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Anti-Inflammatory of Papaya Leaf Extract (*Carica Papaya L*) Towards Membrane Stabilization of Red Blood Cells

Laila Kamilla[1]✉, Sri Tumpuk[2], Maulidyah Salim[3]

[1-3]Poltekkes Kemenkes Pontianak, Indonesia

✉lailakamila79@gmail.com,Phone: +6285750720026

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Abstract

Traditional medical plants are known to society long ago. Apart from easily obtained and inexpensive, it can cure diseases with few side effects than modern medicine. Papaya leaves were used not only because of contained various chemical compounds with pharmacological effects but also alkaloids, flavonoids, saponins, and tannins compounds indicated as anti-inflammatory. This study applied a quasi-experimental design to test the papaya leaves anti-inflammatory activity. The red blood cell stabilization method was employed because analogous to the lysosomal membrane affected the inflammatory process. Purposive sampling was used, creating papaya leaves extract of 50 ppm, 100 ppm, 200 ppm, 400 ppm, 600 ppm, and 800 ppm concentration, made 24 total samples by four times replication. Based on the red blood cell lysis inhibition, the anti-inflammatory activity was measured and was compared with the positive control (diclofenac sodium). The papaya methanol extract result showed the highest anti-inflammatory activity at 800 ppm of 74.29%. The most effective concentration was at 200 ppm of 62.19%. Tukey's test showed p1.000 $\geq$ 0.05, suggesting H_0 was accepted. There was no difference between the anti-inflammatory activity of papaya leaf methanol extract and diclofenac sodium showing stabilization of red blood cell membranes, indicating papaya potentially as an anti-inflammatory.

Keywords: Anti-inflammatory;Papaya Leaves;Membrane Stability;Erythrocytes

INTRODUCTION

The use of natural materials as a traditional medicine in Indonesia is currently increasing, and even some natural materials have been mass-produced on a large scale. According to the Indonesian Ministry of Health, traditional medicine is material or potion material that is in the form of a plant, animal, mineral material, galenic preparations, or a mixture of these materials that has been used for treatment from generation to generation, and can be applied depending on applicable norms in society (Ministry of Health, 2017). The use of traditional medicines is the main choice by the community because the side effects are relatively small if used appropriately without abuse. Another advantage is that the raw material is easy to obtain and the price is cheaper (Tim Cahaya, 2011) (Mahatriny, Payani, Oka, & Astuti, 2014) (Fadilla, 2010).

Research and development of medicinal plants are rapidly growing both at home and abroad, especially in the field of its pharmacological efficacy, one of which is as an anti-inflammatory. Natural materials are expected to contain compounds with high anti-inflammatory activity.

Inflammation is the body's natural protective response that acts against agents that cause cell or tissue damage such as physical trauma, chemicals, or invading pathogenic microorganisms. A series of inflammatory reactions then occur to destroy agents that harm the tissue or prevent further damage. When the inflammatory process occurs, the vascular reaction occurs where fluids, blood elements, and white blood cells, as well as several chemical mediators, are released by cells such as histamine, 5-hydroxytryptamine or serotonin, leukotrienes, and prostaglandins. Inflammation is the body's protection effort to eliminate adverse stimuli and initiate the healing process in tissues that if it is left untreated it can develop into a chronic phase that causes malfunctioning of these tissues or organs (OT, Akilbinu, AA Ayankunle, & EO Awe, 2013).

The use of modern steroids and non-steroidal drugs to treat inflammation is useful for reducing swelling and pain due to inflammation, but it is dangerous if used inappropriately and for a long period can cause a risk of gastrointestinal damage, cardiac toxicity, and more. Anti-inflammatory drugs that have fewer side effects are needed to avoid these risks. (Madhavi, Rao, Vakati, Rahman, & Eswaraiah, 2012) Plants are an alternative that can be used as anti-inflammatory drugs, one of which is the papaya plant.

The use of papaya leaves has been around for a long time. It is one of the types of medicinal plants commonly used by the community empirically as an effort to prevent disease or traditional treatment. Almost all parts of papaya, such as roots, leaves, fruit, seeds, and sap, have medicinal benefits. Papaya leaves themselves are useful for treating acne, increasing appetite, accelerating breastfeeding, treating menstrual pain, and so on. (Setiawan Dalimartha, 2003) (Renata Ayuni, 2012)

The chemical content contained in papaya leaves is the enzyme papain, karpaina alkaloids, pseudocarpaine, glycosides, carposids, saponins, saccharose, dextrose, and levulose (Setiawan Dalimartha, 2003). According to Mangan (2009) the high content of vitamin C, saponins, and alkaloid compounds in papaya leaves can prevent the development of cancer cells.

Phytochemical screening of papaya leaves (Carica papaya L) showed the content of alkaloids, flavonoids, tannins, cardiac glycosides, free and bound anthraquinones, phlobatinin, and saponins. Another study found that the ethanol extract of papaya leaves contains flavonoids, alkaloids, tannins, saponins, anthraquinones, cardenolide, cardiac phenolic glycosides, steroids, and sugars. Flavonoid compounds, steroids, and tannins in free form and tannin-protein complexes are nutritious as an anti-inflammatory (Owoyele, Adebukola, Funmilayo, & Soladoye, 2008) (Imaga et al., 2010) (Setiawan Dalimartha, 2003). Flavonoids are included in the polyphenol group which has antioxidant, anti-inflammatory, anti-carcinogenic, antiviral, anti-tumor properties and can inhibit neurodegenerative diseases (Karakaya & El, 2006).

Terdapat There are various methods used in the study of drugs, chemical constituents, and herbal preparations to demonstrate the presence of anti-inflammatory activity or potential. One of them is the stability of the red blood cell membrane (Oyedapo, Akinpelu, Akinwunmi, Adeyinka, & Sipeolu, 2010). The red blood cell membrane is analogous to the lysosomal membrane and its stability suggests that the extract can also stabilize the lysosomal membrane. Lysosomal membrane stabilization is important in limiting the inflammatory response, by inhibiting the release of lysosomal enzymes from the granules in active neutrophils during the inflammatory process. Enzymes in neutrophils that are released during inflammation (due to the activation of neutrophils) will produce various disorders that can cause inflammation and further tissue damage. Therefore, red blood cell membrane stabilization induced by a hypotonic solution can also be used as a measurement to determine lysosomal membrane stabilization (Kumar et al., 2012). Thus this study aims to examine the activity of papaya leaf extract as an anti-inflammatory.

METHOD

This research is a Quasi Experiment (Quasi-Experimental). The population in this study was papaya (Carica papaya L.) The sample used was papaya leaves that met the inclusion criteria, namely Bangkok papaya, papaya leaves that were about four months old, dark green (leaves 3-5 from the base.), fresh without defects. Determination of the number of samples determined using the Federation formula. The sample treatment was six times four so that the sample total was 24. The research step was initiated by preparing a tool consisting of an analytical balance, blender, filter paper, measuring flask, Erlenmeyer, vaporizer cup, porcelain cup, Becker glass, measuring cup, funnel, test tube, stirring rod, dropper pipette, watch glass, centrifuge tube, micropipette, desiccator, autoclave, oven, centrifuge, vacuum rotary evaporator, water bath, and UV-Visible spectrophotometer. Furthermore, preparing and making research materials, namely: papaya leaves, Aquadest, phosphate buffer 7.4 (0.5 M), Hyposalin (0.25% 0, Isosalin (0.85%), sterile Alsever solution (2.45 grams of dextrose); sodium citrate 2.20 grams; 0.73-gram citric acid dissolved in 100 ml sterile Aquadest), diclofenac sodium and red blood cell suspension (10% v / v). The study consisted of three stages.

Stage 1 extracting papaya leaves. Papaya leaves that fit the criteria are cleaned and chopped. Then dried in a drying cabinet and then blended. The next Simplicia was calculating the water content and then extracting by the maceration method using methanol for 3 x 24 hours by changing the solvent every day. The result of the extraction was evaporated using an evaporator to obtain a thick papaya leaf extract. Furthermore, phytochemical screening is carried out to determine which active substances are in the extract.

Stage 2 was the preparation of 10% red blood cell suspension as much as 10 ml of blood is put into a centrifuge tube containing alsever solution then centrifuged at 3000 rpm for 10 minutes. The supernatant was removed and the blood cell sediment was washed with isosalin solution then centrifuged again, this process was carried out 4 times until a clear isosalin was obtained. Blood volume was measured and resuspended with isosalin to obtain a red blood cell suspension with a concentration of 10%).

Stage 3 was in-vitro anti-inflammatory testing with the red blood cell membrane stabilization method and compared with diclofenac sodium.

No.	Solution	Concentration (ppm)
1	Papaya Leaf Extract (Carica papaya L.	50 100 200 400 600 800
2	Diclofenac Sodium	50 100 200 400 600 800

Furthermore, the measurement is carried out by preparing a test solution consisting of; 1 ml phosphate buffer pH 7.4 (0.15M) 0.5 ml red blood cell suspension, 1 ml sample solution, and 2 ml hyposalin. Positive control solution; 1 ml of pH 7.4

(0.15M) phosphate buffer 0.5 ml of red blood cell suspension, 1 ml of diclofenac sodium solution, and 2 ml of hyposaline. Test control solution; 1 ml of pH 7.4 (0.15M) phosphate buffer, 0.5 ml of isosalin solution as a substitute for red blood cell suspension, 1 ml of the sample solution and 2 ml of hyposalin. Negative control solution; 1 ml of pH 7.4 (0.15M) phosphate buffer 0.5 ml of red blood cell suspension, 1 ml of isosalin solution as a substitute for the sample solution, and 2 ml of hyposaline. Then all the solutions were incubated at 370C for 30 minutes and then centrifuged at 5000 rpm for 10 minutes. The supernatant was then measured for its hemoglobin content with a Uv-Vis spectophotometer at its maximum wavelength. The data analysis technique used the Anova test followed by the Tukey test.

RESULTS AND DISCUSSION

1. Extraction

The papaya leaf extract was made at the Chemical Laboratory of the Yarsi Pharmacy Academy in Pontianak. Papaya leaves prepared as samples were washed, cut into small pieces, then dried, and then blended. Simplisia was then made extract using maceration with 70% methanol solvent for 3 days. Every day the solvent is changed and it is stirred to maximize the process of withdrawing the active substance. The result of maceration is collected and evaporated to remove the remaining solvent and get a thick extract. The extraction results can be seen in the table below:

Tabel 1. Papaya Leaf Extraction Results (*Carica papaya* L.)

Wet weight	Dry simplicia weight	Extract weight	Rendement	Time drying
2000 g	318,39 g	5,53 g	17,44%	0,68%

Based on table 5.1 of 2000 grams of fresh papaya leaves, 318.39 grams of dried simplicia were obtained. From 318.39 grams of simplicia, 55.53 grams of thick papaya leaf extract were obtained with the remaining solvent of 0.68%.

2. Phytochemical Test

The papaya leaf extract was then tested for secondary metabolites to determine the active compounds contained in the methanol extract of papaya leaves. Based on the secondary metabolite test, the methanol extract of papaya leaves contained active compounds in the form of alkaloids, phenols, flavonoids, saponins, tannins, phenols, and terpenoids.

3. Anti-Inflammatory Activities

The method to determine the anti-inflammatory activity by invitro way can be seen from the stabilization of the red blood cell membrane where the researcher took measurements using a UV.Vis spectrophotometer with a maximum wavelength of 413 nm. The results of sample measurements and sodium diclofenac control were then calculated to find the percent stability of red blood cells. The results can be seen in the following table :

Tabel 2. Percentage of Red Blood Cell Membrane Stability from Sample Solution and Diclofenac Sodium

No.	Solution	Concentration (ppm)	Average Stability (%)
1	Papaya Leaf Extract (*Carica papaya* L.)	50	54,65
		100	55,49
		200	62,19
		400	64,95
		600	67,79
		800	74,29
2	Diclofenac Sodium	50	58,76
		100	61,99
		200	66,44
		400	71,23
		600	76,08
		800	83,07

Based on table 2 above, the average percent stability of papaya extract at a concentration of 50 ppm is 54.65%, a concentration of 100 ppm is 55.49%, a concentration of 200 ppm is 62.19%, a concentration of 400 ppm is 64.95%, a concentration of 600 ppm of 67.79%, and a concentration of 800 ppm of 74.29%. Meanwhile, the average percent stability of diclofenac sodium at a concentration of 50 ppm is 58.76%, a concentration of 100 ppm is 61.99%, a concentration of 200 ppm is 66.44%, a concentration of 400 ppm of 71.23%, a concentration of 600 is 76.48%, and a concentration of 800 ppm is 83.07%.

Tabel 3. ANOVA Test Results Percentage of Red Blood Cell Membrane Stability in Papaya Leaf Methanol Extract

	Sum of Square	df	Mean Square	Sig.
Between Groups	1128.66	5	225.73	.000
Within Groups	10.58	18	0.58	
Total	3519.63	23		

Based on the results in table 3, the significance value is 0.000 <0.05 which means that the percent stability data in all test sample groups are significantly different that because the ANOVA test is significantly different so it is followed by Tukey's test which is a continuation test of Anova. The Tukey test aims to determine which group gives a significantly different score from other groups. Tukey test results of methanol extract of papaya leaves with a concentration of 200 ppm and a concentration of 100 ppm of diclofenac sodium can be seen in Table 4 below:

Tabel 4. Tukey Test Results Percentage of Red Blood Cell Membrane Stability in Methanol Extract of Papaya Leaves with a concentration of 200 ppm and Sodium Diklofenak concentration of 100 ppm

(I) Sample	(J) Sample	Mean Difference (I-J)	Sig.
sample concentration 200 ppm	drug concentration 100 ppm	-21500	1.00

The Tukey test results in table 4 show that the extract at a concentration of 200 ppm was not significantly different or identical to the positive control (diclofenac sodium) at a concentration of 100 ppm with a significance value of 1,000> 0.05.

The survival of red blood cells depends on the integrity of the membrane. When these cells are exposed or induced by hypotonic solutions, it causes the formation of oxidative stress that can disrupt the stability of the biomembranes and lead to oxidation of lipids and proteins causing membrane damage characterized by hemolysis. Therefore, the size of hemolysis on the membrane induced by a hypotonic solution can be used as a measurement of anti-inflammatory activity (Kumar et al., 2012).

The results showed a decrease in absorbance along with increased concentration. It can be seen from the small amount of hemolysis that occurs so that the anti-inflammatory activity is greater calculated from the proportion of red blood cell lysis with the formula for the percentage of stability. Anti inflammatory activity is getting strong if that approaches or exceeds positive control.

Accordingly, the extract concentrations of 50 ppm and 100 ppm were not able to inhibit red blood cell lysis properly because the percentage is only 54.65% and 55.49%. But the papaya leaf extract at a concentration of 200 ppm got an increase in the percentage of 62.19%. When compared with diclofenac sodium at a concentration of 100 ppm the inhibition percentage was 61.99%. This shows that papaya leaf extract has potential and is effective as an anti-inflammatory because the percentage value of stability obtained was not significantly different or identical to the positive control.

The ability of papaya leaf extract as an anti-inflammatory is related to the compound content in it. The results of the phytochemical analysis on papaya leaves contained various secondary metabolites such as alkaloids, tannins, flavonoids, saponins, phenols, and steroids. (Owoyele et al., 2008) (Imaga et al., 2010) (Setiawan Dalimartha, 2003). Several studies have shown that there is a relationship between the ability of flavonoid compounds to stabilize membranes and to inhibit enzymatic processes during inflammation. (Middleton, Kandaswami, & Theoharides, 2000). The results of the research by Avula, Pallu, & Reddy, (2010) showed that flavonoids isolated from *Butae monosperma* stem, namely Genistein (4´, 5, 7-*trihidroksi isoflavone*) and Prunetine (4´, 5-*dihydroxy isoflavone*) can inhibit the work of cyclooxygenase and lipooxygenase enzyme in converting arachidonic acid into prostaglandins and leukotrienes, which are inflammatory mediators.

Flavonoids in particular can stop the formation and excretion of substances that cause inflammation due to allergic reactions. Compounds belonging to this group have different effects in dealing with inflammation. The anti-inflammatory mechanism produced by flavonoids can occur through several pathways, one of which is by directly inhibiting the activity of the cyclooxygenase (COX) and lipooxygenase enzymes which cause the inhibition of prostaglandin and leukotriene biosynthesis, which are the end products of the COX and lipoxygenases pathways. This causes the inhibition of leukocyte accumulation and neutrophil degranulation, thereby directly reducing the release of arachidonate by neutrophils and the release of histamine. In normal conditions, leukocytes move freely along the endothelial wall. During inflammation, endothelial-derived mediators and complement factors cause leukocyte adhesion to the endothelial wall. The presence of flavonoid compounds can reduce the adhesion of leukocytes to the endothelium thereby reducing the body's inflammatory response (Nijveldt et al., 2001).

Other compounds that also have anti-inflammatory activity are tannins and saponins, namely their ability to bind to cations, thereby stabilizing erythrocyte membranes and other biological macromolecules (Oyedapo et al., 2010).

CONCLUSION

Papaya leaf extract (Carica papaya) which has anti-inflammatory activity varies according to the concentration (50, 100, 200, 400, 600, and 800 ppm) with 54.65; 55.49; 62.19; 64.95; 67.79; 74.29 respectively. The highest activity was the extract with a concentration of 800 ppm which was 74.29%. The most effective concentration of papaya leaf extract (Carica papaya) is at a concentration of 200 ppm because it shows no difference from Diclofenac Sodium.

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