OPTIMIZATION OF SOLVENT VARIATIONS FROM FRUIT SKIN EXTRACTS BREADFRUIT Artocarpus altilis (Parkinsom ex F.A,Zom) Fosberg ON ANALGETIC ACTIVITY IN VIVO

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Abstract. Breadfruit peel has analgesic properties because it is known to contain flavonoids that can inhibit cyclooxygenase enzymes. This study aims to determine the analgesic activity of 70% ethanol and methanol extracts from the skin of the breadfruit *Artocarpus altilis* (Parkinsom ex F.A.Zom) Fosberg in vivo. The method used in this study was the Sigmund method, in which acetic acid was used as an inducer. 40 white male mice aged 2-3 months, weighing 20–30 grams, were divided into 8 groups, namely negative control (CMC-Na 0.5%), positive control (antalgic), groups of 70% ethanol and methanol extract doses of 100 mg/KgBW, doses of 300 mg/KgBW, and doses of 600 mg/KgBW. The parameters observed were the number of mice stretching every 10 minutes for 1 hour. Stretching data is calculated by percent protection and percent effectiveness. Furthermore, statistical analysis was carried out with SPSS 22, and data were analyzed with one-way ANOVA and post hoc LSD to see significant differences between treatments. The results of the one-way ANOVA test showed that there were significant differences in all groups, with a significant value of 0.05. The results of the posthoc test analysis showed no significant difference between the positive control with 70% ethanol extract and methanol at a dose of 600 mg/KgBB ($P > 0.05$). 70% ethanol and methanol extracts can provide analgesic potency, and the optimal dose, which has nearly the same activity as the positive control, is a dose of 600 mg/kg BW.

Keywords: [Analgesic, *Artocarpus altilis* (Parkinson ex.F.A.Zom) Fosberg, 70% ethanol and methanol]

INTRODUCTION

Pain is a sign that the body is experiencing problems, pain arises due to mechanical or chemical stimulation which can cause damage to tissues and release certain substances called pain mediators such as bradykinin, histamine, serotonin, and prostaglandins (Afrianti, Yenti, & Meustika, 2015). Analgesics are drugs to reduce pain without losing consciousness (Sariana, 2011). Antalgin acts centrally on the brain by relieving pain. However, antalgic is known to have side effects that can result in agranulosis which is fatal for this reason while using this drug it is necessary to carry out regular blood tests (Lukmanto, 1986). Because antalgic drugs have various side effects, the use of drugs from plants has begun to be developed. Based on previous studies, it has been proven that plants from the Moraceae family have analgesic activity. Several studies on analgesic activity in this family, namely, the ethanol extract of jackfruit leaves (Artocarpus heterophyllus Lam.) at a dose of 600 mg/kgBW effectively showed analgesic activity in mice, the ethanol extract of breadfruit leaves (*Artocarpus altilis*) at a dose of 100 mg/kgBW, 200 mg /kgBB can show analgesic activity.

Flavonoid compounds in the Moraceae family are to have analgesic activity whose mechanism of action protects lipid membranes from damage and inhibits the cycle of oxygenase I enzyme which is the first pathway for the synthesis of pain mediators such as prostaglandins. Pain will decrease by inhibiting the production of prostaglandins (Afrianti, Yenti, & Meustika, 2015).In Yusantri's research (2017) it was proven that breadfruit peel contains flavonoids, alkaloids, tannins, triterpenoids, and steroids which dissolve in ethanol, methanol, ethyl acetate, and n-hexane. Therefore this study uses breadfruit peel as analgesic activity. To extract the active substance in the plant, the extraction process is carried out. The effectiveness of extraction is influenced by the sample solvent, type of solvent, and extraction time (Oktavia, 2011). In this study, the selection of solvents was based on the principle of like dissolves like, namely polar solvents will dissolve polar compounds, and non-polar solvents will dissolve non-polar compounds (Afrianti, Oktarina & Kusumawati, 2014). Based on the statement above, a study was carried out to optimize variations of 70% ethanol and methanol solvents from *Artocarpus altilis* breadfruit peel extract (Parkinson ex. F.A.Zom) Fosberg for analgesic activity in vivo.

METHODS

This research is an experimental study using mice as experimental animals. The population used in this study was the skin of the breadfruit *Artocarpus altilis* (Parkinsom ex F.A.Zom) Fosberg obtained from Sendangmulyo Village, Gunem District. The research sample used was 70% ethanol extract and breadfruit peel methanol. Mice cage, scales (Tanita), analytical balance (Ohaus), Sieve no 40 mesh, oven (Memmert), beaker, stir bar, funnel (Herma), measuring flask (Herma), beaker glass (Herma), Erlenmeyer (Herma), measuring cup (Herma), test tube (Iwaki), dropper pipette, test tube rack, simplicial blender (Getra), Ultrasonic (Jinyuanbao Ultrasonic Cleaner), sonde needle, One Med brand 1 mLDisposable Syringe, filter paper, and water bath (DHH-4). Breadfruit peel, 70% ethanol, methanol, glacial acetic acid, distilled water, 0.5% CMC-Na, antalgin, 20% sodium hydroxide, $FeCl_3$, chloroform, ammonia, dragendroff, Mayer, Wagner, and concentrated sulfuric acid. 5 kg of breadfruit peel is washed, then thinly sliced and dried in an indirect way, i.e. by airing it, then crushed using a blender and sifted through a 40 mesh sieve and followed by calculating the drying losses and weight losses of simplicia. Moisture content was determined using the gravimetric method by heating in an oven at 105^{o}C for 3 hours. Extraction using the maceration method, the simplicia powder was macerated with a ratio of 1:4, namely 200 grams of simplicia: 800 mL of solvent. Maceration was carried out for 1 x 24 hours every 6 hours stirring and repeated 3 times. The results of maceration are then collected together and concentrated with a water bath at 40^{o}C.

Phytochemical Screening

1. Identification of Flavonoid Compounds
   As much as 1 mL of breadfruit peel extract was put into a test tube, then heated with 5 mL of distilled water for 5 minutes, then filtered and then measured 1 mL of filtrate and added a few drops of 20% sodium hydroxide solution, if the filtrate formed a yellow color, it contained flavonoids.
2. Identification of Tannin Compounds
   As much as 0.5 grams of concentrated extract was dissolved with 10 mL of distilled water, then filtered and the filtrate was dripped with 2-3 1% FeCl3. A positive result is indicated by the formation of a bluish-black or green color.
3. Identification of Alkaloid Compounds
   A total of 0.5 grams of extract was added to 1.5 mL of chloroform and 3 drops of ammonia. The extract was acidified by adding 2 drops of sulfuric acid two phases are formed in the tube. The acid portion (top layer) was tested with Dragendorff, Mayer, and Wagner reagents, 2-3 drops each. The test result was positive if the Dragendorff reagent formed a red to orange precipitate, Mayer's reagent formed a yellowish-white precipitate and Wagner's reagent formed a brown precipitate.
4. Identification of Steroid and Triterpenoid Compounds
   a. Test Steroids
      As much as 1 mL of breadfruit peel extract was dissolved in 5 mL of chloroform, then added sulfuric acid through the side of the test tube, if a layer with a red color and a layer of sulfuric acid formed a greenish yellow color indicating the presence of steroid compounds.
   b. Triterpenoid Test
      As much as 1 ml of breadfruit peel extract is mixed with 0.5 ml of chloroform, then 1.5 ml of concentrated sulfuric acid is added to form a layer. The color of the reddish-brown precipitate on the coating indicates the presence of triterpenoid compounds.

Analgesic Activity Testing

This study used 24 male mice with a weight of 20-30 g and aged 2-3 months. Mice were first adapted for 7 days in the STIKES Scholar Utama Kudus. Mice were divided into 8 groups with each group consisting of 5 mice and given oral treatment and intraperitoneal induction. The distribution of the test animal groups consisted of the first group being negative control, the second group being positive control, the third group with a 70% ethanol extract dose of 100 mg, the fourth group 70% ethanol extract dose of 300 mg, the fifth group 70% ethanol extract dose 600 mg, the sixth group methanol extract dose

of 100 mg, seventh group of methanol extract dose of 300 mg and an eighth group of methanol extract dose of 600 mg. A total of 500 grams of CMC-Na was weighed and sprinkled into a mortar containing enough warm distilled water, then stirred until it swelled, put in a 100 mL volumetric flask, and added distilled water to 100 mL. Antalgin was converted first from human weight to mice and obtained as much as 1.3 mg for 20 grams of mice. The doses used were 100 mg/KgBW, 300 mg/KgBW, and 600 mg/KgBW. The adapted mice were then weighed and analgetic tests were carried out using the Sigmund method in which acetic acid was the inducer of the mice. Induction was carried out intraperitoneally so that the mice could show writhing by marking the abdomen touching the bottom of the place and both pairs of legs were pulled back, showing stretching the mice were grouped and given treatment and then counted the number of stretches for 1 hour. From the analgetic activity test data, the average of each group was calculated**.** The results obtained were then calculated as the percent protection of the test material and the percent effectiveness of each group. The data obtained from the results of the study were then statistically tested using IBM SPSS Statistics 22. The first tests were the normality test, homogeneity test, One Way ANOVA test, and the Post Hoc Least Significant Different (LSD) test.

RESULTS AND DISCUSSION

Simplisia Drying

The results of drying the simplicia of Artocarpus altilis (Parkinsom ex FAZom) Fosberg breadfruit peels can be seen in table 1.

Table 1. Making Breadfruit Peel Simplicia

Information	Breadfruit skin	Dry simplicia	Breadfruit peel powder	% Drying loss
Heavy	5 Kg	3065 grams	2080 grams	38.70 %
Color	Green	Chocolate	Chocolate	

The skin of the breadfruit from Sendangmulyo Village, Gunem District, Rembang Regency is separated from the fruit and then washed, then a slicing process is carried out which aims to facilitate the drying process to make it faster. The purpose of drying is to simplify the process of making simplicia powder and reduce the water content so that simplicia is not easily overgrown by mold and bacteria. The weight of the breadfruit peel after drying was 3065 grams with a drying shrinkage of 38.70%. Drying shrinkage indicates the amount of water that evaporates or is lost during heating. After the simplicia is dry, it is then blended and sieved using sieve no. 44 with the aim that the powder is selected into fine particles.

Determination of Water Content

The results of the determination of the water content of simplex breadfruit peel Artocarpus altilis (Parkinsom ex FAZom) Fosberg can be seen in table 2.

Table 2. Determination of Water Content

Replication	Initial sample weight (grams)	Dry sample weight (constant) (grams)	% water content
1	1 gram	59,67	6.8%
2	1 gram	61,39	9%
3	1 gram	61,24	0.8%
		Mean±SD	5.53%±4.24

Moisture content is a parameter determination of residual water after the drying process. From the study, the average yield was 5.53%, this indicated that the percentage of water content in the breadfruit peel powder met the simplicial standard, namely ≤ 10%.

Extract Manufacturing

The results of making 70% ethanol extract and methanol extract of Artocarpus altilis (Parkinsom ex FAZom) Fosberg breadfruit peels can be seen in table 3.

Table 3. Extract Preparation

Information	Breadfruit peel powder	Thick extract weight	yield
Ethanol 70%	200 grams	14.38	7.19 %
methanol	200 grams	18.43	9.21 %

Breadfruit peel is extracted using the maceration method, maceration is a suitable method for materials that are not resistant to heat because it can damage the content of secondary metabolites from breadfruit peel, one of which is flavonoids. The solvent used is 70% ethanol and methanol. The purpose of using these solvents is to attract flavonoid compounds which are polar and have several hydroxyl groups so they will dissolve in polar solvents according to the principle of like dissolves like. The maceration results were then crushed and the filtrate obtained was thickened with a water bath at 400C. the yield of a 70% viscous ethanol extract was 14.38 grams with a yield value of 7.19% and a viscous methanol extract of 18.43 grams with a yield of 9.21%.

Phytochemical Screening

Results of phytochemical screening of 70% ethanol extract and methanol of breadfruit peel *Artocarpus altilis (Parkinsom ex FAZom) Fosberg*can be seen in table 4.

Table 4. Phytochemical Screening

Compound	Reagent	Results	information	
			Ethanol 70%	methanol
Flavonoids	NaOH solution	Reddish brown-yellow color	+	+
Saponins	Aquadest	Foam / froth lasted for 3 minutes	-	-
tannins	FeCl31%	Blackish green color	-	+
Alkaloids	• Dragendrof	• Orange precipitate	+	-
	• Wagner	• precipitate chocolate reddish	+	+
	• Mayer	• White precipitate	+	+
Steroids and triterpenoids	Chloroform sulfuric acid	• Greenish yellow	+	-
		• Red	+	+

The results of the phytochemical screening of 70% ethanol extract and methanol of breadfruit peel by maceration method contained flavonoids, tannins, alkaloids, steroids, and triterpenoids. Yusantri's study (2017) also showed the content of secondary metabolites in breadfruit peels, namely flavonoids, tannins, alkaloids, steroids, and triterpenoids.

Analgesic Activity Test Results

ResultsAnalgesic activity test of 70% ethanol extract and methanol of Artocarpus altilis (Parkinsom ex F.A.Zom) Fosberg breadfruit peels can be seen in table 5.

Table 5. Analgesic Activity Test

Test group	Cumulative number of stretches					Average
	1	2	3	4	5	
control (-)	129	135	130	141	136	134,2
control (+)	32	43	29	50	46	40
70% ethanol extract 100	89	105	85	73	65	83,4
70% ethanol extract 300	44	112	93	72	61	76,4
70% ethanol extract 600	64	78	107	63	45	71.4
Methanol Extract 100	83	75	102	98	115	94.6
Methanol extract 300	112	98	73	65	73	84,2
Methanol extract 600	85	79	56	65	75	72

The results of testing the amount of stretching the average mice showed that there was a decrease in the average number of stretching mice in the positive control group by 40 and in the group given 70% ethanol extract dose 600 mg/KgBW by 71.4, the methanol extract dose 600 mg/KgBW by 72 when compared to the group negative control of 134.2 this is due to CMC-Na as an additive including as a thickening agent. The less the average number of stretches shows the better the analgesic effect on a test material.

Percent protection is the ability of the test material to reduce pain caused by intraperitoneal acetic acid induction. The highest percentage of protection was shown by the positive control group of 70.19%, 70% ethanol extract dose of 600 mg/KgBW of 46.79%, and methanol extract dose of 600 mg/KgBW of 46.34%.

Percent effectiveness shows the effect of analgesic activity of the test material with positive control from each group. The percentage of analgesic effectiveness of the test substance at 70% ethanol extract dose of 600 mg/KgBB and methanol extract dose of 600 mg/KgBB gave results close to the percent effectiveness of the positive control, namely 66.66% and 66.02%.

The 70% ethanol extract at a dose of 600 mg/KgBW and the methanol extract at a dose of 600 mg/KgBW provide analgesic effectiveness which is almost equivalent to the positive control (antalgin). Flavonoids as analgesics whose mechanism of action protects the lipid membrane from damage and inhibits the cycle oxygenase I enzyme which is the first pathway of pain such as prostaglandins. Pain will decrease with the inhibition of prostaglandin production.

Data analysis used IBM SPSS Statistics 22. The first test that was carried out was the normality test with the Shapiro-Wilk Test, the results of which showed that the value of P> 0.05. The next analysis is the homogeneity test and a significant value is obtained, namely 0.053> 0.05, which means that the data that has been tested is homogeneous and deserves to be tested by One Way ANOVA to determine whether there is a difference between groups.

The results of the data obtained a significant value (0.000 <0.05), which means that the data in the study there were differences between the treatment groups, due to differences between groups, the data is processed again with a follow-up test, namely the Post Hoc Least Significant Different (LSD) test. The results obtained after the Post Hoc Least Significant Different (LSD) test have a significant value of P <0.05, which means there is a difference between negative control groups with the positive control, positive control group with 70% ethanol extract treatment group, and methanol dose of 100 mg/KgBB, 300 mg/KgBB & 600 mg/KgBB.

The results of the analysis between the positive control group and the treatment group with 70% ethanol extract and methanol doses of 100 mg/KgBW, 300 mg/KgBW, and 600 mg/KgBW showed that there was a significant difference seen from the value of P <0.05. But in the treatment group, 70% ethanol and methanol extract at a dose of 600 mg/KgBW had a P value that was almost close to 0.05.

This shows that the treatment group with 70% ethanol extract and methanol at a dose of 600 mg/KgBW has almost the same analgesic potency as the positive control.

CONCLUSIONS

Breadfruit peel contains flavonoids, alkaloids, tannins, steroids, and triterpenoids. 70% ethanol and methanol extracts from breadfruit peels have the potential for analgesic activity in vivo with an optimum dose of 600 mg/Kg BW. There was no significant difference between 70% ethanol extract and methanol from breadfruit peel at doses of 100 mg/KgBW, 300 mg/KgBW, and 600 mg/KgBW with a P value> 0.05 but a dose of 600 mg/KgBW had the same P.Value. barely close to a significant value.

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