mL. Chloroform extract was found to have little activity (IC₅₀: 0.125 mg/mL). Hexane extract did not show inhibitory activity. The IC₅₀ of DSCG was 0.024 mg/mL. The inhibitory activity of ethanol extract was less potent than DSCG.

To investigate the antihistamine effect of ethanol, chloroform and hexane extracts from *P. alliacea*, the antagonistic effect on the histamine receptor by using ileal strips of guinea pigs was measured. The ethanol extract showed a significant antihistamine action (91.1%) at a dose of 50µg/mL. The chloroform extract was found to have little activity (9.1%) and hexane extract did not show significant antihistamine activity. The results are showed in Table 2.

The antihistamine activity of DSCG was more potent than the extracts (95.7 %). The effects of EtOH extract from *P. alliacea*, at doses of 50, 40, 30, 20 and 10 mg/ml on contractions induced by LTD₄ (10⁻⁸ M) in isolated rat-ileum are showed in Fig 1. The contractions are

Table 1.
Inhibitory effect of extracts on β-hexosaminidase release from RBL2H3 cells induced by DNP-BSA.

Extract	RBL 2H3 cells IC ₅₀ [mg/mL]
Ethanol	0.032
Chloroform	0.125
Hexane	-
DSCG	0.024

Table 2.
Effects of extracts from the leaves of *P. alliacea* in the leukotriene D₄ (LTD₄) tests.

Extract	% LTD ₄
Ethanol	91.1
Chloroform	9.1
Hexane	1.3
DSCG	95.7

Inhibition in % of LTD₄ induced contraction of guinea pig ileal strips at a concentration of 50µg/mL.

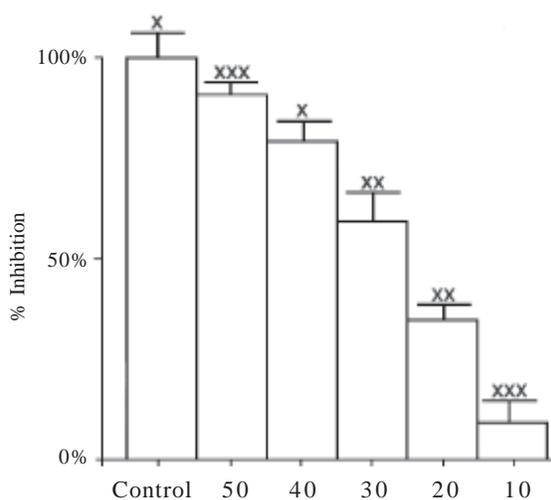


Fig 1. The effects of EtOH extract (50, 40, 30, 20 and 10µg/ml) from *P. alliacea* on contractions induced by LTD₄ (10⁻⁸ M) in isolated rat-ileum. Contractions (%) is expressed as a percentage against control contraction induced by LTD₄ in the absence of samples. Each value shows the mean ± S.E.M. of six animals. *p<0.05, **p<0.01, ***p<0.001 significantly different from the LTD₄-stimulated group.

expressed in % of the maximal contraction obtained in the same tissue before the administration of extract.

The ethanolic extract showed an inhibitory activity dose-dependently and possessed good anti-LTD₄ activity at 50µg/mL (91.1%). Chloroform and hexane extracts were little active in the test (9.1 and 1.3% respectively).

The cysteinyl leukotriene (leukotriene D4) has potent biological actions which significantly contribute to the airway obstruction in asthma. Several of these effects are blocked by drugs known as CysLT1-receptor antagonists. However, there are actions of leukotrienes which are not sensitive to these antagonists, suggesting the presence of additional receptor subtypes.

On isolated smooth muscle preparations kept in organ baths under non-flow conditions, characterised with respect to responsiveness to cysteinyl leukotrienes and sensitivity to purported CysLT1-receptor antagonists, suggests an inhibitory response which cannot be blocked by CysLT1-receptor antagonists. These responses have provisionally been considered to be mediated by CysLT2-receptors. The CysLT1 receptor is blocked by currently available leukotriene antagonists, and the CysLT2 receptor is defined by the absence of selective antagonists. [12] Leukotriene (LT) receptors in the guinea-pig ileum were characterized by Gardiner *et al.*, 1990 [13], these results suggest that two distinct LT receptor types exist on guinea-pig ileum.

One type is predominantly activated by LTD₄ and is antagonized by other structurally distinct LT antagonists and the partial agonist LTE₄. The second type is predominantly activated by LTC₄ and is insensitive to the LT antagonists.

In order to find new antiallergic agents with antagonistic activity against leukotriene D4 (LTD₄) receptors *in vitro*. A method used on isolated pig ileum for studying LTD₄ antagonism [14-19].

In the guinea pig ileum, the ethanol extract of *P. alliacea*, inhibited the contractions produced by leukotriene D4 via activation of CysLT2-receptors in a fashion suggesting competitive antagonism. The contractile response to leukotriene D4 in guinea-pig ileum longitudinal muscle is resistant to CysLT1-receptor antagonists suggests to inhibit responses which cannot be blocked by CysLT1-receptor antagonists. [20,21]. These responses have provisionally been considered to be mediated by CysLT2-receptors suggesting competitive [22].

The leaves of *P. alliacea* has widely been used for the treatment of rhinitis and allergic diseases. The ethanolic extract possess inhibitory effects on LTD₄ induced smooth muscle contraction, and also strongly inhibited the β -hexosaminidase release induced by

antigen (DNP-HAS). which indicates the antiallergic effects. The results demonstrate that the leaves of *P. alliacea* contain biologically active compounds as shown in the test of the plant extract.

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