

# Comparison of Anti-Inflammatory Activity Between Aerobic and Anaerobic Fermented Green Breadfruit Leaf Extract.

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**Abstract---** Inflammation is a local protective response caused by damage to tissue. Plants that have been scientifically proven to have anti-inflammatory properties of *Artocarpus altilis* (parkinson) Fosberg leaves. The biosynthesis process can cause changes in color, content and type of content that is in the breadfruit leaves. One of the biosynthetic processes is fermentation of fresh green leaves into fermented green leaves (HF). The purpose of this study was to determine the anti-inflammatory activity of the ethanol extract of green breadfruit leaves aerobic and anaerobic fermentation, then to determine the doses of ethanol extract of green breadfruit leaves aerobic fermentation and anaerobes which have the best anti-inflammatory activity. Simplisia extraction was carried out by maceration using ethanol as a solvent. The method in the testing process is testing anti-inflammatory activity using udemia inhibition method in rat test animals, and statistical data analysis. The results show that the *Artocarpus altilis* (parkinson) breadfruit leaf plant tested has anti-inflammatory activity. The results showed that anaerobic fermented green breadfruit leaf extract with a dose of 100 mg / kgBW showed the most effective anti-inflammatory effect with a percentage of inflammation inhibition of 96.3%.

**Keywords:** anti-inflammatory, aerobic and anaerobic fermented green, breadfruit leaves

## I. INTRODUCTION

*Artocarpus altilis* is a plant that is used by the Indonesian people as food and is also used as a drug known as breadfruit<sup>1</sup>. Traditionally people use breadfruit leaves for the treatment of liver disease, hypertension, diabetes, heart, kidney, toothache and itching<sup>2,3</sup>. Breadfruit leaves are also effectively used as a skin healer for swelling or itching, because breadfruit leaves contain phenols, quersetins and kaempferol.

Research on breadfruit plants shows the presence of flavonoid compounds, tannins, saponins, steroids / triterpenoids and glycosides<sup>4</sup>. Breadfruit leaves contain artocarpin flavonoid

compounds which have anti-inflammatory and anticancer activity<sup>5</sup>. And breadfruit leaves contain flavonoids which have anti-inflammatory effects by reducing the activation of an inflammatory mediator. <sup>6</sup>Reported that the breadfruit leaf ethanol extract gel was able to inhibit the inflammation of induced mouse feet.

Steroids are organic compounds of non-hydrolyzed sterol fat. Steroids have activities including anti-inflammatory, antidiabetic, antifungal. Steroids are found in almost all plants. Especially breadfruit leaves have steroids <sup>4</sup>. Based on research, breadfruit leaves has been successful in isolation of cytostenone compounds that belong to steroid secondary metabolites<sup>7</sup>.

The biosynthesis process can cause changes in color, content and type of content that is in the breadfruit leaves. One of the biosynthetic processes is fermentation of fresh green leaves into fermented green leaves (FGL). The process of changing from fresh green leaves to fermented green leaves was chosen because with this process it is expected that the compounds present in fresh green breadfruit leaves will turn into new compounds and can increase levels of the compounds in the fermentation process in breadfruit leaves. Leaf fermentation is done by stacking the leaves for 5 days after the washing process<sup>7</sup>.

From the results of research conducted by <sup>8</sup> the administration of fallen dry and fermented green leaf extract in male white rats wistar strain has anti-inflammatory activity, fermented green leaf extract gives the best anti-inflammatory results with inhibition percentage of 71.82% , compared to fallen dry.

Based on the results that have been studied, the research continues to see whether the differences in the aerobic and anaerobic fermentation processes carried out on breadfruit leaves can increase anti-inflammatory activity.

## II. EXPERIMENTAL

### A. *Plants Material*

Fresh green breadfruit leaves *Artocarpus altilis* (Parkinson) Fosberg obtained from the Batununggal District, Bandung. Determination was carried out at the Taxonomy Herbarium Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjajaran University, Bandung. Making simplicia is done in two ways, namely aerobic and anaerobic methods, the method is as follows: material in the form of fresh green breadfruit leaves as much as 80 leaves are collected and cleaned with water then drained, from 80 leaves divided into two stacks by aerobic and anaerobic methods. Aerobic fermentation is carried out as a pile of 40 leaves for 5 days in a room that still has air and light, the leaves are taken from pile 2 to pile 39. Anaerobic fermentation carried out as much as 40 leaves accumulation and put into an airtight plastic container, removed air using a vacuum and allowed to stand for 5 days, the leaves taken from the fermentation is the 2nd to 39th pile. After both methods are finished the leaves are dried by aerating.

### B. *Crude drug characterization*

Characterization of simplicia quality includes determination of total ash content, determination of water soluble extract, determination of ethanol soluble content, determination of drying shrinkage, determination of water content. The method is carried out in accordance with the methods established by the Indonesian Ministry of Health in 2000 in the Indonesian *Materia Medika* book.

### C. *Phytochemical screening*

Phytochemical screening was performed toward all extracts. Magnesium ribbon), HCl and amylalcohol for flavonoid compound.  $\text{FeCl}_3$  10% used for phenolic compound, gelatine for tannin, Dragendorf and Mayer reagents for alkaloid, Potassium hydroxide 5% for quinone, vanillin 10% in  $\text{H}_2\text{SO}_4$  for monoterpene and sesquiterpene, Lieberman-Burchard for steroid and triterpenoid<sup>9,10,11</sup> Saponins showed by a stable foam formation for 10 min after shaking of water extracts<sup>12</sup>.

### D. *Extraction*

Simplicia powder of 600 grams, from aerated and anaerobic fermented green breadfruit leaves which has been dried, as much as 300 grams, each extracted using maceration method, by means of simplicia put into a closed container added 96%

ethanol solvent as much as 1: 10, for 3x24 hour with a change of solvent for 3 times. Liquid extract is stored every day for 3 days by changing solvents. Then the liquid extract was evaporated with a rotary vaporator so that a thick extract was obtained.

### E. *Preparation of Test Animals*

Before use, the rats were adapted for  $\pm 7$  days and given food and drink in a controlled manner every day. Before testing, the rats were first satisfied for 18 hours and were still given a drink, the rats were grouped into 5 groups, each group consisted of 5 mice consisting of:

Group I: control group, given a carrier (2% PGA suspension).

Group II: the comparison group was given the diclofenac sodium drug 0.9 mg / kgBW.

Group III: fermented green breadfruit leaf extract with a dose of 50 mg / kgBW.

Group IV: were given fermented green breadfruit leaf extract at a dose of 100 mg / kgBW.

Group V: fermented green breadfruit leaf extract with a dose of 150 mg / kgBW.

### F. *Anti-inflammatory Testing*

Antiinflammatory effect testing is done by measuring mouse foot edema using a digital plethysmometer. The way it works is as follows: first the plethysmometer is cleaned first and calibrated using a surfactant solution. The rat's feet are marked as a measurement of the volume of the rat's feet. The volume of the rat's feet was measured as the initial volume ( $V_0$ ), that is the volume of the foot before being given the drug and induced with a carrageenan solution. Then all groups of mice were injected subplantar on the hind legs of the rat with carrageenan 0.1 ml. After the inflammation is formed the volume of the rat's feet is measured by dipping it into a plethysmometer. Then 1 hour after administration of carrageenan to each control group mouse was given an oral 2% PGA suspension. Each rat in the test group was given ethanol extract of breadfruit leaves in the form of a suspension, and the comparison group was given a sodium diclofenac suspension orally. The volume of the feet of mice was measured by dipping into a plethysmometer for an interval of 1 hour for 5 hours after injection of carrageenan solution. Anti-inflammatory activity of breadfruit leaf ethanol extract is shown by the percentage of edema formation. This treatment was carried out on ethanol extracts of green breadfruit leaves aerobic and anaerobic fermentation. The volume of the foot of the control group and the test group was compared, and calculated statistically so that it could be concluded whether a significant difference was obtained.

### III. RESULT

#### Taxonomic Determination

The taxonomic determination of breadfruit plants was carried out in "Herbarium Jatinangor", Plant taxonomy laboratory, department of biology FMIPA UNPAD, number of determination : 138/HB/02/2017. The plants used was *Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg as Family Moraceae. Fig 1 shows the picture of breadfruit leaves.



Figure 1. Breadfruit leaves

#### Crude drug characterization

Characterization of crude drug that was carried out include water content, water soluble materials, total ash content, ethanol soluble materials and loss on drying. The results can be seen in table 1.

Table 1. The results of the quality characteristics of simplicia breadfruit leaves aerobic and anaerobic fermentation.

Characterization	Simplicia		MMI (2000)
	Aerobic (%)	Anaerobic (%)	
Total ash content	13,5	20,5	≤ 10
Ethanol soluble materials	12	13	≥ 6,3
Water soluble materials	18	23	≥ 18
Loss on drying	12	12	≤ 10
Water content	12	18	≤ 10

The results of characterization on the aerobic and anaerobic breadfruit leaves on the water soluble extract and ethanol soluble extract met the requirements, while the ash content, drying shrinkage and water content did not meet the quality requirements of Indonesian medica materia. Determination of simplicia ash content of aerobic

breadfruit leaves 13.5% and anaerobic 20.5%. The results of the ash content are not in accordance with the literature  $\leq 10$  this is because the fermentation process carried out produces different compounds, one of which is organic and inorganic minerals, apart from being caused by the fermentation process, the process of taking leaves that are not sourced from cultivated plants affects the mineral content of plants .

Determination of water content, determination of water content is important to avoid simplicia overgrown with fungus so as to reduce the quality of simplex. And the results obtained for aerobic fermentation leaves are 12% while the anaerobic fermentation leaves are 18%. Both of them exceeded the literature limit, which is  $\leq 10$ . This is when the fermentation process produces water vapor, because there is still a respiration process on green breadfruit leaves which are stacked for 5 days and vacuumed (for anaerobic fermentation leaves) so that the resulting water content is very high.

#### Phytochemical screening

Phytochemical screening of fermented breadfruit leaves showed the presence of alkaloid, flavonoids, phenols, terpenoids/steroids, saponin and quinones. For the results of flavonoids, orange or yellow changes occur on the amyl alcohol layer. The addition of magnesium and hydrochloric powder causes the reduction of flavonoids to give red colour. Flavonoid compounds are a group of the largest phenol compounds found in nature. Flavonoids can act as antioxidants as free radical catchers because their hydroxyl groups donate hydrogen to free radicals. In testing the saponin compound the results were positive, the froth as high as 1-1.5 cm was marked. This froth occurs because of the presence of a hydrophilic group which binds to water while hydrophobia binds to air.

The positive phenol test results are indicated by changes in blackish green when reacted with  $FeCl_3$ . The color change is due to the presence of hydroxyl phenolic groups in the compound.

Testing of terpenoids/steroids is indicated by formation of red or green colour when reacted with Liberman-Burchard which shows a positive steroid content. The results of the negative mono/sesquiterpenes test formed purple when reacted with vanillin sulfate. Tests for the positive quinone are marked with red color when reacted with 5% KOH.

Based on the results of phytochemical screening all extracts tested showed the same result.

**Extraction**

The production of aerated and anaerobic green breadfruit leaf extracts was carried out through a maceration process using 96% ethanol solvent. This maceration method was chosen because it is a method suitable for compounds that are not heat resistant (thermolabile), so as to reduce the damage to compounds contained therein. The choice of ethanol 96% solvent because it is a universal solvent and can attract all the compounds contained in the simplicia leaves of green breadfruit aerobic and anaerobic fermentation. From the extraction of 300 g of green breadfruit simplified aerobic and anaerobic fermentation leaves, each simplicia was performed 3 times, to the total amount using 96.2 ethanol as much as 11.2 L. In the aerobic maceration process and anaerobic maceration carried out for 3 days, and replacement of ethanol 96% was carried out after the breadfruit leaves were soaked for 24 hours, then ethanol solvent was added again as much as 1.8 L. And obtained thick extract for aerobic fermented green breadfruit leaves as much as 16 g with 5.33% extract rendement and green anaerobic fermented leaves as much as 17 g with extract yield of 5.66%. The yield of anaerobic fermentation is higher than that of

aerobic fermentation, this is because the secondary metabolites contained in anaerobic fermentation are compounds that have high polarity correlated with the determination of water soluble and ethanol extracts where the anaerobic fermentation results are higher than aerobic fermentation.

**Anti-Inflammatory Activity Testing**

Antiinflammatory testing of aerobic and anaerobic fermented green breadfruit leaf extract, using the rat foot inflammation method using the induction of chemical compounds. The chemical compound used in this study is carrageenan. The type of carrageenan used is kappa carrageenan, carrageenan was chosen because it can release prostaglandins after being injected into test animals, carrageenan plays a role in the formation of edema in the inflammatory model, carrageenan also does not cause permanent tissue damage. In the process of edema formation, carrageenan will induce cell injury with the release of mediators that initiate the inflammatory process. Udem caused by carrageenan can last for 6 hours and gradually diminish within 24 hours.

**TABLE 2. THE RESULT OF AVERAGE EDEMA VOLUME**

Group	T0	TK	T1	T2	T3	T4	T5
Negative control	0,514	1,210	1,266	1,280	1,278	1,246	1,254
Diklofenac sodium	0,642	0,960	0,856	0,732	0,666	0,664	0,652
Aerobic 50 mg	0,604	1,098	1,164	1,164	1,054	0,966	0,822
Aerobic 100 mg	0,576	0,968	0,964	0,916	0,870	0,814	0,760
Aerobic 150 mg	0,584	1,059	1,0625	1,023	0,967	0,9225	0,872
Anaerobic 50 mg	0,598	1,018	0,992	0,890	0,832	0,800	0,750
Anaerobic 100 mg	0,568	0,840	0,806	0,752	0,690	0,652	0,610
Anaerobic 150 mg	0,666	1,012	0,942	0,872	0,790	0,758	0,686

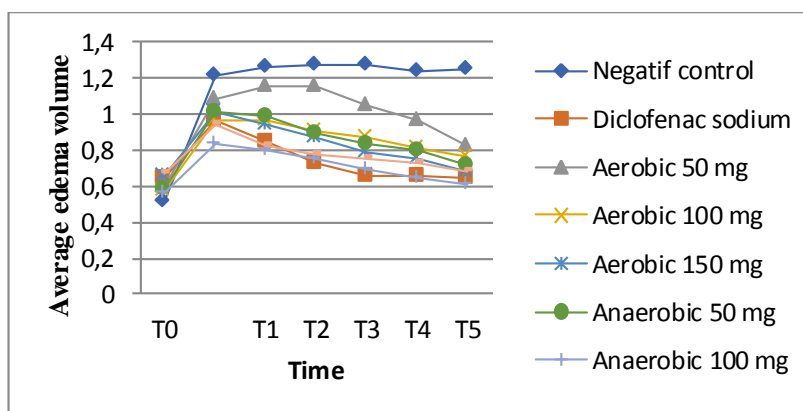


Figure 2. Graph of average edema volume with time.

The first preliminary test was an average edema edema volume test which was to test that whether the 1% carrageenan used actually produced edema and could also be used to determine the edema model, it could be seen from the increase in edema volume at the mouse feet at 1 o'clock, when induced carrageenan. 1% carrageenan was already able to cause edema, this is shown by the edema volume

graph shows that the volume of mouse feet induced with 0.1 ml carrageenan increased. Testing inflammation of the soles of mice after induced carrageenan each treatment for 5 hours can be seen in table 3.

**TABLE 3. PERCENT INFLAMMATION**

Group	T0	TK	T1	T2	T3	T4	T5
Negatif control	0	135,40	146,30	149,02	148,63	142,41	140,96
Diklofenac sodiu	0	49,53	33,33	14,01	3,73	3,42	1,55
Aerobic 50 mg	0	81,78	92,71	92,71	74,50	59,93	36,09
Aerobic 100 mg	0	68,05	67,36	59,02	51,04	41,31	31,94
Aerobic 150 mg	0	51,95	41,44	30,93	18,61	13,81	3,00
Anaerobic 50 mg	0	70,23	65,88	48,82	39,13	33,77	25,41
Anaerobic 100 mg	0	40,47	22,91	16,96	11,90	9,82	4,38
Anaerobic 150 mg	0	47,88	41,90	32,39	21,47	14,78	7,39

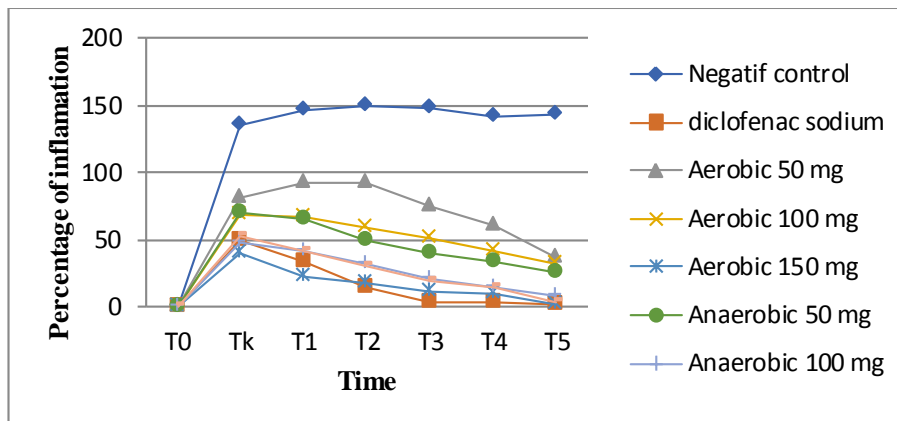


Figure 3. Inflammation percent graph with time

Figure 3. above there is a graph showing the decrease in inflammation after inducing carrageenan. All ethanol extracts of green breadfruit leaves aerobic and anaerobic fermentation with a dose of 50 mg / kgBB, 100 mg / kgBB and 150 mg / kgBB can reduce the edema volume of mouse feet, from 1 to 5 hours, and the most effective extract reduce the volume of inflammation namely anaerobic extract dose of 100 mg / kgBB then

anaerobic extract dose of 150 mg / kgBB. In testing the reduction of inflammation of the ethanol extract of green breadfruit leaves aerobic and anaerobic fermentation contains flavonoids which are known to have anti-inflammatory activity, especially inhibiting the cyclooxygenase enzyme. With the inhibition of the enzyme cyclooxygenase, prostaglandin release is also inhibited. Test for percent inhibition of mouse feet after induced carrageenan in each treatment for 5 hours. The result can be seen in table 4.

TABLE 4. RESULT OF PERCENT INHIBITION

Group	T0	TK	T1	T2	T3	T4	T5
Negatif control	0	0	0	0	0	0	0
Diklofenak sodium	0	63,41	77,21	90,59	97,48	97,59	98,01
Aerobic 50 mg	0	39,59	36,62	37,78	49,87	57,91	74,93
Aerobic 100 mg	0	49,74	53,95	60,39	65,66	70,98	77,81
Aerobic 150 mg	0	61,63	71,67	79,24	87,47	90,30	95,91
Anaerobic 50 mg	0	48,13	54,96	67,23	73,67	76,28	82,34
Anaerobic 100 mg	0	70,10	84,33	88,61	91,99	93,10	96,34
Anaerobic 150 mg	0	64,63	71,35	78,26	85,54	89,61	94,75

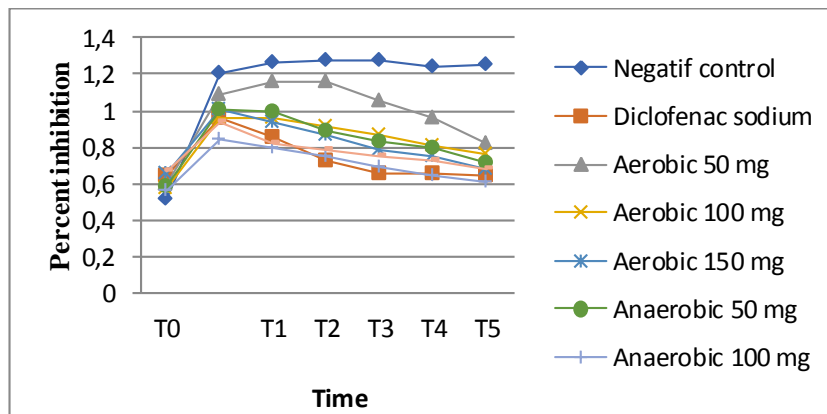


Figure 4. Percentage of inhibition with time

Based on observations, the average inflammation inhibition showed that edema inhibition increased up to the 5th hour. Ethanol extract of anaerobic breadfruit leaves 100 mg / kgBB showed the greatest inflammation inhibition which was 96.3% at the 5th hour. The results of this study showed that all doses have anti-inflammatory effects. The edema presentation is calculated according to data volume formed at each hour and its dose. From the presentation data it can be concluded that the ethanol extract of green breadfruit leaves aerobic and anaerobic fermentation has anti-inflammatory effects although it is not as good as Na-diclofenac drug.

Data obtained from the anti-inflammatory test were then statistically analyzed using the one way ANOVA test to show a significant difference between each treatment because the p value <0.05. In the Post Hoc method the analysis of the volume of edema was obtained. Decrease in the volume of rat feet given ethanol extract of green breadfruit leaves aerobic fermentation 50 mg / kgBB was not significantly different from negative control at 1-2 hours, whereas ethanol extract of green breadfruit leaves aerobic fermentation dose of 100 mg / kgBB and 150 mg / kgBB was significantly different with negative control at 1-5 hours, for anaerobic

fermented green breadfruit leaves ethanol extract dose 50, 100 and 150 mg / kgBB significantly different from the negative control group at 1-5 hours. When compared with the positive control aerobic extract dose of 50 mg / kgBB was not significantly different from the positive control at 5 hours, while the aerobic extract dose of 100 mg / kgBB was not significantly different from the positive group at 1.4,5 hours. Furthermore aerobic extract 150 mg / kgBB was not significant with positive control at 1-5 hours. For anaerobic extract a dose of 50 mg / kgBB was not significantly different from positive control at 4.5 hours. And for anaerobic extract dose of 100 mg / kgBB was not significantly different from positive control at 1,2,3,4,5 hours, for anaerobic extract 150 mg / kgBB was not significantly different from positive control at 1, 3, 4 and 5 hours.

#### IV. CONCLUSION

The most effective anti-inflammatory activity was shown in the group given anaerobic fermented green breadfruit leaves ethanol extract at a dose of 100 mg / kgBW.

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