

# Inhibition on Lipoxygenase of the Flavonoids from *Selaginella Labordei*

## Abstract

LOX inhibitory activities of six Flavonoids compounds from medicinal plants of *Selaginella labordei* are investigated. Six Flavonoids have been isolated from the active fraction, and the 50% inhibitory concentration (IC<sub>50</sub>) of the each sample against LOX in vitro was determined, using a ultraviolet spectrophotometer with kinetics analysis software. The six Flavonoids all have obvious inhibitory action on lipoxygenase. Among them, two kinds of Flavonoids were more active in inhibiting lipoxygenase than others the IC<sub>50</sub> of amentoflavone was 7.03 $\mu$ M and robustaflavone was 8.05 $\mu$ M. Six Flavonoids are active compounds in inhibiting lipoxygenase from *S. labordei*.

**Keywords:** *Selaginella labordei*; Flavonoids; lipoxygenase

## Research Article

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**Xu Jiacheng, LI Li and Chen Keli\***

Traditional Chinese Medicine Resource and Compound Prescription, Hubei University of Chinese Medicine, China

**\*Corresponding author:** Chen Keli, Key Laboratory of Ministry of Education for Traditional Chinese Medicine Resource and Compound Prescription, Hubei University of Chinese Medicine, Wuhan 430065, PR, China, Email: kelichen@126.com

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## Introduction

*Selaginella labordei* Hieron. ex Christ is a rare medicinal plant from *Selaginellaceae* with activities in anti-tumor [1], anti-virus[2], inhibiting xanthine oxidase [3-4] and lipoxygenase (LOX) [5]. LOXs are involved in the biosynthesis path of leukotrienes, prostaglandins and induce the oxidation of fatty acid, and also involved in the pathologic process of inflammation, mutagenesis and cancer [6]. It is found that the ethyl acetate extracts from the whole herb of *S. labordei* showed obvious inhibitory activities against LOX according to our investigation [5]. But the concrete active compounds inhibiting LOX have not been known yet. So, six kinds of Flavonoids compounds were isolated from the active fraction ethyl acetate extract of *Selaginella labordei* under the direction of activity test [3,7]. In this paper, lipoxygenase inhibitory activities in vitro of 6 Flavonoids compounds isolated from *S. labordei* were determined, using a ultraviolet spectrophotometer with kinetics analysis software, so that to provide with experimental basis for clinical applications of this herb.

## Materials

### Reagents and apparatus

Lipoxygenase and linoleic acid were purchased from Sigma Chemical Co.(St.Louis,MD). Quercetin from National Institute for the Control of Pharmaceutical and Biological Products. Dimethyl sulfoxide (DMSO) and Tween-20 from Amresco Inc (USA). All other reagents used were of analytical grade. DU640 Spectrophotometer with dynamics/time software (Beckman, USA).

### Plant materials and compounds

The plant materials of *Selaginella labordei* were collected

in September, 2005 from Lushan Mountain of Jiangxi Province, China, and authenticated by Professor Chen Keli, and the plant specimens were deposited at the herbarium of the Hubei University of Traditional Chinese Medicine. The air-dried and powdered plant material was extracted at room temperature with EtOH, then extracted with EtOAc, separated and purified to afford the total Flavonoids by defatting and macroporous resin method. The total Flavonoids was further separated and refined to give six flavone compounds. The purity of each of the six Flavonoids was more than 97% quantitated by HPLC. The structures of these compounds (Figure1) were elucidated by means including UV, IR, 1H-NMR, 13C-NMR, HSQC, HMBC, HR-TOFMS, EI-MS, DEPT and COSY [3,7,8].

### Preparation of test solution

Phosphate buffer (0.2 M, pH 7.0): Dissolve K<sub>2</sub>HPO<sub>4</sub> 11.34 g, KH<sub>2</sub>PO<sub>4</sub> 4.75 g, in 500mL water. Enzyme solution (11945units/mL): Dissolve 2.7 mg lipoxygenase (110,600units/mg) in 1mL phosphate buffer as stock solution, Get 40 $\mu$ L stock solution into 960 L phosphate buffer for assay. In the process of testing the solution must be keep at low-temperature with ice pack. Substrate solution: 0.01 M substrate stock solution: 157.2 $\mu$ L linoleic acid, 157.2 $\mu$ L Tween-20 and 10mL deionised water, was clarified by adding 1mL of 1 M NaOH and made to 50mL with water (Made fresh daily); Substrate buffer solution: substrate stock solution was diluted with 0.2 M phosphate buffer to give 3.3mM substrate, flush with oxygen for 2 min.

### Sample solution and reference substance solution

Weigh accurately all the above samples and positive reference substance quercetin and then dissolve respectively in DMSO (final concentrations<1%), and diluted appropriately in the experiment to afford various concentration of solutions.

## Methods

### Determination of LOX activity

Experiment was made, as described in related literature [9], the concentration of lipoxygenase being 11945 units/mL, and increase value in absorbance at 234 nm was recorded at 5 s intervals for 5 min. In any appropriate long period of time, absorbance was increased linearly with time, and the slope was reaction rate (dA/min). The greater slope shows the stronger of enzyme activity.

### Sample determination

The 6 Flavonoids samples were dissolved in DMSO to obtain various concentrations of solutions. To get appropriate quantity of solution into 2 mL quartz cuvettes, the above procedure was repeated and increase value in absorbance at 234 nm was recorded at 5 s intervals for 2 min. Each sample was determined 3 times in parallel, and reaction rate was recorded respectively, and inhibition rate of samples was calculated with averages.

### Blank control

Blank sample contains same amount DMSO in quartz cuvettes without lipoxygenase, and the above procedure was repeated, recording the changing value of absorbance.

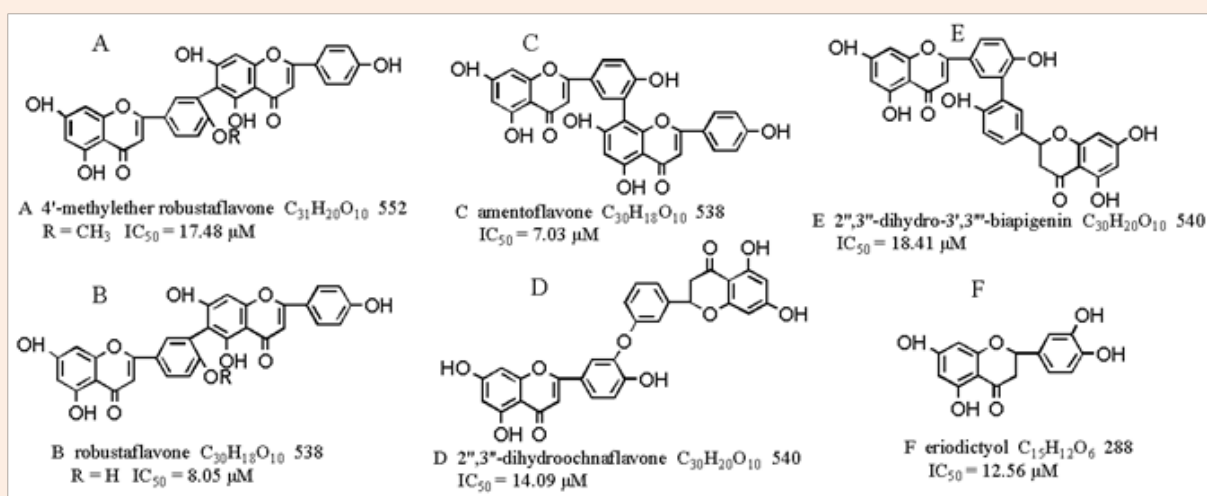
### Results

Data processing was carried on with the use of SPSS 13.0 statistical package. Firstly, inhibitory rate (IR) of samples on LOX was calculated according to the following formula:  $IR = [1 - (R_s - R_o) / (R_p - R_o)] \times 100\%$ , IR signifies inhibitory rate,  $R_s$ ,  $R_o$ ,  $R_p$  expressing respectively reaction rate of sample, blank and LOX in the formula. Then, regression equation was computed, taking inhibitory rate (IR) as a dependent variable and sample concentration (C) as the independent variable. Because IR and C exist the linear relationship, the 50% inhibitory concentration (IC<sub>50</sub>) of the sample was computed directly according to regression equation. The experimental results are shown in Table 1.

**Table 1:** Inhibition on LOX by six flavone compounds.

| Sample | Sample Name                         | Regression Equation        | IC <sub>50</sub> (μg/mL) | IC <sub>50</sub> (μM) |
|--------|-------------------------------------|----------------------------|--------------------------|-----------------------|
| A      | 4'-methylether obustaflavone        | $I_R = -6.937 + 5.902C$    | 9.65                     | 17.48                 |
| B      | obustaflavone                       | $I_R = -1.638 + 11.925C$   | 4.33                     | 8.05                  |
| C      | amentoflavone                       | $I_R = -1.934 + 13.739C$   | 3.78                     | 7.03                  |
| D      | 2'',3''-dihydrochonaflavone         | $I_R = -12.785 + 8.253C$   | 7.61                     | 14.09                 |
| E      | 2'',3''-dihydro-3'',3'''-biapigenin | $I_R = -2.036 + 5.235C$    | 9.94                     | 18.41                 |
| F      | eriodictyol                         | $I_R = 5.012 + 12.729C$    | 3.53                     | 12.56                 |
| X      | quercetin                           | $I_R = -11.811 + 519.095C$ | 0.12                     | 0.40                  |

Note: Correlation coefficient  $r > 0.98$ , of all samples.



**Figure 1:** The structures of six flavone compounds.

## Conclusions

From Table 1, it is shown that six Flavonoids have obvious inhibitory action on lipoxygenase. Among them, two kinds of Flavonoids were more active in inhibiting lipoxygenase than others, the IC<sub>50</sub> of amentoflavone was 7.03 μM and robustaflavone was 8.05 μM. So the six Flavonoids are the active compounds inhibiting lipoxygenase from *Selaginella labordei*. The inhibiting action on LOX of Flavonoids from *Selaginella labordei* is one of the mechanisms of anti-tumor and anti-virus action of the herb.

It is shown From Figure 1 that (B) robustaflavone and (A) 4'-methylether robustaflavone belong to homologue, the difference in their structures only displays in (B) 4'-OH and (A) 4'-OMe. It is indicated from the experimental data that the activity inhibiting LOX is probably reduced to one half after the H in (B)4'-OH is replaced by Me(-CH<sub>3</sub>), and their IC<sub>50</sub> respectively are 8.05μM and 17.48μM. This is only a preliminary inference to the relations between structure and effect, when further experimental study in more Flavonoids is obtained, also combined with computer auxiliary medicine design, and finally construction effect relations of LOX inhibitors will be confirmed and clarified.

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