ABSTRACT

Several extracts from Philippine medicinal plants used for asthma and other inflammatory diseases were evaluated for their ability to inhibit the action of 15-lipoxygenase. The inhibitory activity was tested spectrophotometrically using quercetin as positive control. Eleven species belonging to 11 families displayed varying inhibitory activities. Commelina diffusa and Euphorbia hirta showed the highest inhibitory activity at 51.3% and 48.5%, respectively. These plants may contain new 15-lipoxygenase inhibitors.

Keywords: Asthma, inflammation, lipoxygenase, medicinal plants, plant extracts.

INTRODUCTION

Lipoxygenases (LOX) constitute a heterogeneous family of lipid peroxidizing enzymes which catalyze the dioxygenation of polyunsaturated fatty acids containing the 1,4-cis,cis system to their corresponding hydroperoxy derivatives.[1] The primary lipid targets of LOX activity are arachidonic acid(AA) and linoleic acid(LA). The metabolism of LA and AA by LOXs produces biologically active pro-inflammatory mediators implicated in the development of asthma.[2] Hence, inhibition of LOX activity and identification of bioactive extracts are of pharmacological interests. In this paper, we evaluated the 15-lipoxygenase inhibitory action and analyzed the phytochemical contents of plants that are traditionally used to treat inflammatory conditions like asthma in the Philippines. The use of different herbal preparations is still being used in the Philippines. The country is rich in folklore that attributed medicinal benefits to quite a number of plants.[3] However, a considerable number of plants still needs to be scientifically validated, hence, much work is still needed to investigate the bioactivity and phytochemicals of these plants. The present research was undertaken to determine the potential scientific basis for the use of these plants in traditional medicine.

MATERIALS AND METHODS

Plant material

Fresh leaves of Amaranthus viridis, Bambusa spinosa, Commelina diffusa, Crataeva religiosa, Ediota alba, Euphorbia hirta, Isotoma longiflora, Monochoria vaginalis, Pistia stratiotes, Plumeria rubra and Premna odorata were collected from the University of the Philippines, Diliman Campus and submitted to the Dr. Jose Vera Santos Herbarium, Institute of Biology, University of the Philippines, Diliman for authentication. Voucher specimens for each plant were also deposited.

Plant extraction

The leaves were washed with running water and allowed to drip dry. The air-dried samples were weighed then homogenized for overnight soaking in methanol using clean glass jars. The crude methanolic extracts were concentrated in vacuo using a rotary evaporator (Heidolph).

Phytochemical analysis

The phytochemical screening methods used were based on Edeoga[4] and Harborne.[5] Qualitative test
for terpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds, alkaloids and cardiac glycosides were performed.

**15-Lipoxygenase inhibitory assay**

The spectrophotometric assay used is generally based on the 15-LOX catalyzed reaction between oxygen and a polyunsaturated fatty acid with a 1,4-diene-type structure. Briefly, inhibition of 15-LOX was carried out as described by Wangensteen[6] with modifications using a reaction medium containing 237 U/mL of soybean 15-LOX-1 (Sigma), 33 uM LA, 2 M borate buffer at pH equal to 9 at ambient temperature. The metabolism of LA by 15-LOX-1 leads to the formation of 13-S-hydroperoxyoctadecadienoic acid (13-HPODE) which is hydrolyzed to 13-S-hydroxyoctadecadienoic acid (13-HODE). The enzyme inhibitory effect was tested by adding the crude methanol extract of the sample (dissolved in 10% DMSO in borate buffer) to the incubation mixture. The LOX activity was monitored as an increase of the absorbance at 234 nm, which reflects the formation of 13-HPODE from LA, every 30 seconds for 5 minutes and done in triplicates.

The value for % inhibition of enzyme activity was calculated as:

\[
\text{Activity} = \frac{(\Delta A_{1}/\Delta t) - (\Delta A_{2}/\Delta t)}{\Delta A_{2}/\Delta t} \times 100\%
\]

where \( \Delta A_{1}/\Delta t \) and \( \Delta A_{2}/\Delta t \) are values for increase in \( A_{234} \) for sample without test sample and with test sample, respectively.

**RESULTS AND DISCUSSION**

Acute inflammation in the lung and airway is fundamentally important to host defence, but chronic or excessive inflammation leads to several common respiratory and airway diseases, including asthma[7]. Asthma has the highest morbidity amongst inflammatory lung diseases[8] and has become the most common chronic disease among children according to the World Health Organization. Although asthma cannot be cured, appropriate management can control the disease. Current therapeutic targets of this inflammatory disease focus on blocking the initiating mediators of the inflammation. One of the therapeutic strategies being studied for asthma management involves controlling the activity of lipoxygenases.

The *in vitro* inhibition of soybean 15-LOX activity was used to evaluate the activities of the plant extracts in this study. Inhibition of soybean 15-LOX is generally regarded as predictive inhibition of the mammalian enzyme.[9,10] Based on the result, most tested methanolic plant extracts showed LOX inhibitory activity with *C. diffusa* showing the highest activity at 51.3% followed by *E. hirta* with 48.5% inhibitory effect at a concentration of 167 µg/ml. All samples however did not exhibit higher activity compared with the positive control quercetin at 62.4% at a concentration of 17 µg/ml as shown in Table 2. The preliminary result of this study validates the traditional use of the plant extracts as remedy for respiratory inflammation specifically asthma.[3]

<table>
<thead>
<tr>
<th>Local name</th>
<th>Scientific name</th>
<th>Family</th>
<th>Traditional uses²</th>
<th>Plant part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kolitis</td>
<td><em>Amaranthus viridis</em></td>
<td>Amaranthaceae</td>
<td>Poultice for inflammation, boils and abscesses</td>
<td>Leaves</td>
</tr>
<tr>
<td>Kawayan</td>
<td><em>Bambusa spinosa</em></td>
<td>Gramineae</td>
<td>Emmenagogue, anthelminthic, antispasmodic, coughs, asthma, food</td>
<td>Roots, Leaves, young shoots</td>
</tr>
<tr>
<td>Alikbangon</td>
<td><em>Commelina diffusa</em></td>
<td>Commelinaceae</td>
<td>Swelling, burns, boils</td>
<td>Leaves</td>
</tr>
<tr>
<td>Salingbobog</td>
<td><em>Crataeva religiosa</em></td>
<td>Capparaceae</td>
<td>Stomach ache, swelling, rheumatism</td>
<td>Leaves, bark</td>
</tr>
<tr>
<td>Tinta-tinta</td>
<td><em>Eclipta alba</em></td>
<td>Asteraceae</td>
<td>Hepatitis, wound healing, asthma, skin diseases</td>
<td>Leaves</td>
</tr>
<tr>
<td>Gatas-gatas</td>
<td><em>Euphorbia hirta</em></td>
<td>Euphorbiaceae</td>
<td>Asthma, sedative, hemostatic</td>
<td>Leaves</td>
</tr>
<tr>
<td>Estrella</td>
<td><em>Isotoma longiflora</em></td>
<td>Campanulaceae</td>
<td>Asthma, wounds</td>
<td>Plant</td>
</tr>
<tr>
<td>Gating uwak</td>
<td><em>Monochoria vaginalis</em></td>
<td>Pontederiaceae</td>
<td>Boils, asthma, coughs, toothache</td>
<td>Leaves, roots</td>
</tr>
<tr>
<td>Kiapo</td>
<td><em>Pistia stratiotes</em></td>
<td>Araceae</td>
<td>Diuretic, coughs, asthma skin disease</td>
<td>Leaves</td>
</tr>
<tr>
<td>Kalachuchi</td>
<td><em>Plumeria rubra</em></td>
<td>Apocynaceae</td>
<td>Swelling, asthma, purgative</td>
<td>Leaves</td>
</tr>
<tr>
<td>Alagaw</td>
<td><em>Premna odorata</em></td>
<td>Verbenaceae</td>
<td>Coughs, headache</td>
<td>Leaves</td>
</tr>
</tbody>
</table>
C. diffusa exhibited the highest activity at 51.3%. It has shown antifungal activities against Trychophyton species and evaluated for its wound healing action. It was evaluated against 5-LOX where it exhibited 27% inhibition. The difference between the three mammalian lipoxygenases, 5-, 12-, and 15- is the carbon position where they catalyze arachidonic acid oxygenation.

E. hirta showed moderate inhibition at 48.5%. This plant has been previously evaluated against a wide range of activities which includes anti-diabetic, mutagenicity, antiviral, antibacterial and antifungal.

In the literature, natural compounds reported as 15-LOX inhibitors comprise of flavonoids isolated from orange peels, and from the leaves of Orthosiphon spicatus, terpenoids as 15-LOX inhibitors have also been isolated from sponges. The 15-LOX inhibitory activity of extracts from the leaves and seeds of Coriandrum sativum have been shown to be positively correlated to its phenolic content. In order to check for the presence of flavonoids, terpenoids, and other secondary metabolites in the plant samples a phytochemical screening was done. The result for the phytochemical screening is shown in Table 3. Phytochemical screening of plant extracts reveals the presence of flavonoids, and terpenoids, which have been proven in previous studies to be responsible for the suppression of LOX activity. Qualitative test for terpenoids, saponins, tannins, flavonoids, steroids, phenolic compounds, alkaloids and cardiac glycosides were performed. C. diffusa and E. birta were both positive for the presence of flavonoids and terpenoids. It is possible that the compounds responsible for the observed bioactivity belongs to the said family and specifically inhibits 15-LOX.

To the best of our knowledge, this is the first report of the evaluation of the 15-lipoxygenase inhibitory activity of the tested medicinal plant extracts. This work has scientifically validated the use of the plant extracts in folkloric medicine and C. diffusa and E. birta could contain new 15-lipoxygenase inhibitors.

**ACKNOWLEDGEMENT**

This project was funded by the Natural Sciences Research Institute of the University of the Philippines Diliman.
REFERENCES


