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# Anti-inflammatory and antispasmodic activity of *Ipomoea imperati* (Vahl) Griseb (Convolvulaceae)

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## Abstract

*Ipomoea imperati* (Convolvulaceae) lives on the sandy shores of the Brazilian coast and in other areas of the world. The anti-inflammatory activity of a methanol-water extract of the leaves of *I. imperati* was investigated in experimental models of acute and subchronic inflammation. Topical application of the extract (10 mg/ear) inhibited mouse ear edema induced by croton oil (89.0 ± 1.3% by the lipid fraction with an $IC_{50}$ of 3.97 mg/ear and 57.0 ± 1.3% by the aqueous fraction with an $IC_{50}$ of 3.5 mg/ear) and arachidonic acid (42.0 ± 2.0% with an $IC_{50}$ of 4.98 mg/ear and 31.0 ± 2.0% with an $IC_{50}$ of 4.72 mg/ear). Phospholipase $A_2$, purified from *Apis mellifera* bee venom, was also inhibited by the extract (5.0 mg/ml lipid and aqueous fraction) *in vitro* in a dose-dependent manner (85% by the lipid fraction with an $IC_{50}$ of 3.22 mg/ml and 25% by the aqueous fraction with an $IC_{50}$ of 3.43 mg/ml). The methanol-water extract of *I. imperati* (1000 mg/kg) administered by the oral route also inhibited the formation of cotton pellet-induced granulomas (73.2 ± 1.2% by the lipid fraction and 56.14 ± 2.7% by the aqueous fraction) and did not cause gastric mucosal lesions. *I. imperati* extracts (10 mg/ml) also inhibited in a dose-dependent manner the muscle contractions of guinea pig ileum induced by acetylcholine and histamine ($IC_{50}$ of 1.60 mg/ml for the lipid fraction and 4.12 mg/ml for the aqueous fraction). These results suggest the use of *I. imperati* as an anti-inflammatory and antispasmodic agent in traditional medicine.

**Key words**
- Anti-inflammatory activity
- Antispasmodic activity
- Arachidonic acid
- Croton oil
- Medicinal plants
- Phospholipase $A_2$

## Introduction

Natural products have served as a source of drugs for centuries, and about half of the pharmaceuticals in use today are derived from natural products (1). Dependence on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health care (2).

*Ipomoea littoralis* (Convolvulaceae), currently known as *I. imperati* (Vahl) Griseb, is a member of the *Ipomoea* section Batatas, and is the only species in the section that is native and endemic to the old world (3). *I. imperati* blooms from December to April and has white flowers with a yellow throat apex and a purple throat base as a particularly distinctive characteristic (4). This plant grows well on sandy seashores in tropical climates, especially in Atlantic coastal areas and it is used in traditional medicine for the

treatment of inflammation (the leaves being employed to treat furunculosis), swelling and wounds, as well as a diuretic (5). The extract of *I. imperati* is usually obtained from its leaves, which are also used to treat pains after childbirth and for stomach problems. A clinical study has reported an analgesic effect of this extract (5). In addition, other species of the family Convolvulaceae such as *I. pes-caprae* also have been reported to have anti-inflammatory, antispasmodic and antihemolytic properties (6).

In the present study, we examined the anti-inflammatory and antispasmodic activity of an alcohol-water extract of *I. imperati* and of its aqueous and lipid subfractions following oral and topical administration in several animal models *in vivo*, and also *in vitro* using the enzyme phospholipase $A_2$ ($PLA_2$).

## Material and Methods

### Animals

Fasted male Wistar rats (180 ± 10 g), male Swiss mice (25 ± 5 g) and male guinea pigs (200-350 g) obtained from the breeding colony of the Institute of Biology, University of São Paulo (IB/USP) were used. The animals had free access to water. All experiments were approved by the Ethics Committee of the Physiology Department, IB/USP, São Paulo.

### Plant material

The leaves of *I. imperati* were collected by one of the authors (A.C.B. Paula) along the seashore at Boracéia, São Paulo State, Brazil, in January and February from 1995 to 1998. The specimens were identified by Dr. José Rubens Pirani (University of São Paulo, São Paulo, Brazil) and Dr. Rosângela Simão Bianchini (Botanical Institute, São Paulo, Brazil) and were deposited in the Botanical Institute under voucher number SP 351848.

### Extraction and fractionation procedures

The leaves of *I. imperati* (Vahl) Griseb (2.65 kg) were extracted with 40% methanol. After filtration, solvents were evaporated under vacuum (40ºC). Partition using water:dichlorometane (1:1, v/v) separated polar (80 g) and apolar (65 g) compounds. Lyophilization was used for the aqueous fraction and evaporation *in vacuo* at 40ºC (lipid fraction) was used to obtain the two fractions used for the pharmacological tests.

### Acute toxicity in animals

Acute toxicity was studied in groups (N = 10) of male albino mice (18-22 g). *I. imperati* (lipid and aqueous fraction) was injected intraperitoneally and mortality was recorded for 24 h (7).

### Mouse ear edema induced by arachidonic acid and croton oil

The *in vivo* anti-inflammatory activity of *I. imperati* was assessed in the mouse ear edema model using arachidonic acid and croton oil to induce inflammation (8). Control mice received only the irritant agents, whereas experimental mice also received the extract (2.5, 5.0, 7.5 and 10.0 mg/ear of both fractions) applied topically together with the irritant agent. Arachidonic acid and croton oil were dissolved in acetone to concentrations of 100 and 10 mg/ml, respectively. Each mouse received 0.5 mg of arachidonic acid/ear or 200 µg of croton oil/ear in the left ear.

The drugs were applied topically to the inner surface of the ear with an automatic pipette in a volume of 5 µl (arachidonic acid) or 20 µl (croton oil). The right ear (control) received 20 µl of acetone alone (vehicle).

The mice were killed by cervical dislocation 1 and 6 h after arachidonic acid and after treatment with croton oil, respectively. Each ear was perforated with a metal punch (a 6-

mm diameter disc) and edema was assessed by subtracting the weight of the disc from the right control ear from the weight of the disc from the left treated ear. Hydrocortisone (217.0 µg/ear) was used as the positive control.

Drug effects were calculated as percent inhibition using the following equation:

$$\frac{\text{(weight of left minus right control ears) - (weight of left minus right treated ears) x 100}}{\text{(weight of left control ear)}}$$

**Cotton pellet granuloma**

Dental cotton rolls (Johnson and Johnson, New Brunswick, NJ, USA) were cut into 5-mm pieces and sterilized in groups of four pellets (160 mg). Rats were anesthetized and the pellets were implanted subcutaneously at four symmetrical positions in the abdomen (9,10).

Two hours after implantation of the cotton pellets, the animal groups (N = 10 each) were treated orally with carboxymethylcellulose (0.2 mg/kg), mineral water (1 ml), dexamethasone (0.2 mg/kg), or the lipid and aqueous fractions of the extract of *I. imperati* at doses of 10, 30, 100, 300 or 1000 mg/kg. Administration was then continued daily for 6 days. On the seventh day, the animals were killed by cervical dislocation, and the granulomas were removed, dried (60ºC), and weighed. The difference between the initial and final dry weight corresponded to the weight of the granulomatous tissue formed. The animals sacrificed after subchronic treatment had their stomachs excised and opened along the greater curvature to determine the number of lesions.

**Phospholipase activity**

The inhibition of $PLA_2$ activity (purified from *Apis mellifera* bee venom) by the *I. imperati* extract was assayed by measuring the decrease in the pH of the incubation mixture using a pH electrode in a closed stirring chamber. The assay medium contained 4 mM sodium taurocholate, 12 mM calcium chloride and 7 mM phosphatidylcholine dipalmitate (Sigma, St. Louis, MO, USA). According to Ref. 11, this technique is reliable down to pH 5.0. In the present study, the mean initial pH of the phospholipid mixture was 8.0. Positive controls were set up using purified $PLA_2$ (0.33 µg/ml) from bee venom. The total incubation volume was 2.5 ml. The four different concentrations used in these *in vitro* experiments were 2.5, 3.0, 3.5 and 5.0 mg/ml of both fractions. In the case of the lipid fraction we used propylene glycol as an apolar solvent and mineral water as a polar solvent.

**Isolated ileum**

Guinea pigs were sacrificed by cervical dislocation and exsanguinated. Ileum segments (2 cm each) were prepared and mounted to record isometric contractions using force transducers (Grass FTO3) connected to a Narco Bio Systems polygraph. A 30-min period for stabilization was followed by a 10-min period during which basal activity was recorded. The test substances (lipid and aqueous fractions of the extract dissolved in carboxymethylcellulose at doses of 1.0, 3.0, 5.0 and 10.0 mg/ml) were added to the bath in a volume of 50 µl; one concentration was tested per ileum segment.

In the same experiments, the preparations were electrically stimulated with supramaximal rectangular pulses (20 V, 1.0 ms, 0.1 Hz) and the effects of non-cumulative extract concentrations were evaluated as described above.

The influence of the extract on the contractions induced by acetylcholine (1 µM) and histamine (1 µM) was studied in preparations preincubated for 10 min with the fraction. The fractions of the *I. imperati* extract were tested at their corresponding $IC_{50}$ for the inhibition of electrically induced contractions. The effect of these fractions

was assessed by comparing the contractile responses to acetylcholine and histamine obtained in the absence and in the presence of extract components (1.0, 3.0 and 10.0 mg/ml) (12).

### Statistical analysis

Results are reported as means ± SEM and were analyzed statistically by analysis of variance (ANOVA) followed by the Tukey test. P values of less than 0.05 were considered significant.

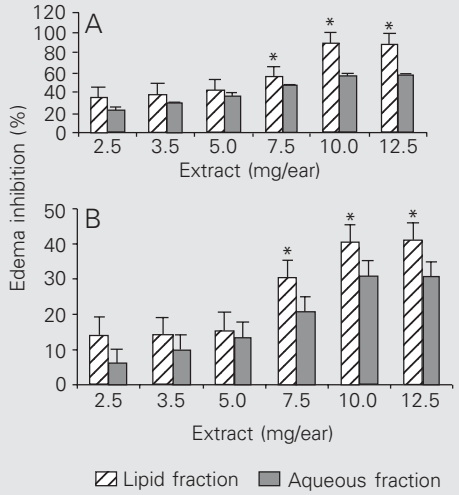


Figure 1. Effect of topical application of *Ipomoea imperati* lipid and aqueous fractions on croton oil- (200 µg/ear) (A) and arachidonic acid-induced (0.5 mg/ear) (B) mouse ear edema. The positive control dexamethasone (217 µg/ear) induced a maximum inhibition of 52.0 ± 1.0% and acetone was the negative control. Each column is the mean of 5 mice, and the bars indicate the SEM. *P<0.05 compared to control (Tukey test).

Table 1. Inhibitory action of the lipid and aqueous fractions of *Ipomoea imperati* leaf extract on phospholipase $A_2$ activity.

| Extract | Concentration (mg/ml) | Inhibition (%) |
|---|---|---|
| Lipid fraction | 2.5 | 15.52 ± 1.3 |
| | 3.0 | 17.63 ± 1.0 |
| | 3.5 | 72.99 ± 2.5 |
| | 5.0 | 81.27 ± 1.4 |
| Aqueous fraction | 2.5 | 4.66 ± 1.2 |
| | 3.0 | 11.11 ± 2.3 |
| | 3.5 | 12.47 ± 1.8 |
| | 5.0 | 25.77 ± 1.9 |

Data are reported as the mean % inhibition ± SEM for 5 rats. Lipid and aqueous fractions of *I. imperati* were significantly different (lipid: 3.5 and 5.0 mg/ml ≠ 2.5 and 3.0 mg/ml; aqueous: 3.5 and 5.0 mg/ml ≠ 2.5 mg/ml, 5.0 mg/ml ≠ 3.0 mg/ml) (P<0.05, Tukey test).

## Results

### Acute toxicity study in animals

The methanol-water extract of *I. imperati* at doses up to 2000 mg/kg did not produce any mortality in male albino mice (N = 10) up to 24 h after intraperitoneal injection.

### Effect of *Ipomoea imperati* on croton oil- and arachidonic acid-induced mouse ear edema

The mouse ear edema reached a maximum at 6 h after croton oil application and 1 h after arachidonic acid application. The lipid and aqueous fraction, 10 mg/ear of *I. imperati* extract, significantly inhibited swelling, considerably reducing the vascular permeability response to croton oil and arachidonic acid application. *I. imperati* inhibited the inflammation induced by croton oil in a concentration-dependent manner. The maximum inhibition induced by the lipid and aqueous fractions was 89.0 ± 1.3% with an $IC_{50}$ of 3.97 mg/ear and 57.0 ± 1.3% with an $IC_{50}$ of 3.5 mg/ear, respectively (P<0.05), with a confidence interval (CI) of 2.51-6.27 and 2.98-6.09 mg/ear, respectively. The positive control dexamethasone (217 µg/ear) induced a maximum inhibition of 52.0 ± 1.0% with an $IC_{50}$ of 28.3 µg/ear and a CI of 26.2-30.45 µg/ear (Figure 1A).

Similarly, *I. imperati* extracts (10 mg of lipid or aqueous fractions/ear) dose dependently inhibited the inflammation induced by arachidonic acid (0.5 mg/ear) by 42.3 ± 2.0 and 31.0 ± 2.0%, respectively. The $IC_{50}$ was 4.98 mg/ear, with a CI of 2.94-5.08 mg/ear, and 4.72 mg/ear with a CI of 3.94-6.08 mg/ear, respectively (P<0.05) (Figure 1B).

Both fractions of *I. imperati* extract (5.0 mg/ml) had a significant inhibitory activity against $PLA_2$ (0.33 µg/ml bee venom) (Table 1). The lipid fraction inhibited $PLA_2$ activity by 85%, but the aqueous fraction only inhibited it by 25% (P<0.05). The $IC_{50}$ for both fractions against $PLA_2$ activity was 3.22 and

3.43 mg/ml, respectively, and the CI was 2.47-3.98 and 2.83-4.04 mg/ml, respectively, which are not proportional to the weight of the extract.

The extract exhibited significant inhibitory activity on the formation of granulation tissue in the cotton pellet test. The lipid and aqueous fractions of *I. imperati* (at the dose of 1000 mg/kg) inhibited the inflammatory process by 73.2 ± 1.2% ($IC_{50}$ of 93.7 mg/ear and CI of 68.1-128.95 mg/ear) and by 56.14 ± 2.7% ($IC_{50}$ of 102.5 mg/ear with a CI of 72.10-145.59 mg/ear), respectively, 6 days after implantation of the cotton pellet (Table 2). The lipid fraction (1000 mg/kg) was as effective as dexamethasone (0.20 mg/kg) in inhibiting this inflammation by 73%. No gastric mucosal lesions were observed after 6 days in the rats used in these experiments. Other doses used in this experiment proved to be effective, but maximum inhibition was obtained with 1000 mg/kg (Table 2).

The methanol-water extract of *I. imperati* dose dependently inhibited the tone and amplitude of the contractions of guinea pig ileum in electrically stimulated preparations. The lipid fraction had an $IC_{50}$ of 1.60 mg/ml and caused a maximum inhibition of 85.0 ± 1.0% (CI of 0.72-3.58 mg/ml), while the aqueous fractions had an $IC_{50}$ of 4.12 mg/ml and caused a maximum inhibition of 81.0 ± 1.53% ($P<0.05$; Figure 2) with a CI of 2.13-7.97 mg/ml.

The contractions in response to acetylcholine and histamine (1 µM, each) were antagonized by the *I. imperati* methanol-water extract (10 mg/ml). The $IC_{50}$ for the lipid and aqueous fractions against acetylcholine were 3.48 mg/ml (CI of 1.23-5.68) and 1.84 mg/ml (CI of 0.28-2.34 mg/ml), respectively, and maximum inhibition was 68.0 ± 0.9% for both fractions. With histamine, the $IC_{50}$ was 4.03 mg/ml (CI of 0.67-6.10 mg/ml) and 1.69 mg/ml (CI of 0.9-3.45 mg/ml) for the lipid and aqueous fractions, respectively. The maximum inhibition for both fractions was 57 ± 0.86% ($P<0.05$; Figure 3).

Table 2. Effect of the oral administration of *Ipomoea imperati* leaf lipid and aqueous extracts on rat granuloma tissue formation.

| Treatment | Dose (mg/kg) | Dry granuloma weight (mg) | | Inhibition (%) |
|---|---|---|---|---|
| | | Initial | Final | |
| Carboxymethylcellulose | 0.2 | 298.0 ± 3.1 | 492.0 ± 2.0 | – |
| *Ipomoea imperati* (lipid fraction) | 10 | 240.0 ± 0.8 | 220.0 ± 1.3 | 20.3 ± 1.3 |
| | 30 | 239.0 ± 1.2 | 204.0 ± 2.0 | 35.1 ± 1.8* |
| | 100 | 245.0 ± 1.9 | 194.2 ± 1.0 | 51.2 ± 1.9* |
| | 300 | 243.0 ± 0.9 | 181.1 ± 0.89 | 62.2 ± 1.8* |
| | 1000 | 241.7 ± 1.9 | 168.4 ± 1.8 | 73.2 ± 1.2* |
| *Ipomoea imperati* (aqueous fraction) | 10 | 239.0 ± 1.2 | 225.9 ± 1.6 | 13.1 ± 0.98 |
| | 30 | 242.0 ± 1.5 | 223.8 ± 1.2 | 18.2 ± 1.4 |
| | 100 | 250.0 ± 1.2 | 220.0 ± 1.4 | 30.5 ± 1.6* |
| | 300 | 243.0 ± 0.9 | 200.5 ± 1.0 | 42.5 ± 1.8* |
| | 1000 | 241.9 ± 1.8 | 185.8 ± 1.8 | 56.1 ± 2.7* |
| Dexamethasone | 0.2 | 240.3 ± 2.3 | 166.3 ± 1.5 | 74.0 ± 3.2* |

Data are reported as the means ± SEM for 10 rats. Lipid fractions 30, 100, 300 and 1000 mg/kg of both fractions and the positive control (dexamethasone) were significantly different (*$P<0.05$, Tukey test).

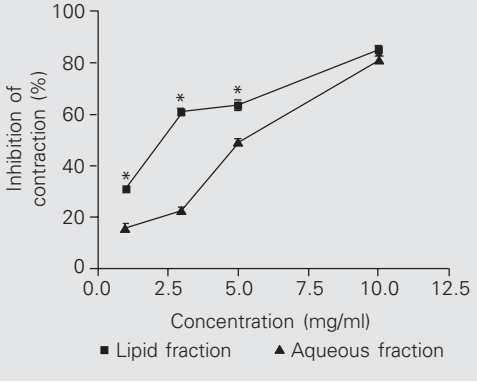


Figure 2. Dose-dependent inhibition of contractions by *Ipomoea imperati* extracts in electrically stimulated isolated guinea pig ileum. Each point represents the mean ± SEM of 5 experiments. Lipid fractions (1.0, 3.0 and 5.0 mg/ml) were significantly different from the aqueous fraction (*$P<0.05$, Tukey test).

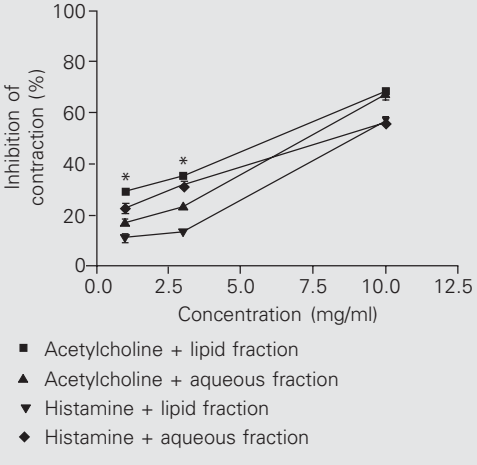


Figure 3. Dose-dependent inhibition of 1 µM acetylcholine- and 1 µM histamine-induced contractions in guinea pig ileum by *Ipomoea imperati* extracts. Data are reported as means ± SEM for 5 experiments. *$P<0.05$ compared to the aqueous fraction (Tukey test).

## Discussion

A large number of herbal drugs are reputed to be of excellent medicinal value, and are used for the treatment of several ailments. In folk medicine, various indigenous drugs are used in single and/or in combined form to treat different types of inflammatory and arthritic conditions, with considerable success (13).

In the present study we showed that the lipid fraction of the methanol-water *I. imperati* leaf extract contains the active agent against topical inflammation induced by croton oil and arachidonic acid. However, the positive control dexamethasone was effective only in inhibiting croton oil-induced topical inflammation. It is known that the anti-inflammatory effect of dexamethasone depends on the model used. Lee et al. (14) reported that dexamethasone (0.05 mg/ear dissolved in acetone) applied topically 16 h before the induction of mouse ear edema inhibited by 92% the inflammation induced by 12-O tetradecanoyl phorbol acetate (TPA). It has been established that inflammation induced by TPA/croton oil is related to the activation of $PLA_2$, which releases arachidonic acid from the cell membrane. Arachidonic acid, in turn, is metabolized to prostaglandins and leukotrienes. Substances able to inhibit edema could be inhibitors of cyclooxygenase (COX) and/or 5-lipoxygenase (15). However, other agents are involved in edema generation such as serotonin and bradykinin, which act directly through specific receptors ($5HT_2$, $B_2$) present in the endothelial cells of postcapillary venules. On the other hand, pretreatment with antagonists of protein kinase C may suppress the inflammation induced in guinea pig illeum. However, the inflammation observed in this model is related to the activation of $PLA_2$. Bresnick et al. (16) stated that $PLA_2$ catalyzes the sn-2 hydrolysis of phospholipids liberating free fatty acids, predominantly arachidonic acid, and lysophospholipids. These products can have biological actions or be further metabolized to form a variety of proinflammatory lipid mediators including prostaglandins, leukotrienes, or platelet-activating factor and thus the inhibition of $PLA_2$ by pharmacological agents should have led to an anti-inflammatory effect.

The mechanism of croton oil-induced inflammation involves an increase in $PLA_2$ activity (17,18), which in turn leads to the release of arachidonic acid and subsequent biosynthesis of leukotrienes and prostaglandins (19,20), thus also involving the lipoxygenase pathway. It has been firmly established that arachidonic acid metabolites act as mediators of the inflammatory response via COX and lipoxygenase activity, and have therefore been a target for the development of therapeutic agents (21).

Enzyme inhibition assays are important tools in the search for new drugs. Our experimental work also concerns the effects of *I. imperati* extracts on several *in vivo* and *in vitro* eicosanoid-releasing systems, to investigate whether they contribute to the anti-inflammatory activity of these compounds. The anti-inflammatory action of *I. imperati* extracts on arachidonic acid-induced inflammation involved inhibition of the $PLA_2$ enzyme and probably of COX II (COX-2), the key enzyme involved in prostaglandin biosynthesis. So $PLA_2$ and probably the COX-2 enzyme of arachidonate metabolism can be inhibited by *I. imperati*, suggesting that this plant can provisionally be classified as a "dual inhibitor", with greater $PLA_2$ than COX-2 activity. The step preceding arachidonic acid liberation, which is catalyzed by $PLA_2$, can be a target of the drugs (22).

Since endothelial cell contraction increases vascular permeability, and since this permeability is inhibited by vasodilating agents, the antispasmodic activity of *I. pes-caprae* can be assumed to contribute to the anti-inflammatory action of this plant (6). The cited investigators based their hypo-

thesis on the tentative theory that endothelial cell contraction causes increased vascular permeability and on the fact that agents such as ß-adrenoceptor agonists and calcium antagonists inhibit this permeability with general relaxant properties.

During the process of acute inflammation vascular leakage is a cardinal effect believed to result from the actions of certain inflammatory mediators, such as histamine, 5-hydroxytryptamine, bradykinin, $LTE_4$ and platelet-activating factor, on the postcapillary venules. The mechanism of this leakage was proposed to be due to mediator-induced contraction of endothelial cells. The cells are thereby pulled apart from each other and create gaps, allowing blood components to leak into the interstitial compartment creating edema (23). Pongprayoon et al. (24) suggested that endothelial cells and smooth muscle cells share a similar contractile mechanism. Vasoconstriction has been implicated in the dermatitis caused by jellyfish stings, leading to local vascular insufficiency and gangrene. Agents with a direct vasodilatory action, e.g., papaverine ($IC_{50}$ values of 30 µg/ml) have been recommended for the treatment of such toxin-induced dermatitis because of their nonspecific antispasmodic action.

*I. imperati* extracts have local and systemic anti-inflammatory actions in mice and rats, respectively. The pharmacological mechanism involved in this anti-inflammatory effect may be related to the inhibition of $PLA_2$ and COX-2. Additional experiments are necessary to demonstrate the inhibition of COX-2 by *I. imperati*. The extract also presented a nonspecific antispasmodic activity on the isolated ileum, inhibiting histamine and acetylcholine. In the acute toxicity assay, 1 mg/kg of *I. imperati* methanol-water extract caused no mortality in mice after 24 h.

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