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Research Article

INHIBITION OF LIPOXYGENASE ACTIVITY FROM *AVERRHOA CARAMBOLA* L. LEAF EXTRACTS

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ABSTRACT

Objective: Inflammation is a protective response to tissue injury caused by physical trauma, harmful chemicals, or microbiological substances. Leukotriene, which originates in the lipoxygenase pathway, is a mediator of inflammation. *Averrhoa carambola* L. leave contains flavones, including apigenin, which exhibits potential anti-inflammatory activity through the inhibition of lipoxygenase activity. The present study aimed to examine the lipoxygenase inhibition activity of *A. carambola* leaf extracts.

Methods: *A. carambola* leaf extracts were obtained using multilevel maceration using the solvents n-hexane, ethyl acetate, and ethanol. Subsequently, total flavonoid contents in the three extracts were determined using the colorimetric method. Apigenin content in the most active extract was determined using high-performance liquid chromatography.

Results: The extract with the highest lipoxygenase inhibition activity was the ethyl acetate extract with an IC_{50} value of 10.17±0.83 µg/mL followed by the n-hexane and ethanol extracts with IC_{50} values of 40.99±0.51 and 35.61±0.66 µg/mL, respectively. Total flavonoid content from the n-hexane, ethyl acetate, and ethanol extracts was 3.01, 24.24, and 8.24 mg quercetin equivalent/g extract, respectively. A qualitative test using thin-layer chromatography showed that all extracts contained apigenin. The most active (ethyl acetate) extract contained 5.39% apigenin with a high-performance liquid chromatography method.

Conclusion: The ethyl acetate extract of *A. carambola* leaf is the most active extract to inhibit lipoxygenase activity. Flavonoid content has a strong correlation to the inhibitory activity of each extract.

Keywords: Inflammation, *Averrhoa carambola* L., Lipoxygenase, Apigenin.

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INTRODUCTION

Inflammation is a protective response against tissue injuries caused by physical trauma, harmful chemicals, or microbiological substances [1]. The process of inflammation is initiated following a stimulus that damages the cell; subsequently, the cell releases phospholipids, which contain arachidonic acid. Phospholipase activity results in the separation of arachidonic acid from phospholipids, after which arachidonic acid is activated by lipoxygenase. Arachidonic acid is then converted into unstable forms (hydroperoxide and endoperoxide) that are then metabolized into leukotrienes (LTs), which are associated with an inflammatory response [2]. LTs are inflammatory mediators formed in the lipoxygenase pathway. The inhibition of LT formation helps in reducing the incidence of inflammatory responses.

Inflammation is commonly treated using a class of synthetic drugs known as nonsteroidal anti-inflammatory drugs (NSAIDs) as well as steroids. However, NSAIDs can cause undesirable side effects, particularly gastrointestinal disorders such as abdominal pain and gastric ulcers, as well as anemia due to bleeding in the digestive tract [3]. Thus, there is a need for the development of natural medicines to overcome the side effects of inflammatory drugs currently available in the market. Star fruit (*Averrhoa carambola* L.) is widely cultivated in Indonesia, particularly on a large scale in Depok. This plant has been reported to contain natural flavonoids, particularly flavones, namely, apigenin, which has potential anti-inflammatory activity [4].

Based on the results of a previous study, *A. carambola* leaf extracts can be empirically used as an external medication to treat chickenpox, ringworm, and headaches by applying them to the affected parts. In addition, the decoction of *A. carambola* leaves relieves angina, oliguria, ulcers, bacterial skin infections (pyoderma), postpartum edema, gastroenteritis, and traumatic wounds [5], while ethyl acetate fractions from *A. carambola* leaves inhibit edema formation and cell migration in the ear skin of mice smeared with *Croton tiglium* L [6]. The findings above demonstrate that the ethyl acetate fraction of *A. carambola* leaves has anti-inflammatory properties. Based on these data, we aimed to assess the inhibition activity of *A. carambola* leaf extracts against lipoxygenase.

MATERIALS AND METHODS

Materials

n-Hexane, ethyl acetate, and ethanol extracts of *A. carambola* leaves were obtained from the Laboratory of Phytochemistry and Pharmacognosy, Faculty of Pharmacy, Universitas Indonesia.

Extraction

Leaves powder of *A. carambola* was extracted using multilevel maceration using a non-polar solvent (n-hexane), semi-polar solvent (ethyl acetate), and polar solvent (70% ethanol) at a ratio of 1:10 (sample: solvent). The maceration time used for one cycle was 24 h, with occasional stirring during the first 6 h and then remaining for 18 h. This process was repeated 4 times. Then, the extract was evaporated using a rotary vacuum evaporator for 4 days or a water bath at 60°C for 7–8 days to obtain a thick and concentrated extract.

Lipoxygenase inhibition assay

The inhibition of lipoxygenase activity was determined using soybean lipoxygenase (final concentration, 75 U/mL), linoleic acid substrate (final concentration, 75 µM), 200 mM boric buffer (pH=9), and methanol. Apigenin was used as a positive control. For the assessment, a mixture of 50 µL of apigenin standard solution or sample of n-hexane, ethyl acetate and ethanol extracts of *A. carambola* leaves (various concentrations); 1650 µL borate buffer (pH=9.0); and 1000 µL linoleic

acid (substrate), was incubated for 10 min at 25°C. Subsequently, 300 µL of lipoxygenase was added to the mixture, which was incubated again for 15 min at 25°C. Finally, 1000 µL of methanol was added and the absorbance was measured using a UV–Vis spectrophotometer at a wavelength of 234 nm. Spectrophotometric measurements were conducted in triplicate. The percentage of lipoxygenase inhibition was calculated using the following formula [7]:

$$\text{Lipoxygenaseinhibition}(\%) = \frac{(A-B)-(C-D)}{(A-B)} \times 100\%$$

A = Absorbance of blank solution with lipoxygenase.
B = Absorbance of blank solution without lipoxygenase.
C = Absorbance of positive control or sample solution with lipoxygenase.
D = Absorbance of positive control or sample solution without lipoxygenase.

The percentage of lipoxygenase inhibitions obtained was plotted onto a graph to obtain a linear regression equation in the form of $y=a+bx$, which was used to determine IC_{50} values.

Determination of total flavonoid content

Total flavonoid content was determined using the $AlCl_3$ colorimetric method by adding 0.5 mL standard quercetin to the extracts, followed by the addition of 1.5-mL ethanol P, 0.1-mL P 10% $AlCl_3$, 0.1-mL 1M sodium acetate, and 2.8-mL distilled water. The mixture was shaken and allowed to stand for 30 min at room temperature and then its absorbance was measured at the maximum absorption wavelength (430 nm). Blank measurements were similarly conducted without the addition of $AlCl_3$. The absorbance values of the quercetin standard obtained were used to plot a calibration curve, which were used to determine the total flavonoid content of the extract.

Calculation of apigenin content in the ethyl acetate extract

Ethyl acetate extract was analyzed using high-performance liquid chromatography (Waters, Milford, MA, USA) using a SunFire C_{18} column (4.6 × 150 mm; Waters) at a flow rate of 1 mL/min and an injection volume of 20.0 µL. A photodiode array detector (Waters) was used for detection at a wavelength of 340 nm. The mobile phase used was acetonitrile: water (45:55) with 0.1% formic acid.

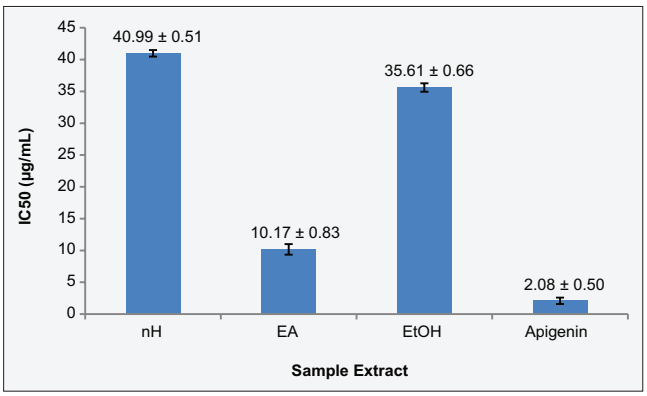

Fig. 1: Graph indicating the difference among IC_{50} values of all extracts and standard against lipoxygenase activity. nH=sample from extraction with n-hexane, EA=sample from extraction with ethyl acetate, EtOH=sample from extraction with an ethanol solvent

RESULTS AND DISCUSSION

Extraction

A. carambola leaf extracts were obtained using multilevel maceration. Multilevel extraction using different polar solvents is employed to separate less polar compounds from polar compounds. Hexane retains non-polar compounds such as triterpenoids, steroids, fats, and chlorophyll, whereas ethyl acetate retains semi-polar compounds such as flavonoids. On the contrary, ethanol retains polar compounds such as alkaloids, tannins, glucides, and flavonoids [8]. The ratio of sample to the solvent used for extraction was 1:10 [9]. The yield of the extract obtained after extraction is indicated in Table 1.

Lipoxygenase inhibition assay

The underlying principle of the present method determining lipoxygenase inhibition activity is the production of hydrogen peroxide by lipoxygenase [10], which is characterized by a decrease in absorbance in the presence of the inhibitor. Here, apigenin was used as the positive control as an inhibitor. According to Deschamps *et al.* [11], the higher the concentration of apigenin used, the more electrons are donated to reduce the active sites of iron in the lipoxygenase enzyme. Thus, the susceptible electrons are not oxidized to form hydroperoxy-9 and 11-octadecadienoic acid products. The lipoxygenase inhibition assay results based on apigenin showed an IC_{50} value of 2.08 µg/mL, while the n-hexane, ethyl acetate, and methanol extracts of *A. carambola L.* leaves had IC_{50} values of 40.99±0.51, 10.17±0.83, and 35.61±0.66 µg/mL, respectively [Fig. 1].

Determination of total flavonoid content

Total flavonoid content was determined using quercetin as the standard. A calibration curve was plotted based on the absorbance values of quercetin obtained using a UV–Vis spectrophotometer. The calibration curve yielded the following linear regression equation: y=0.082x–0.006 (r=0.9994). This regression equation was used to determine total flavonoid content in extracts. Total flavonoid content is expressed as quercetin equivalent (QE), which is the number of milligrams equivalent to quercetin in 1 g sample. The n-hexane, ethyl acetate, and ethanol extracts contained 3.01, 24.24, and 8.24 mg QE/g sample, respectively. Total flavonoid content in the ethyl acetate extract was higher than that in the n-hexane and ethanol extracts; therefore, we concluded that lipoxygenase activity was inhibited by flavonoids. The results also demonstrated that higher total flavonoid level was associated with lower IC_{50}.

Determination of apigenin content

Apigenin content was determined only in the extract that showed the highest lipoxygenase inhibition activity using apigenin as a standard. Using the obtained regression equation, the value obtained in the determination of ethyl acetate extract samples was plotted in the graph to obtain apigenin content. Apigenin content in the *A. carambola* leaf ethyl acetate extract was 5.39% based on the calculation included in Table 2. Chromatogram profile from high-performance liquid chromatography analysis is shown in Fig. 2.

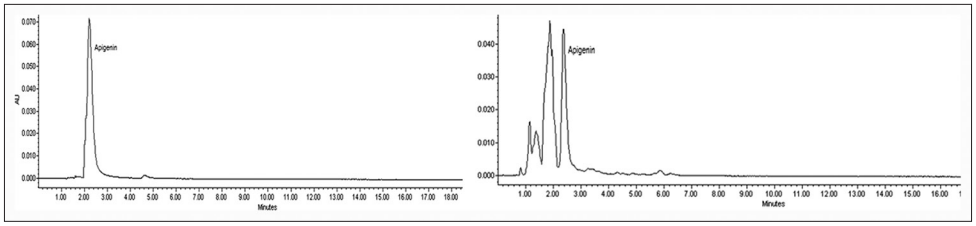

Fig. 2: Chromatogram profile from high-performance liquid chromatography analysis. Mobile phase: Acetonitrile:water (45:55) with 0.1% formic acid

Table 1: Yield extraction of star fruit leaf (*Averrhoa carambola* L.)

Extraction solvents	Dried sample (g)	Extract (g)	Yield (%)
n-Hexane	500	9.46	1.89
Ethyl acetate		15.08	3.01
Ethanol		147.92	29.58

Table 2: Apigenin content of ethyl acetate extract

Sample concentration (µg/mL)	Area	Linear regression	Apigenin content
200	558027	y=53899x−22633, r=0.999	5,39

CONCLUSION

The ethyl acetate extract was the most effective in inhibiting lipoxygenase activity with an IC_{50} value of 10.17±0.83 µg/mL, total flavonoid content of 24.24 mg QE/g extract, and 5.39% apigenin. Flavonoid content has a strong correlation to the inhibitory activity of each extract.

ACKNOWLEDGMENTS

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CONFLICTS OF INTEREST

We declare that we have no conflicts of interest.

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