FLAVONOID LEVEL ANALYSIS OF ETHANOL EXTRACT OF PAPAYA LEAVES (*Carica Papaya* L.) and ITS APPLICATION AS AN ANTI-PAIN IN TEST MICE (Mus *Musculus*)

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A B S T R A C T

Papaya leaf (*Carica papaya* L.) is one of the plants that is commonly used by the community as a traditional herbal medicine because it contains secondary metabolite compounds that have pharmacological activities such as alkaloids, flavonoids, vitamin C, vitamin A, vitamin B1, sacrose, dextrose, levulose, tannins, proteins, carbohydrates, etc. This study aims to determine the levels of flavonoids contained in papaya leaf extract (*Carica papaya* L.) and its application as an anti-pain tested in mice (*Mus musculus*). The research methods used were extraction by maceration method, analysis of flavonoid levels by UV-Vis Spectrophotometry method and its application in test animals was carried out in vivo. Based on the results of the study, the level of flavonoids in papaya leaves (*Carica papaya* L.) from ethanol extract in coastal areas was 63.89 mgQE/g or 6.39% and ethanol extract in mountainous areas was 74.80 mgQE/g or 7.48%. Furthermore, the most effective analgetic activity of ethanol extract of papaya leaves (*Carica papaya* L.) was found at the maximum dose concentration.

INTRODUCTION

Medicine using plants has existed since ancient times which is known as traditional medicine and produces herbal medicine. Many plants have been recorded to have content as medicinal therapeutic ingredients, but public awareness of this is still minimal and most of them are only because they have been used for generations. An example is the papaya plant (*Carica papaya* L.) (Octavianus *et al*, 2014). The side effects of herbal medicine are smaller if used correctly and appropriately, both the right dosage, the time of use, the quality of the ingredients, and the feasibility of the medicinal plants used. There are herbal plants that are used as analgesics (anti-pain), for example papaya leaves (*Carica papaya* L.). Papaya leaves (*Carica papaya* L.) are known to have empirical properties for analgetic (anti-pain) (Darwis, 2017).

The papaya plant (*Carica papaya* L.) is one of the plants that is easy to live anywhere. In Baubau City, Southeast Sulawesi Province, there are many papaya leaf plants (*Carica papaya* L.), where people often use them for herbal medicine. In general, papaya leaves (*Carica papaya* L.) are commonly consumed in the form of herbal medicine by the local community (Hidayah *et al*, 2020). In ancient times, papaya leaves (*Carica papaya* L.) were used as pain relievers during menstruation. The substances contained in papaya leaves that are useful as pain relievers are flavonoids and karpain alkaloids (Herbie, 2015). Analgetics are drugs that have the function of reducing pain, because the way they work passes through the central nervous system which changes the response to pain (Susanto and Fitriana, 2017).

Papaya leaves (*Carica papaya* L.) are known to contain flavonoid compounds, where these flavonoids are beneficial for analgetic therapy (anti-pain) by inhibiting the action of the enzyme cyclooxygenase, which is an enzyme that plays a role in the main pathway of pain mediator synthesis. So that the production of prostaglandins carried out by arachidonic acid is reduced, thereby reducing pain (Sianturi and Rachmatiah, 2020).

Several previous studies on papaya leaves (*Carica papaya* L.) regarding its benefits as an anti-pain through animal tests have been conducted, such as the study of Afrianti *et al* (2014) has proven that papaya leaves (*Carica papaya* L.) containing flavonoids are able to inhibit the inflammatory process that causes pain. This is strengthened by the research of Patel *et al* (2014), stating that if the

results of the phytochemical test of papaya leaves are proven to contain flavonoids which can be anti-pain by the working process of inhibiting the enzyme cyclopsigenase, by inhibiting cyclooxygenase it can suppress the production of prostaglandins which causes pain to be reduced. Furthermore, research conducted by Dermiati *et al* (2018) proved that papaya leaf ethanol extract has an analgesic effect on male mouse test animals.

Although many studies on papaya leaves (*Carica papaya* L.) have been found, research and search for medicinal raw materials continue to be carried out in several areas to get the best quality and best effects. So research was carried out in Baubau City, Southeast Sulawesi Province because papaya leaves (*Carica papaya* L.) are very easy to find and people in the local area make this plant as an herbal medicine or herb for generations to relieve pain. Therefore, papaya leaf extract has the potential to be used as a plant that has analgetic (anti-pain) properties. The anti-pain properties of papaya leaves are caused by secondary metabolite compounds, one of which is flavonoids. Researchers are interested in analyzing the flavonoid levels of ethanol extract of papaya leaves (*Carica papaya* L.) and testing its analgesic (anti-pain) effect on mouse test animals (*Mus musculus*).

METHODOLOGY

Tools

The tools used in this study were glass jars, analytical scales (*Kern*), digital scales (*SF-400*), animal cages, oral needles (*Canula*), stirring rods (*Pyrex*), horn spoons, beakers (*Pyrex*), glass funnels (*Pyrex*), bunsen, tripods, petri dishes, test tubes (*Pyrex*), tube brushes, measuring flasks (*Iwaki*), micro pipettes (*One Med*), *stopwatc*, 1 cc spoit (*One-Med*), vial container, pestle casket, spatula, oven (*Memmert* UN 260), chocolate bottle, mesh strainer 10, blender (Miyako), gloves, *rotary evaporator* (*B-One*) and UV-Vis spectrophotometry (*B-One*).

Materials

The ingredients used are papaya leaf ethanol extract (*Carica papaya* L.), mefenamic acid medicine 500 mg (HJ), potassium acetate 5% (*Colloid*), ethanol 96% (*Rofa*), aquadest (*Waterone*), quercetin (*Sigma Coa*), acetic acid 1% (*Rofa)*, *AlCl3* 10% (*Catologue*), Na.CMC 0.5% (*Aloin*), HCl concentrate (*Hawkins*), magnesium powder (Mg) (*Xingkang*), methanol pa (*Rofa*), 70% alcohol (*One Med*), handscoon, label paper, filter paper (*Healthcare*) and masks.

Test Animals

The experimental animal used in this study is a male white mouse (*Mus musculus*) with an average weight of 20-25 grams.

Working Procedure

Sampling

Samples were taken from two different places, namely Bukit Wolio Indah Village, Wolio District (mountains) and Sulaa Village, Betoambari District (coastal) in Baubau City.

Sample Preparation

Papaya leaf samples are cleaned of small impurities, then chopped or separated from the stem and dried without direct sunlight, then blended and filtered until smooth.

Extract Manufacturing

The fine powder of the sample is weighed as much as 250 grams, then the powder is placed in a glass jar, then 3 liters of ethanol is added until all the powder is submerged and stirred for 30 minutes until homogeneous, then let it sit for 3 days until a precipitate appears, after that it is separated between the pulp and the filtrate, the filtrate is evaporated by a rotavapor machine at a temperature of 500C to obtain a concentrated extract, then put it in the oven to dry the extract at 400C.

Flavonoid Compound Test

Papaya leaf extract is taken as much as 0.5 mL, then add 2 mL of hot 50% methanol and then poured into a test tube, after that it is mixed with enough Mg metal powder and dripped with HCl as many as 10 drops, if a red, orange, yellow or green solution is formed, it is stated to contain flavonoids.

Creation of Reagent Indicators

The preparation of quercetin solution (1000 ppm) is measured with quercetin powder as much as 25 mg, then mixed with methanol p.a volume of 25 mL. The manufacture of 10% Aluminum chloride (AlCl3) reagent is weighed with 1 gram of powder and then mixed with 10 mL of aquadest. The manufacture of 5% Potassium Acetate reagent is measured with 0.5 grams of powder and then dissolved with 10 mL of aquadest.

Flavonoid Level Analysis

Determination of the standard curve of quercetin

The 1000 ppm quercetin solution is broken down into several concentration series, then diluted to 100 ppm. The dosage series is made and then put into a 5 ml vial in a pipette of 0.5 ml, 1 ml, 1.5 ml, 2 ml, and 2.5 ml (10, 20, 30, 40 and 50 ppm). Then it is added with 0.5 ml of AlCl3, 0.5 ml of potassium acetate and sufficient with aquadest in each vial up to 5 mL. Each test solution was incubated for 30 minutes, and readings were made at a wavelength of 431 nm.

Determination of total flavonoid levels

A total of 10 mg of concentrated extract mixed with 10 ml of ethanol, then a solution concentration of 1000 ppm is obtained. A total of 0.5 mL of 1000 ppm extract solution is added with 0.5 mL of AlCl3, 0.5 mL of potassium acetate and added with aquadest in each vial up to 5 mL. Each test solution was left for 30 minutes, and readings were taken at a wavelength of 431 nm.

Analgetic Activity Test in Mice

Preparation of 0.5% NaCMC Solution (negative control)

Na.CMC powder was weighed as much as 0.5 grams, then heated aquadest as much as 100 mL with Bunsen fire. After heating, the powder is put into the hot aquadest and ground until homogeneous.

Extract Suspension Manufacturing

Papaya leaf ethanol extract was measured at doses of 200 mg/kgBB, 400 mg/kgBB and 800 mg/kgBB, respectively, then put into labeled vial bottles. Then, a 0.5% b/v Na.CMC solution is added according to the specified dose calculation.

Mefenamic Acid Suspension Manufacturing (positive control)

Mefenamic acid was weighed as much as 0.0013 grams, put into a labeled vial bottle. Then, a 0.5% b/v Na.CMC solution is inserted according to the specified dose calculation.

Preparation of 1% acetic acid solution

A measuring flask with a volume of 100 mL was prepared, then 25 mL of aquadest was pipetted into the flask, then 1 mL of 100% acetic acid was pipetted into the flask. After that, enough aquadest to the limit of the terra, then stir until homogeneous.

Treatment of Test Animals of Mice (*Mus musculus*)

Before taking action, the test animal was first fasted for approximately 8 hours so that it could be ensured that there were no food residues left in the mice's stomach so that the research process could run smoothly. For 30 minutes, all test animals were induced to use a 1% acetic acid solution to obtain pain due to pain which was administered intraperitoneally (i.p.). Furthermore, it will be given oral treatment as below.

The test animals were grouped into 5 groups randomly for each group of 3 mice consisting of:

Group 1: administration of 0.5% Na.CMC suspension of 1 mL (as a negative control).

Group 2: administration of 200 mg/kgBB extract in 1 mL of 0.5% Na.CMC solution.

Group 3: administration of suspension of extract at a dose of 400 mg/kgBB in 1 mL of 0.5% Na.CMC solution.

Group 4: administration of suspension of extract at a dose of 800 mg/kgBB in 1 mL of Na.CMC 0.5% solution.

Group 5: administration of mefenamic acid suspension 500 mg/kgBB in 1 mL of 0.5% Na.CMC solution (as a positive control)

Then each group, per mouse was placed on top of *the plate form* and observed squirming movements due to the pain effect caused by 1% acetic acid every 5 minutes for a duration of 1 hour. Observation of the squirming of mice is seen when it starts to feel pain, its body stretches and then its stomach presses against the floor. The results were accumulated for 60 minutes as a code of writhing movements of experimental animals. The strength of analgesic activity was observed from the ability of sample inhibition to reduce squirm in experimental animals (Afrianti *et al*, 2014).

RESULTS & DISCUSSION

Research Results

This study used 250 grams of papaya leaf powder in coastal areas and 250 grams of papaya leaf powder in mountainous areas soaked with ethanol for 3 liters each using a maceration process, so that

dry extracts of 72 grams of coastal papaya leaves and 65 grams of mountain papaya leaves were obtained.

Results of Phytochemical Screening Test for Flavonoid Compounds

Table 1. Identification of Flavonoids in Samples

Sample	Serbuk Mg + HCl 10%	Information
P1	Yellowish-green color	Positive reaction (+)
P2	Yellowish-green color	Positive reaction (+)

Source : Primary Data, 2024

Information:

P 1 : Papaya Leaf (Carica papaya L.) coastal area
P 2 : Papaya Leaves (Carica papaya L.) in mountainous areas

The results of Table 1 prove that ethanol extracts of papaya leaves (P1) and papaya leaves (P2) contain flavonoid compounds due to yellowish-green color change after adding Mg and HCl 10%.

Results of Flavonoid Level Analysis

Table 2. The results of the measurement of the absorbance of the quercetin standard solution at a wavelength of 431 nm.

Quercetin Standard Solution (ppm)	Absorbansi
10	1,331
20	2,024
30	2,619
40	2,983
50	3,053

Source : Primary Data, 2024

In the quantitative test of flavonoid compounds, quercetin is used as a standard solution with various concentrations, namely 10 ppm, 20 ppm, 30 ppm, 40 ppm and 50 ppm.

Table 3. Results of flavonoid levels in the sample

Sample	Rep.	Abs.	Up to Flavonoids (mgQE/g Extract)	Up to (%) b/b
P 1	Simply	0,202	54,95	5,50
	Double	0,226	65,86	6,59
	Triple	0,237	70,86	7,09
	Average		63,89	6,39
P 2	Simply	0,245	74,50	7,45
	Double	0,240	72,23	7,22
	Triple	0,252	77,68	7,77
	Average		74,80	7,48

Source : Primary Data, 2024

Description :

P 1 : Papaya Leaf (*Carica papaya* L.) coastal area
P 2 : Papaya Leaves (*Carica papaya* L.) in mountainous areas

From the results of Table 3, it was stated that the flavonoid content of papaya leaves (P1) was 63.89 mgQE/g or 6.39% and papaya leaves (P2) was 74.80 mgQE/g or 7.48%. This states that papaya leaves (P2) have higher flavonoid compounds than papaya leaves (P1). Therefore, extracts from mountainous areas can be continued to be tested for analgetic activity in mice (*Mus musculus*).

Results of Analgetic Activity Test in Mice

Table 4. Analgetic Activity Data on Test Animals of Mice (*Mus musculus*)

Oral Administration	The first minute-											
	5	**10**	**15**	**20**	**25**	**30**	**35**	**40**	**45**	**50**	**55**	**60**
Group. 1	L	L	L	L	L	L	L	L	L	L	L	L

Group. 2	L	L	L	L	L	L	M	M	M	N	N	N
Group. 3	L	L	L	L	M	M	M	M	N	N	N	N
Group. 4	L	L	L	M	M	M	N	N	N	N	N	N
Group. 5	L	L	L	M	M	M	M	M	N	N	N	N

Source : Primary Data, 2024
Information:

Group 1 : Administration of Na.CMC 0.5% (as a negative control)
Group 2 : Giving an extract concentration of 200 mg (Dose 0.004 g/kgBB)
Group 3 : Administration of extract concentration 400 mg (Dose 0.008 g/kgBB)
Group 4 : Administration of 800 mg extract concentration (Dose 0.016 g/kgBB)
Group 5 : Administration of drug concentration of 500 mg (Dose 0.0013 g/kgBB/ as a positive control)
L Effect : Still in pain (wriggling)
M Effect : Reduced pain (lack of squirming)
N Effect : Movement returns to normal (healed)

From the results of table 4, it was stated that group 4 with an extract concentration of 800 mg/kgBB was better to provide its analgetic effect from all groups, including positive control.

Discussion

Research on papaya leaves (*Carica papaya* L.) was taken from the city of Baubau with two different locations, namely in mountainous areas and in coastal areas. The location of the mountainous area taken is in Bukit Wolio Indah Village and the coastal area taken is in Sulaa Village in Baubau city. After the leaf samples are collected from two different location points, the leaf samples are separated from insects and dirt that stick to them, then washed thoroughly with running water and then dried without being exposed to direct sunlight so that the moisture content contained in the leaf samples decreases until it changes color to greenish-brown, which is a sign that the leaf samples are ready for extraction.

Papaya leaf extract is processed using the maceration method with ethanol solvents, the selection of ethanol because it can filter polar and non-polar compounds without being toxic. Soaking is carried out for 3 days, followed by filtration to separate the filtrate from the dregs. The extract is then compacted using a rotary evaporator until it becomes thick brownish-green. The yield was calculated, with a yield of 28.2% for coastal areas and 26.0% for mountainous areas, to determine the total compounds contained in solvents without determining the type of compound (Ahmad *et al,* 2015).

Qualitative tests to detect flavonoids in plants were carried out using magnesium (Mg) and hydrochloric acid (HCl) powder reagents. After the addition of reagents, there is a yellowish-green color change, which indicates the break of glycoside chains and reduced flavonoids so that flavonoids can be identified (Mutmainnah, 2017). The test results showed that ethanol extracts of papaya leaves from coastal and mountainous areas experienced the same color change, indicating that both were detected to contain flavonoids.

A quantitative test of total flavonoids by UV-Vis spectrophotometry method was carried out as a tool to measure flavonoid levels in ethanol extract of papaya leaves (Carica papaya L.). This method was chosen because flavonoids have a conjugated aromatic system that produces a strong absorption band against the ultraviolet spectrum and visible light.

In this study, the quercetin used was concentrated at 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm as standards in determining sample levels. Quercetin was chosen because it is a flavonoid that has a carbonyl group in the C-4 atom and a hydroxyl group in the C-3 or C-5 atom, which is adjacent to the structure of flavones and flavonols (Aminah et al., 2017).

The calibration curve was determined by measuring the absorbance of a standard solution by UV-Vis spectrophotometry using a wavelength of 431 nm. In total flavonoid measurements, AlCl3 is added to the sample to form a complex that causes the wavelength to shift to the visible region, as evidenced by the stronger yellow color. Potassium acetate is added to maintain the wavelength in the

visible region. The sample was incubated for 1 hour before being measured so that the reaction reached perfection, which ultimately led to optimal color intensity (Salmia, 2016).

Based on the measurement results, it can be stated that the more concentration is used, the more absorbance is obtained. The relationship between the standard quercetin level and its absorption results in a linear regression equation y = 0.044x + 0.0811 with a correlation coefficient (r) of 0.9239. From this equation, the total flavonoid content for P1 extract (coastal papaya leaf) is 63.89 mgQE/g or 6.39%, while P2 extract (mountain papaya leaf) is 74.80 mgQE/g or 7.48%. Based on the research of Alzanado et al. (2022), 96% ethanol extract of papaya leaves (*Carica papaya L*.) has a total flavonoid content of 94.174 mg/g extract or 9.41%. Meanwhile, Nugroho (2019) found that the total flavonoid content in papaya leaf ethanol extract was 6.8357%.

The difference in the percentage of flavonoid levels in papaya leaf samples was caused by temperature variations during sampling, so its physiological function depended on the condition of the plant (Hanani, 2014). The results of the study found that ethanol extract of papaya leaves (*Carica papaya L*.) from coastal and mountainous areas both contained flavonoids, but the levels of flavonoids in mountainous areas were higher than in coastal areas. Flavonoids function as analgesics that can minimize pain by preventing the formation of inflammation. The mechanism of action of flavonoid analgesics is to prevent the activity of the enzyme cyclooxygenase, which ultimately reduces the production of prostaglandins in the arachidonic acid pathway, which prevents inflammation and minimizes pain (Sianturi and Rachmatiah, 2020).

Therefore, further research was conducted to test the effectiveness of anti-pain activities in mouse test animals. The ethanol extract of papaya leaves (*Carica papaya L*.) to be tested is a sample originating from mountainous areas, because the levels of flavonoids are more than those from coastal areas. Before the treatment, the test animals were not given any food consumption for 8 hours, but only given a drink so that the extract could react optimally. The analgetic effect of papaya leaf extract was tested by inducing mice using 1% acetic acid intraperitoneally. 1% acetic acid causes severe irritation to the mucosa of the abdominal cavity, so the mice show a pain response such as pulling the legs back and the abdomen touching the floor.

From the data obtained, it can be seen that the analgetic effect on Kel.3 with a dose of 0.008 g and the suspension of mefenamic acid is almost the same, although the suspension of mefenamic acid is more effective because it still shows an anti-pain effect a few hours after the test. Meanwhile, Kel.4 with a dose of 0.016 g/kgBB showed better analgetic activity compared to mefenamic acid suspension, because the concentration of ethanol extract of papaya leaves (*Carica papaya L*.) was higher. Kel.4 and mefenamic acid suspension have equal potential in reducing pain response in mice. The anti-pain effects seen a few hours after treatment indicate that papaya leaf ethanol extract has promising potential. Previous research has shown that mefenamic acid has better analgesic activity due to its synthetic compounds that directly address pain. However, long-term use can result in negative side effects. Therefore, papaya leaf ethanol extract can be considered safer for long-term use than mefenamic acid, as it is derived from natural ingredients.

The results of this study showed that the analgesic effect of Kel.4 was longer and better than that of other groups, including a positive control, mefenamic acid. The best dose for the optimum analgetic effect is 0.016 g/kgBB. This result is better than the study of Octavianus *et al*. (2014), which found the best dose of 2.4 g/kgBB, where lower doses already showed good analgetic effects. Research by Afrianti *et al*. (2014) tested analgetic activity in mice with doses of papaya leaf ethanol extract (*Carica papaya* L.) of 100 mg/kgBB, 300 mg/kgBB, and 600 mg/kgBB. The results showed the best dose at 600 mg/kgBB, with analgetic effects visible at 25 minutes and peaking around 40 to 60 minutes. The results of our study were similar, showing analgetic activity began to be seen at 20 minutes and peaked at 35 minutes at maximum dose.

CONCLUSION

From this study, it was concluded that the flavonoid content in ethanol extract of 96% papaya leaves (*Carica papaya* L.) was 6.39% in coastal areas and 7.48% in mountainous areas, indicating that papaya leaves in the mountains had a higher flavonoid content. In addition, ethanol extract of papaya leaves with a concentration of 800 mg/kgBB showed the best analgetic effectiveness in mice (*Mus musculus*). The limitations of this study are the lack of appropriate dosage in testing and the relatively short time of the anti-pain effectiveness test.

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