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Comparison of Anti-Inflammatory Activity between Fermented and Dried Breadfruit Leaves Extract *(Artocarpus Altilis)*

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Abstract

Breadfruit leaves (*Artocarpus altilis*) have been empirically used to treat inflammation. Biosynthesis process such as fermentation can possibly changes the composition of secondary metabolites in breadfruit leaves. The aim of this study was to compare the anti-inflammatory activity between fermented and natural-dried breadfruit leaves extract. Fermented and dried breadfruit leaves were extracted with soxhlet device using methanol solvent. The anti-inflammatory activity of both extracts were evaluated using carrageenan (1% w/v) foot edema model of inflammation in male Wistar rats. The edema was measured with plethysmometer every 1 hour for 6 hours. The results showed that both extracts exhibited anti-inflammatory activity. 50 mg and 100 mg fermented leaves extracts exhibited 37.17% and 71.82% inhibition, while the same concentrations of dried extract exhibited 39.50% and 57.72%, inhibiton respectively. In conclusion, the best anti-inflammatory activity was shown by fermented breadfruit leaves extract.

Keywords: *Artocarpus altilis*, anti-inflammatory, breadfruit, fermentation

Introduction

Indonesia has the highest biodiversity in the world. Of 30 thousands of plants species, more than 1000 species have been used for medical treatment purpose. Breadfruit plant (*Artocarpus altilis*) is one of the plants that has been empirically used to treat inflammation, skin diseases, and liver cirrhosis.[1,2]

Previous study showed that breadfruit leaves (60 mg/kg) had potent anti-inflammatory effect.[3] A study conducted by Riasari *et al* showed that cytostenone which was successfully isolated from the breadfruit leaves is considered responsible for its anti-inflammatory activity.[3-5] Biosynthesis process, such as fermentation can possibly changes the composition of secondary metabolites in breadfruit leaves.[6]

Currently, there is limited information whether the fermentation process could enhance anti-inflammatory activity of the breadfruit leaves. Therefore, this study was conducted to compare anti-inflammatory activity between fermented and dried breadfruit leaves extract.

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Table 1. % Inhibition of Breadfruit Leaves Extract on Inflammation

Dosage Treatment	T1(%)	T2(%)	T3(%)	T4(%)	T5(%)	T6(%)
Positive control	57.93	63.82	43.05	47.11	59.60	77.56
50 mg fermented leaves extract	41.11	27.51	27.51	32.69	37.17	37.17
100 mg fermented leaves extract	47.19	67.12	67.12	69.83	71.82	71.82
50 mg dried leaves extract	27.08	10.26	30.21	35.19	39.50	39.50
100 mg dried leaves extract	34.37	41.67	50.24	54.16	57.72	57.72

Methods

Plant determination was conducted at Herbarium, School of Biotechnology Science, Bandung Institute of Technology. Instruments used in this study included soxhlet extraction device, rotary evaporator, and plethysmometers. Materials used in this study included natural-dried breadfruit leaves, fresh breadfruit leaves, methanol solvent, male Wistar rats, 0.9% sterile NaCl, carrageenan, PGA, sodium diclofenac and distilled water. Fresh breadfruit leaves were washed, dried, and fermented for 5 days. After the fermentation process was complete, the leaves were dried at room temperature.

Fermented and natural-dried breadfruit leaves were assayed to determine ash content, percentage of ethanol soluble compounds, and percentage of water soluble compounds. Extraction was performed using soxhlet device at 60 ^{0}C. A total of 163.31 g of fermented breadfruit leaves were extracted with 1.8 l of methanol solvent. Extract of dried breadruit leaves was obtained by extracting 150 g of leaves with 1.8 l of methanol solvent. During extraction, the leaves were wrapped with filter paper and placed in the soxhlet device. The methanol solvent was placed into a flask and heated at approximately 60 ^{0}C. The solvent was evaporated and underwent condensation in the cooling device, before extracting the plant. The liquid extract was then concentrated using a rotary evaporator to obtain thick extracts. Phytochemical screening was performed to determine the composition of secondary metabolites in both extracts. The compounds tested included alkaloids, flavonoids, tannins, phenolics, triterpenoids, steroids, quinones, monoterpenes, sesquiterpenes, and saponins.

The anti-inflammatory activity of both extracts were evaluated using carrageenan (1% w/v) foot edema model of inflammation in the male Wistar rats. Carageenan was used as inducer of inflammation since it has more sensitive response to drugs.[7,8] The edema was measured with plethysmometer every 1 hour for 6 hours. Analysis of variance (ANOVA) was conducted. $P<0.05$ defined statistical significance. The activities of the extracts were compared with standard drug, sodium diclofenac.

Results and Discussion

Results of the plant determination showed that plant used in this study was *Artocarpus altilis* (Parkinson) Fosberg. Phytochemical screening showed that natural-dried breadfruit leaves contained flavonoids, steroids, triterpenes, monoterpenes, sesquiterpenes and phenols, while fermented breadfruit leaves contained flavonoids, steroids, monoterpenes, sesquiterpenoids, phenols, quinones and tannins. Our study showed that fermentation process resulted in

different secondary metabolites. Ash content in natural-dried breadfruit leaves was 37%, while in fermented breadfruit leaves was 20.50%. Determination of ash content is important to examine content of mineral and anorganic elements. Water soluble content in dried breadfruit leaves was 2%, while in the fermented leaves was 21.9%. In dried breadfruit leaves, ethanol soluble content was 2.2%, while in the fermented leaves was 4.95%. These tests were important to provide information related to dissolved compounds and to select appropriate solvent for extraction. This finding showed that fermented breadfruit leaves contained more polar compounds.

Soxhlet extraction was performed because secondary metabolites in breadfruit leaves were heat-resistant. Methanol was selected because it had better polarity compared to other solvents, thus it could extract more secondary metabolites. We obtained 8.91 g (5.94%) thick extract of natural-dried breadfruit leaves and 20.94 g (12.82%) thick extract of fermented breadfruit leaves.

% inhibition against inflammation can be seen in Table 1. We found that strongest inhibition was shown by 100 mg of fermented leaves extract (71.82%). The lowest inhibition was shown by 50 mg of fermented leaves extract (37.17%) and 50 mg of dried leaves extract (39.50%). Based on statistical analysis, 100 mg of fermented leaves extract had the best anti-inflammatory activity compared to other extracts. Geranyl flavonoids and its derivatives isolated from the breadfruit leaves were considered responsible for its anti-inflammatory activity.[9,10]

Conclusion

The strongest anti-inflammatory activity was exhibited by fermented breadfruit leaves extract.

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Conflict of interest

None declared.

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