ORIGINAL ARTICLE

Fractionation of an ethanolic extract of purple leaves (*Graptophyllum pictum*) with antioxidant and lipoxygenase activity inhibition assay

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ABSTRACT

Graptophyllum pictum offers therapeutic potential that has received attention from researchers around the world. The purple leaf is native to New Guinea and has been widely distributed, including Indonesia. This study aims to determine the antioxidant activity and potential inhibition of the lipoxygenase (LOX) enzyme in the hexane fraction, ethyl acetate fraction, and water fraction of purple leaf. Samples were extracted by maceration using 96% ethanol, followed by multilevel fractionation using ethyl acetate, hexane, and water solvents. The fractions were determined for their antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl method, and their potential in LOX enzyme inhibition was analyzed using an ultraviolet-vis spectrophotometer. The ethyl acetate fraction showed the highest antioxidant activity with inhibition concentration (IC_{50}) of 17.23 μg/mL; LOX inhibition was also demonstrated by the highest ethyl acetate fraction with IC_{50} 133.47 μg/mL, followed by the hexane fraction, and then the water fraction. These results suggest purple leaves with ethyl acetate fraction can be a new drug innovation with antioxidant and anti-inflammatory properties, and this study can be used as an evaluation material for further drug development.

Key words: Drug development, Indonesia herbs, multilevel fractionation, purple leaves, stress oxidative

INTRODUCTION

Indonesia takes great pride in the abundant variety of medicinal plant species that can be harnessed for herbal medicine in the health industry. *Graptophyllum pictum* is found in Indonesia due to its fertile growth in the tropics. It is known as the "purple leaf" and is referred to as a caricature plant because of its colorful leaves. Purple leaves are reported to have active ingredients such as flavonoids, phenolics, alkaloids, tannins, saponins, glycosides,[1,2] steroids, anthraquinones, coumarins, and sugars.[3] A study by Makkiyah *et al*.[2] Ismail and Maddiah[4] showed the presence of flavonoid metabolite content in purple leaves, which has a high potential as an antioxidant. The flavonoid in purple leaves has anti-inflammatory activity,[5] as shown *in vivo* decrease in cyclooxygenase (COX) and superoxide dismutase levels.[6]

When there is an imbalance between antioxidants and reactive oxygen species (ROS), oxidative stress builds. Antioxidants bind free radicals produced by phagocytic

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leukocytes during inflammation. ROS contributes to prostaglandins' formation and arachidonic acid's conversion into pro-inflammatory intermediates through COX and lipoxygenase (LOX).[7,8] In addition, LOX can oxidize fatty acids such as linoleic acid, an unsaturated fat, thereby increasing the risk of chronic inflammatory diseases, including rheumatoid arthritis, autoimmune diseases, psoriasis and ankylosing spondylitis, and high blood pressure and heart disease.[9]

The extraction process of plant extracts is influenced by the diverse active compounds found in plants. Fractionation is a method used to separate main compounds from other groups, allowing for a better understanding of phytochemical properties because it can be a reference in estimating the nature of the polarity of the compounds to be separated.[10]

Numerous studies have examined the fractionation of purple leaves, yielding varying. These studies aim to establish the effectiveness of hexane, ethyl acetate, methanol, water, and butanol solvents in fractionating purple leaves. An essential point in isolating target components is the choice of solvent. Various solvents have been reported for their ability to obtain purple leaf target compounds, including fractions with ethanol solvent, showing antibacterial activity.[11] n-hexane is used as an effective solvent in producing compounds with analgesic properties and shows the highest cytotoxic.[12,13] The ethyl acetate fraction showed antioxidant properties with high flavonoid levels, and cytotoxic properties against MCF-7 cells were produced from hexane, ethyl acetate, and water fractions.[3] The ethyl acetate fraction and water fraction had inhibitory power through carrageenin-induced rat edema test.[5,14] From the various studies related to the type of solvent used to evaluate the bioactivity of purple leaves, until now, no information has been found regarding the evaluation of antioxidant activity with LOX enzyme inhibition of purple leaf fractions. This is important because discovering antioxidant or anti-inflammatory profiles can be scientific evidence for developing natural materials and becoming a solution for human diseases or metabolic disorders. Therefore, this study aims to obtain fractions of the ethanol extract of purple leaves and assess their antioxidant activity and LOX enzyme inhibition. This research is anticipated to contribute to the drug development in the healthcare industry significantly.

MATERIALS AND METHODS

Plant material

Purple leaf was authenticated with the number BMK0164092016 by Taopik Ridwan and was taken from the Tropical Biopharmaca Research Center, Bogor Agricultural University, Indonesia. Sampling was harvested in July 2023. The flowchart of this study is shown in Figure 1.

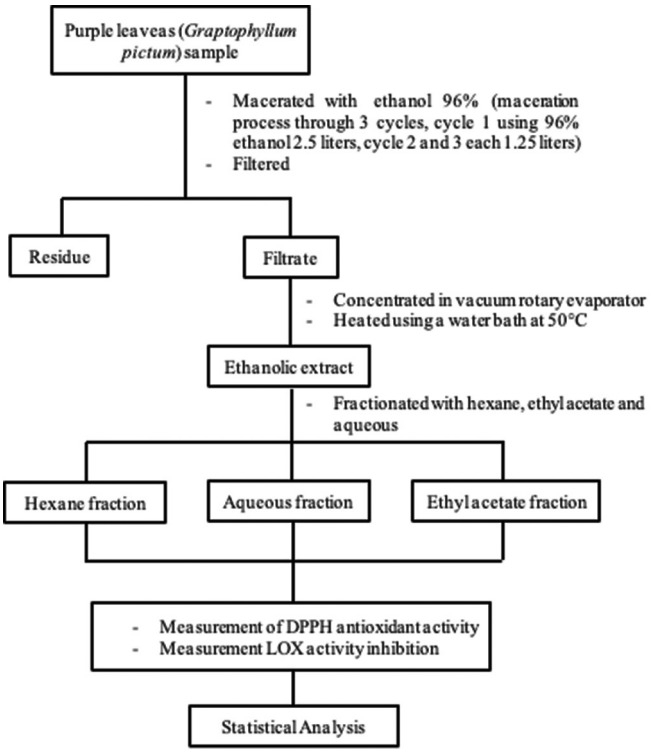

Figure 1: Flow chart of the stages of this research work

Preparation and extraction of plant extract, multilevel fractionation

The sample extraction process refers to the research of Rustini and Arianti.[15] Fractionation was carried out referring to the procedure of Ibrahim (2019) in Sulistyowati *et al*. with modification.[16] Fractionation was carried out by dissolving 10 g of purple leaf extract into 100 mL of warm distilled water at 50°C and sonicating using an ultrasonic device to increase the solubility of the extract. The solution was put into a separatory funnel, and then, 100 mL of hexane was added and shaken until the water phase partitioned into the hexane phase. When the hexane phase was saturated, the hexane was collected in a beaker. Next, the extract in the aqueous phase was added back with the same amount of hexane. The fractionation process with hexane was carried out four times until a clear solvent was obtained. Fractionation was continued with ethyl acetate with the same procedure. Each fraction was then evaporated to obtain hexane, ethyl acetate, and water fractions.

2,2-diphenyl-1-picrylhydrazyl antioxidant activity assay

Antioxidant activity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH)-free radical scavenging method. This method refers to the research of Selvia *et al*.[17]

Lipoxygenase inhibition assay

The LOX inhibition method refers to the research of Listiyani *et al*.[18] LOX inhibitory activity testing against baicalein solution was performed with the same procedure

with the concentration that has been prepared. Blank and blank control were made without adding a sample, and the sample control was made without adding an enzyme. The inhibition concentration (IC_{50}) value was determined using an equation derived from the percentage inhibition value and the concentration tested. The formula used to calculate the percentage of inhibition is:

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

A: Absorbance of blank

B: Absorbance of control blank

C: Sample absorbance

D: Absorbance of control sample.

Statistical analysis

The data were provided as mean ± standard deviation. The data were analyzed using the IBM SPSS Statistic Version 22 (SPSS Inc; IBM, Chicago IL, US). The data were analyzed using one-way ANOVA with significance level ($P < 0.05$).

RESULTS

This study resulted in an extract weight of 18.571 and a yield of 9.3%. The fractionation results were significantly different ($P < 0.05$). Table 1 shows the weight of each fraction produced.

2,2-diphenyl-1-picrylhydrazyl antioxidant activity

Table 2 and Figure 2 show the results of the DPPH antioxidant test expressed in terms of IC_{50} with ascorbic acid as the comparator compound. The ethyl acetate fraction has the highest antioxidant capacity, with the lowest IC_{50} value of 17.23 µg/mL, compared to ascorbic acid capacity of 2.58 µg/mL, followed by the hexane and aqueous fractions.

Optimization of enzyme concentration and substrate concentration linoleic acid

Based on the results [Figure 3], the optimum condition for LOX to bind with the linoleic acid substrate is at a concentration of 6.25 U/mL; this is indicated based on the highest absorbance. Furthermore, when the enzyme concentration was increased to 7.5 U/mL, the product produced decreased, as indicated by the decrease in the measured absorbance value. Figure 4 shows the optimum substrate concentration at 60 µM. When the concentration was increased to 70 µM, absorbance decreased, which means that the product produced decreased.

The lipoxygenase inhibition activity

This LOX inhibitory activity indicates the potential of purple leaves as an anti-inflammatory. The LOX inhibition results for each fraction were not significantly different ($P = 0.06$), as shown in Table 2. The ethyl acetate fraction had the highest LOX inhibition (IC_{50} 133.47 µg/mL), followed by the hexane fraction (IC_{50} 153.8 µg/mL), and the water fraction had the lowest LOX inhibition with the highest IC_{50} value (IC_{50} 162.84 µg/mL), while baicalein showed an IC_{50} value (0.22 µg/mL) [Figure 5].

DISCUSSION

This study separates plant extracts into many fractions based on their polarity properties. The solvent is hexane, which has very low polar properties; ethyl acetate is semipolar, and aqueous is polar.[19] This current study suggests that purple

Table 1: Fraction weight from the fractionation results

Solvents fraction	Weight (g)
Hexane	4.902±0.0015[c]
Ethyl acetate	0.730±0.0015[a]
Aqueous	0.929±0.0131[b]

Significant differences ($P<0.05$) were found between letters in the same column after analysis of variance and Tukey comparison test

Table 2: The half-maximal inhibitory concentration value of 2,2-diphenyl-1-picrylhydrazyl scavenging and lipoxygenase inhibition of fraction

Fractions/ standards	DPPH radical scavenging, IC_{50} µg/mL ($P=0.25$)	LOX inhibition, IC_{50} µg/mL ($P=0.06$)
Hexane	69.49±8.07	153.8±15.94
Ethyl acetate	17.23±4.27	133.47±9.29
Aqueous	75.10±6.98	162.84±23.51
Ascorbic acid	2.58±0.85	ND
Baicalein	ND	0.22±0.23

Significant differences with value $P>0.05$ are shown in parentheses.
DPPH: 2,2- diphenyl-1-picrylhydrazyl, ND: No determined, IC_{50}: Half-maximal inhibitory concentration, LOX: Lipoxygenase

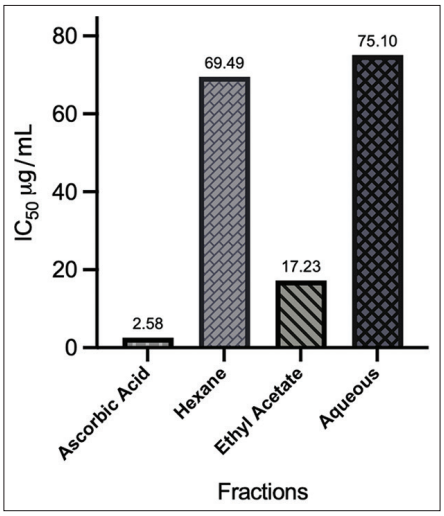

Figure 2: Graph of inhibition concentration of antioxidant activity of purple leaves fraction

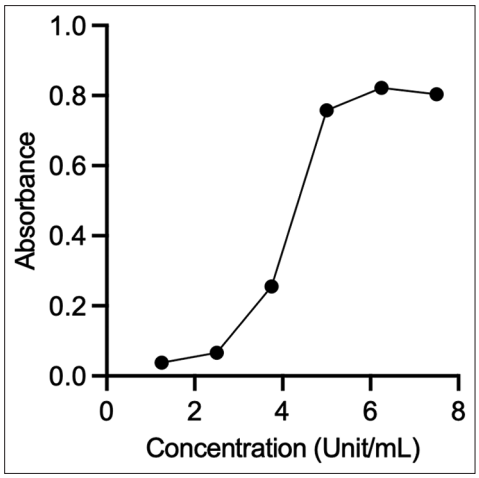

Figure 3: Enzyme concentration optimization result curve

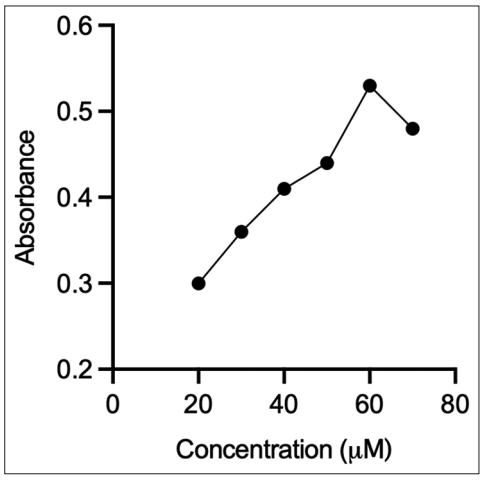

Figure 4: Optimization of substrate concentration linoleic acid

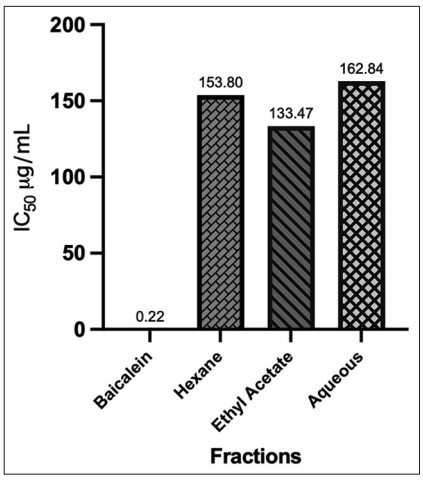

Figure 5: Graph of inhibition concentration of lipoxygenase inhibition of purple leaves fraction

leaves' antioxidant activity and LOX enzyme inhibitory activity are mostly produced from semipolar fractions. Compounds that can be attracted to semipolar solvents are flavonoids, saponins, alkaloids, steroids, and phenolics.[20-22] Antioxidants are a group of compounds that can minimize free radicals and ROS that occur within cells. The capacity of substances to function as antioxidants is usually evaluated using the DPPH free radical technique. In this study, all fractions showed the ability to inhibit DPPH free radicals through IC_{50} values. The ethyl acetate fraction showed the highest antioxidant activity and was followed by aqueous and hexane. As is known, ethyl acetate is semipolar and can extract compounds that are also semipolar in nature.[23] Phenolic and flavonoid active compounds are the active compounds behind antioxidant activity; these compounds work through their ability to reduce the formation of free radicals.[20,24]

LOX is a class of oxidative enzymes that produce pro-inflammatory mediators known as leukotrienes or anti-inflammatory mediators known as lipoxins at their active sites.[25] Medicinal plant bioactives may act as LOX enzyme inhibitors. Inhibition of LOZ enzyme is important, because LOX is an enzyme that can convert arachidonic acid into leukotrienes and eicosanoids that cause allergic and inflammatory reactions. Thus, LOX inhibitors will prevent the production of leukotrienes, and in consequence, the inflammatory process reduces.[26] Plant LOX is known to be inhibited by antioxidant compounds, such as polyphenolic compounds or flavonoid content.[14] In the results of this study, the ethyl acetate fraction has the highest LOX inhibition; the lowest IC_{50} value indicates this. This is considered a correlation between purple leaf flavonoid compounds and their activity in inhibiting LOX enzymes. This result is in line with Subardini *et al*., which gives ethyl acetate as the mechanism of LOX enzyme inhibitor fraction.[14] However, there is an antagonistic result that the research of Ozaki *et al*.[5] suggested ethanol extract (50%) of purple leaves from the aqueous fraction providing better anti-inflammatory properties compared to semipolar solvents. The reason for the different results might be the 50% ethanol concentration used to extract purple leaves. The study utilized an *in vivo* anti-inflammatory testing mechanism to evaluate the fractions on carrageenan-induced rat edema and the vascular permeability test and writhing test induced by acetic acid, which may contribute to the varied results. There are many studies that resulted in similar facts. According to previous research by Makkiyah *et al*.,[27] purple leaves extracted with ethyl acetate solvent contain xanthohumol metabolites, which are flavonoids and correlate to antioxidants and anti-inflammatory properties.[28] Jiangseubchatveera *et al*.[3] and Rustini and Arianti[15] presented the highest antioxidant activity also from the ethyl acetate fraction. This study provides the latest information on the bioactivity of purple leaves, so it is important to further innovate purple leaves by isolating target compounds that are efficacious as antioxidant and anti-inflammatory and can support applications in the health industry.

CONCLUSION

All fractions showed antioxidant activity and LOX inhibition. The ethyl acetate fraction exhibited the highest antioxidant capacity (IC_{50} 17.23 µg/mL), followed by the hexane fraction (IC_{50} 69.49 µg/mL) and the water fraction (IC_{50} 75.10 µg/mL). The ethyl acetate fraction inhibited LOX the most (IC_{50} 133.47 µg/mL), followed by the hexane fraction (IC_{50} 153.8 µg/mL), and the water fraction (IC_{50} 162.84 µg/mL). The results of all investigations show that ethyl acetate fraction is the best in providing antioxidant and anti-inflammatory activities, so this can be the latest innovation in the future health industry's development.

Author contributions

SA performed all the procedures in the laboratory under the supervision of FAM, who wrote the manuscript. EPR and DLCP reviewed the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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