Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(14)60229-X

15-Lipoxygenase inhibition of *Commelina benghalensis, Tradescantia fluminensis, Tradescantia zebrina*

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PEER REVIEW

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Comments

In general, this work is interesting. The authors evaluated the 15-lipoxygenase inhibitory activity of the methanol leaf extracts of *C. benghalensis, T. fluminensis* and *T. zebrina,* and came to a conclusion that the fraction of *T. fluminensis* truly exhibited inhibition activity against 15-lipoxygenase activity.
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ABSTRACT

Objective: To evaluate the 15-lipoxygenase inhibitory activity of the methanol leaf extracts of *Commelina benghalensis, Tradescantia fluminensis* (*T. fluminensis*) and *Tradescantia zebrina*.
Method: The inhibitory activity was evaluated using a spectrophotometric assay by observing the increase in absorbance at 234 nm due to the formation of the product 13-hydroperoxyoctadecadienoic acid. The extracts were also tested for the presence of terpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds, alkaloids and cardiac glycosides.
Results: All the extracts inhibited the action of 15-lipoxygenase at a concentration of 0.2 μg/mL. *T. fluminensis* and *Tradescantia zebrina* exhibited higher than 50% inhibition with *T. fluminensis* at 87.2%. *T. fluminensis* was partitioned with ethyl acetate and hexane and their IC_{50} values were determined at 8.72 μg/mL and 98.04 μg/mL, respectively.
Conclusions: *T. fluminensis* is a potentially good source of 15-lipoxygenase inhibitors.

KEYWORDS
Inflammation, Medicinal plants, Plant extracts, 15-Lipoxygenase

1. Introduction

Asthma is a chronic medical condition which affects millions of people across the globe. Asthma is characterized by the inflammation of the airway and bronchoconstriction with symptoms like wheezing, cough, dyspnea, chest tightness, and breathlessness[1]. The people afflicted with this kind of disease are more sensitive to certain stimuli. Environmental and genetic factors may trigger asthma. Environmental factors can be allergens, respiratory and viral infections, diet, hygiene, and air pollution. The genetic factor may determine not only the cause of asthma but may also the cause of the severity of the said disease. Together with the environmental factors, asthma poses a great threat to numerous people[2].

Asthma can be treated by bronchodilator therapy and controller therapy depending on the type and severity of asthma[3]. One drug therapy for asthma and other related inflammatory diseases is the use of leukotriene antagonists.

Leukotriene is one of the classes of products of the oxidative pathway of metabolism of the arachidonic acid[4]. It has an essential function in the manifestations of asthma

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Foundation Project: Supported by the University of the Philippines Diliman through the Natural Sciences Research Institute (Grant No. CHE-09-2-02).

Article history:
Received 25 Nov 2013
Received in revised form 2 Dec, 2nd revised form 11 Dec, 3rd 18 Dec 2013
Accepted 25 Jan 2014
Available online 28 Mar 2014

and other nasal allergies by initiating the receptors in the inflammatory cells[5]. The metabolism of arachidonic acid provides potent proinflammatory and hypersensitivity mediators for a number of bronchial diseases such as asthma. These mediators are catalysed by the lipoxygenase enzyme family[6,7]. There are three classes of enzyme that specifically catalyses arachidonic acid in mammals: the 5-, 12- and 15-lipoxygenase[6-8]. They are expressed in different tissues including liver, kidney and adipose tissues[9]. Inhibition of soybean 15-lipoxygenase is generally regarded as predictive inhibition of the mammalian enzyme[10-12].

Several works have been done to look for new anti-inflammatory compounds. A number of synthetic compounds have been evaluated for their 15-lipoxygenase inhibitory action[13-18]. A limited number of studies have been carried out regarding the 15-lipoxygenase inhibition of plant extracts. Acetone and polar lipid fractions of pumpkin seeds were found to inhibit soybean lipoxygenase[19]. Friedelin isolated from the bark of *Commiphora berryi* inhibited soybean lipoxygenase with an IC_{50} of 35.8 μmol/L[20]. *Cassia alata* extract was found to inhibit lipoxygenase with an IC_{50} of 90.2 μg/mL[21]. *Guava* leaf extracts inhibited the catalytic activity of a leucocyte-type 12 lipoxygenase[22]. Kushecarpin A from *Zizyphus oxyphylla* and isolates from *Desmos cochinchinensis* have been shown to suppress lipoxygenase[23,24]. The Commelinaceae family which is a medicinal herb has been reported to have anti-inflammatory, febrifungal and diuretic properties[25]. This study aims to evaluate the bioactivity against 15-lipoxygenase of three different species under the Commelinacea family, namely, *Commelina benghalensis (C. benghalensis)*, *Tradescantia fluminensis* (*T. fluminensis*) and *Tradescantia zebrina (T. zebrina)*.

2. Materials and methods

2.1. Plant material

Fresh leaves of *C. benghalensis*, *T. fluminensis* and *T. zebrina* were collected from the University of the Philippines, Diliman Campus and submitted to Dr. Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman for authentication.

2.2. Plant extraction

The *C. benghalensis*, *T. fluminensis* and *T. zebrina* leaves were washed with running water and allowed to drip dry. The air-dried samples were weighed and then homogenized for overnight soaking in methanol using clean glass jars. The crude methanolic extracts were concentrated in vacuo using a rotary evaporator (Heidolph). The methanol extract of *T. fluminensis* was further partitioned using hexane and ethyl acetate.

2.3. Phytochemical analysis

The phytochemical screening methods used were based on Edeoga and Harborne[26,27]. Qualitative test for terpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds, alkaloids and cardiac glycosides were performed.

2.4. Preparation of reagents and extracts

About 14 221.059 IU/mL of enzyme solution was prepared from soybean 15-Lipoxygenase (Sigma Cat. L7395) using 1 mg enzyme and 16.05 mL phosphate buffer, and the solution was kept on ice throughout the assay. For the substrate solution, a stock of 1 984.6 μmol/L linoleic acid was prepared (Sigma cat. No. 1376). A total of 10 μL linoleic acid was added to 50 μL absolute ethanol, with 60 μL of 0.5 mol/L NaOH and 16.05 mL phosphate buffer. For the positive control, a stock solution of 7.48×10^{-4} mol/L quercetin solution was prepared by weighing 0.005 65 g quercetin (Sigma) and adding 200 μL DMSO. The volume was then adjusted to 5 mL using the prepared borate buffer. For the samples, 20 mg plant extracts was weighed and dissolved in 50 μL DMSO. The volume was adjusted to 250 μL with the addition of 200 μL phosphate buffer.

2.5. 15-Lipoxygenase inhibitory assay

The assay was based on the procedure done by Wangensteen with slight modifications[28]. Briefly, the activity of 15-lipoxygenase is observed as it catalysed the reaction between oxygen and linoleic acid. The increase in absorbance at 234 nm was due to the formation of the product 13-hydroperoxyoctadecadienoic acid from the reaction of oxygen and linoleic acid. The spectrophotometric assay was performed using a UV-Vis double-beam spectrophotometer (Schimadzu model 1800). For the blank solution, 10 μL solvent control (50 μL DMSO in 200 μL phosphate buffer) was placed in a test tube with 200 μL linoleic acid and 2 790 μL phosphate buffer. A blank was left in the blank sample cuvette holder throughout the assay. A total of 50 μL lipoxygenase was transferred to a test tube with 2 740 μL phosphate buffer, and then 10 μL of the test solution was added. The resulting mixture was then incubated for 5 min. After which, 200 μL linoleic acid was added to the mixture and then the absorbance read at 234 nm a minute at 30 seconds interval.

2.6. Data analysis

From the absorbance versus time plot, the slope which represented the enzyme activity was obtained. The percent lipoxygenase inhibitory was then computed using the equation below:

$$\text{Activity}=\frac{\left(\frac{\Delta A1}{\Delta t}-\frac{\Delta A2}{\Delta t}\right)}{\left(\frac{\Delta A1}{\Delta t}\right)}\times 100$$

where $\frac{\Delta A1}{\Delta t}$ and $\frac{\Delta A2}{\Delta t}$ are the slopes of the solvent control and test samples respectively.

2.7. Statistical analysis

All data obtained was subjected to One-way analysis of variance followed by Dunnett's *post hoc* test at α=0.05. This was done to identify the sample groups with means that are significantly different at 95% confidence interval from the mean of the solvent control group.

3. Results

Dried leaves used were 48.46 g *C. benghalensis*, 857.98 g *T. zebrina* and 25.09 g *T. fluminensis*. The samples gave 12.9% *C. benghalensis*, 0.078% *T. zebrina* and 3.06% *T. fluminensis* methanol extracts.

The methanol extracts were evaluated for their lipoxygenase inhibitory activity as shown in Table 1. All extracts showed significant inhibition with *T. fluminensis* exhibiting the highest activity at 87.18%.

Table 1
Lipoxygenase inhibitory activity of the methanol extracts.

Sample	% Inhibition
Solvent	--
Quercetin	63.33*
T. zebrina	64.44*
C. benghalensis	33.61*
T. fluminensis	87.18*

Quercetin at 3.8×10^{-3} μg/mL and plant samples at 0.2 μg/mL. The data represents a mean of three individual experiments and those conditions that resulted in a significant change (P<0.05) from appropriate control values are denoted by an asterisk.

The methanol extracts were then qualitatively tested for the various phytochemicals they contain. As shown in Table 2, *T. zebrina* was tested positive for the presence of saponins, phenolic compounds and flavonoids tests, while *C. benghalensis* showed the presence of terpenoids and flavonoids tests and *T. fluminensis* to the flavonoids and steroids tests.

Table 2
Results of the phytochemical analysis.

Phytochemical	*T. zebrina*	*C. benghalensis*	*T. fluminensis*
Tannins	−	−	−
Saponins	+	−	−
Terpenoids	−	+	−
Flavonoids	+	+	+
Cardiac glycosides	−	−	−
Phenolic compounds	+	−	−
Steroids	−	−	+
Alkaloids	−	−	−

+ present, − absent.

The plant sample with the highest inhibitory activity was then partitioned using distilled hexane and ethyl acetate. The percentage yields were 1.03% hexane extract and 1.08% ethyl acetate extract. The resulting extracts were then evaluated for their lipoxygenase activity as shown in Table 3. The ethyl acetate extract exhibited higher activity at 87% than the hexane extract at 56%. This may be due to the different phytochemicals they contain. The ethyl acetate and hexane fractions of *T. fluminensis* were positive for the presence of flavonoids.

Table 3
Lipoxygenase inhibitory activity of *T. fluminensis* extracts.

Sample	% Inhibition	Flavonoids
Solvent	--	−
Quercetin	70.89*	+
Hexane	56.00*	+
Ethyl acetate	87.41*	+

Quercetin at 3.8×10^{-3} μg/mL and plant samples at 0.2 μg/mL. The data represents a mean of three individual experiments and those conditions that resulted in a significant change (P<0.05) from appropriate control values are denoted by an asterisk.

For better comparison as to which extract had a more potent inhibitory activity against the 15-lipoxygenase enzyme, the IC_{50} was obtained for each fraction. The IC_{50} for the hexane fraction was determined to be 98.04 μg/mL while the ethyl acetate fraction was 8.72 μg/mL, indicating a more potent and better inhibitory action.

4. Discussion

C. benghalensis, *T. fluminensis* and *T. zebrina* methanol leaf extracts gave significant lipoxygenase inhibition. The three extracts showed positive results for the presence of flavonoids. Flavonoids are polyphenolic compounds with a flavones structure of two benzene rings joined by a γ-pyrone ring. The structure has several variations of substitution and there are now 4 000 characterized flavonoids[29]. They possess a wide spectrum of bioactivity and they inhibit a number of enzymes[30]. Flavonoids have been shown

to inhibit lipoxygenase activity and the presence of such constituents may be responsible for the inhibitory activity of the extracts[31].

T. fluminensis methanol extract showed the highest inhibitory activity at 87.18%. Its ethyl acetate fraction gave higher inhibitory activity at 87.40% than its hexane fraction at 56.00%. It is possible that the flavonoids present in the ethyl acetate fraction have higher lipoxygenase inhibitory action and may contain planar structures. The structure-activity relationship of similar flavonoids quercetin, luteloin, catechin and taxifolin have been investigated. Quercetin and luteloin showed superior inhibitory activity over catechin and taxifolin because of the planarity of the molecules[12]. The IC_{50} of the ethyl acetate fraction of *T. fluminensis* was determined to be 8.72 μg/mL while that of *Cassia alata* extract was 90.2 μg/mL[18], indicating a more potent and better inhibitory action for the former.

There are few literatures that deal with the study of the phytochemical constituents of the Commelinaceae family. The most common phytochemical constituents studied were the glycoflavones, flavonols, and phenolic acids[32]. Another recent study for the phytochemical constituents of Commelinaceae is that of Ogbebor and Edeoga[33]. Based on their study, alkaloids and saponins are present in a species under the said family. In comparison with the results, some constituents did not exhibit a positive result which may be due to insufficient amount of the active compounds present in concentration used in the tests. Another cause may be the presence of interfering compounds thus producing negative results[34].

Further isolation and purification work will be done to determine the bioactive compound or compounds responsible for the lipoxygenase inhibition. The pure compounds will also be chemically modified for structure-activity relationship studies.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This project was funded by the University of the Philippines Diliman through the Natural Sciences Research Institute with grant No. CHE-09-2-02.

Comments

Background

The manuscript describes evaluation of plant extracts that exhibit 15-lipoxygenase inhibitory activity. Three methanol leaf extracts employed in the study were from *C. benghalensis*, *T. fluminensis* and *T. zebrina*. Further fractionation based on polarity of *T. fluminensis* extract was performed and tested for inhibitory activity.

Research frontiers

The research aimed to evaluate the 15-lipoxygenase inhibitory activity of the methanol leaf extracts of *C. benghalensis*, *T. fluminensis* and *T. zebrina* and the authors found that *T. fluminensis* is a potentially good source of 15-lipoxygenase inhibitors.

Related reports

Numerous lipoxygenase inhibitors have been searched from plant sources. Some are also referred to in the manuscript.

Innovations and breakthroughs

This research concluded that *T. fluminensis* is a potentially good source of 15-lipoxygenase inhibitors which will be useful to other researchers.

Applications

The ethyl acetate fraction of *T. fluminensis* looks interesting if the inhibition is valid (not sure about the source of enzyme). Further identification of purified compounds could be applicable and fruitful for practical use.

Peer review

In general, this work is interesting. The authors evaluated the 15-lipoxygenase inhibitory activity of the methanol leaf extracts of *C. benghalensis*, *T. fluminensis* and *T. zebrina*, and came to a conclusion that the fraction of *T. fluminensis* truly exhibited inhibition activity against 15-lipoxygenase activity.

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