ANALGETIC EFFECTIVENESS TEST OF 96% ETHANOL EXTRACT OF KECOMBRANG (ETLINGERA ELATIOR (JACK) R.M. SMITH) LEAVES ON SWISS WEBSTER MENCITES

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Abstract: *Pain is an unpleasant experience accompanied by tissue damage that can be overcome by administering analgesic drugs. The use of analgesic drugs can have side effects if used excessively, thus treatment with traditional plants becomes an alternative to analgesic therapy. Torch ginger leaf has potential as an analgesic because it has flavonoid compounds, alkaloids, tannins, and saponins as well as active compounds of chlorogenic acid. The purpose of this study was to prove the analgesic effectiveness of 96% ethanol extract of torch ginger leaves on male mice of the Swiss Webster strain. This study used 25 male mice which were grouped into 5 groups. K(-) was given 1% CMC Na, K(+) was given mefenamic acid 1.3 mg, and P(1), P(2), and P(3) were given 96% ethanol extract of torch ginger leaves with a dose of 6 mg, 12 mg, and 24 mg/20 gBW mice. Each test animal was given 1 mL/gBW of mice orally, after 30 minutes the mice were induced by 1% acetic acid as much as 1 mL/gBW of mice, then the number of mice wriggled for 30 minutes with 5-minute intervals. In the results of the normality test and homogeneity test, the value ($p>0.05$) means that the data is normally distributed and homogeneous, then continued with the One Way Anova test, the value is ($p<0.05$), then the Tukey test is carried out with the result that there is no significant difference. between the K(+) group and the treatment group at a dose of 24 mg/20 gBW mice, so it can be concluded that the most optimal 96% ethanol extract of torch ginger leaves provides analgesic effectiveness in male Swiss webster strain mice induced by 1% acetic acid is at a dose of 24 mg. /20 gBB mice.*
Keywords: *Torch ginger leaf (Etlingera elatior (Jack) R.M. Smith), Analgesic effectiveness, stretching method.*

INTRODUCTION

Pain according to The International Association for the Study of Pain is an unpleasant sensory and emotional experience accompanied by actual and potential tissue damage (Tudang, Wuisan & Najoan, 2013). Analgesic drugs can be used to reduce or eliminate pain without changing consciousness (Auliah, Latuconsina & Thalib, 2019). The use of analgesic drugs can have side effects if used excessively (Wardoyo & Oktarlina, 2019). Thus, it is hoped that treatment with traditional plants will become an alternative to analgesic therapy, one of which is the Etlingera elatior (Jack) R.M. Smith) plant. The leaves of Etlingera elatior (Jack) R.M. Smith itself can have potential as an analgesic because it has an active compound Chlorogenic Acid which is an antioxidant with two phenolic groups capable of capturing free radicals through proton transfer to reduce pain with analgesic properties (Chan, Lim & Tan, 2011). The results of phytochemical screening conducted in the research of Burhan, Rahim & Regina (2016) found that ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves positively contains alkaloids, flavonoids, tannins, terpenoids, and saponins.

Flavonoid compounds can be analgesic, according to research by Amri & Mamboya (2012) on papain enzymes, flavonoids can inhibit the enzyme cyclooxygenase I which plays a role in prostaglandin biosynthesis as a mediator of pain formation, so that inhibition of COX I will cause inhibition of the onset of pain. Tannin compounds can be analgetic, according to research conducted by Hesturini, Herowati & Widodo (2017) on the analgetic test of gandarusa leaf extract that the mechanism of action of tannins as analgetics by stimulating lipomodulin protein biosynthesis which can inhibit the enzymatic work of phospholipase, which is the enzyme responsible for the release of arachidonic acid and blocking the cyclooxygenase and lipooxygenase pathways so that its metabolites, namely prostaglandins, leukotrienes, prostacyclin and thromboxane, cannot be formed.

Saponin compounds are classified into triterpenoids Saponin compounds can be analgetic, according to Tamimi, Queljoe & Siampa (2020) in the analgetic test of moringa leaf extract that saponins are classified into triterpenoids and steroid saponins which are anti-inflammatory, analgesic, and cytotoxic. Alkaloid compounds can also be analgetic, according to research by Tamimi, Queljoe & Siampa (2020) on the analgetic effect test of moringa leaf extract that alkaloid compounds have a function as inhibitors of important phases in prostaglandin biosynthesis, namely in the cyclooxygenase pathway in the arachidonic acid metabolic pathway which can inhibit the onset of pain.

METHODS

Research Type and Design :

This research is a type of animal experimental research with a simple experimental design (Posttest Only Control Group Design).

Population, Number of Samples, and Characteristics of Respondents :

This study used 25 male Swiss Webster mice aged 2-3 months with a body weight of 20-30 grams obtained from the Islamic University of Sultan Agung Semarang.

Time and Place of Research :

This research was conducted at the Pharmacology Laboratory of the Cendekia Utama Kudus College of Health Sciences in March-April 2021.

Research Tools and Materials :

The tools used in this study are mice scales, dark glass jars, filter paper, rotary evaporator, stirring rod, analytical scales, water bath, test tube, glass funnel, beaker glass, measuring cups 10 mL and 100 mL, measuring flask 100 mL, dropper pipette, stopwatch, mortar and stamper, mice sonde, cannula, 1 cc syringe, blender, gloves, sieve no. 60, aluminum foil, moisture balance, Meyer reagent, ethanol 96%, HCl 2N, distilled water, Meyer reagent. 60, aluminum foil, moisture balance, and macerator tools, while the materials used in this study are Etlingera elatior (Jack) R.M. Smith leaves, 96% ethanol, 2N HCl, distilled water, Meyer reagent, Bouchard at reagent, drag-drop reagent, Lieberman burchard reagent, FeCl3 1%, concentrated HCl, Mg powder, CMC Na 1%, mefenamic acid tab 500 mg, 1% acetic acid, NaCl 0.9%.

Material Collection and Simplisia Preparation Stage :

Etlingera elatior (Jack) R.M. Smith leaves were taken from the Colo area of Jalan Kudus Muria kilometer 18 Dawe Kudus. Kecombrang leaves used are green leaves and large (Renaninggalih,

Mulkiyam & Sadiyah, 2014). Leaf collection is done by using a knife taken randomly from several trees. The collected Etlingera elatior (Jack) R.M. Smith leaves are then carried out at the simplistic-making stage, namely by wet sorting, washing, kneading, dry sorting, packing, storage, and making powdered simplisia (Depkes RI, 2017).

Preparation of 96% Ethanol Extract of Etlingera elatior (Jack) R.M. Smith Leaf

Simplisia powder of Etlingera elatior (Jack) R.M. Smith leaves was weighed as much as 500 grams and then put into a maceration container and added 96% ethanol as much as 5 liters until the simplisia was completely submerged. The maceration container was closed and stored for 3 days in a place protected from direct sunlight while occasionally stirring, and then repeating maceration for 2 times treatment. The filtrate of 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves was then collected and evaporated using a rotary evaporator until a thick extract was obtained (Depkes RI, 2017).

Phytochemical Screening of 96% Ethanol Extract of Etlingera elatior (Jack) R.M. Smith Leaf

a. Alkaloid Identification

10 mg of thick extract of f Etlingera elatior (Jack) R.M. Smith leaves was put into a test tube, 2 mL of 96% ethanol and stirred, 5 mL of 2 N HCl, and then 9 mL of distilled water. Heated for 2 minutes and then filtered using filter paper to obtain f Etlingera elatior (Jack) R.M. Smith leaf extract. Taken 3 drops of the filtrate were obtained and then added 2 drops of Meyer reagent to produce a white or yellow precipitate. Furthermore, 3 drops of the filtrate obtained were taken, then 2 drops of Bouchardat reagent were added to produce a black-brown precipitate. Taken 3 drops of the filtrate obtained and then added 2 drops of Dragendrof reagent produces a brick red precipitate. Alkaloids are considered positive if there are at least 2 or 3 precipitates from the above experiments (Kusumawati, Supriningrum & Rozadi, 2015).

b. Flavonoid Identification

10mg of thick extract of Etlingera elatior (Jack) R.M. Smith leaves was put into a test tube with 2 mL of 96% ethanol then stirred, added 2 mg of Mg powder and 3 drops of concentrated HCl. If a red, yellow, or orange color is formed, it indicates the presence of flavonoids (Darmawi, Saleh & Kartika, 2015).

c. Identification of Tannins

10 mg of thick extract of Etlingera elatior (Jack) R.M. Smith leaves were put into a test tube added 2 mL of 96% ethanol then stirred, added 1 to 2 drops of 1% FeCl3 solution. If a dark blue and blackish-green color is formed, it indicates the presence of tannins (Kusumawati, Supriningrum & Rozadi, 2015).

d. Saponin Identification

10 mg of thick extract of Etlingera elatior (Jack) R.M. Smith leaves was put into a test tube added 2 mL of 96% ethanol stirred, 5 mL of hot water, and shaken for 15 minutes, then added 1 to 2 drops of HCl 2 N. If permanent foam is formed, it indicates the presence of saponins (Kusumawati, Supriningrum & Rozadi, 2015).

e. Identification of Terpenoids

10 mg of thick extract of Etlingera elatior (Jack) R.M. Smith leaves is put into a test tube added 2 mL of 96% ethanol and then stirred, added 3 drops of Lieberman-Burchard reagent. if a red or purple color is formed, it indicates the presence of terpenoids (Darmawi, Saleh & Kartika, 2015).

Analgesic Effectiveness Testing

The method used in this study is the writhing test. Test animals were randomly grouped into 5 groups, each group of 5 test animals, then fed for 18 hours but still given a drink. Each group was given treatment orally as much as 1 mL / gBB mice with the specified dose level.

a. Group 1: Negative control 1% b/v CMC Na suspension.
b. Group 2: Positive control mefenamic acid suspension 1.3 mg/20 gBB mice.
c. Group 3: Suspension of 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves at a dose of 6 mg/20 gBB mice.
d. Group 4: Suspension of 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves at a dose of 12 mg/20 gBB mice.
e. Group 5: Suspension of 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves at a dose of 24 mg/20 gBB mice.

After the mice were treated, 30 minutes later the pain indicator was induced intraperitoneally using 1% v/v acetic acid as much as 1 mL / gBB mice, after a few minutes the mice would wriggle with both

pairs of legs forward and backward, and the abdomen pressed against the cage floor (Ministry of Health of the Republic of Indonesia, 2016). This study is in line with the research of Winarti & Wantiyah (2011) on the analgetic test of temu kunci rhizome extract using the writhing test method by counting the number of writhing mice during 30 minutes of observation with an interval of 5 minutes.

Calculation of % Wriggle Protection and % Analgesic Effectiveness

a. % Wriggle Protection
   % Wriggle protection = (100 - (P/K x 100)) %
   Description:
   P: Cumulative number of writhing of test animals after administration of the prescribed drug
   K: Cumulative number of writhing of negative control test animals (CMC Na 1%).

b. % Analgesic Effectiveness
   Analgesic effectiveness = % P / % KP x 100%
   Description:
   P: % Protection of writhing in each treatment group
   KP: % Protection of positive control (mefenamic acid) (Kemenkes RI, 2016)

Data Analysis

Data analysis was performed statistically using the SPSS 23 program. Pharmacological data on the number of writhing mice obtained were analyzed by testing the normality of the data and the homogeneity of the data. The results of the data normality test and data homogeneity showed that the data were normally distributed and homogeneous ($p>0.05$), then continued with the parametric test, namely the One Way Anova test with the criteria ($p<0.05$) which means there is a significant difference between the treatment groups, and continued with the Tukey test to determine the comparison of mean values between groups is statistically different or not.

RESULTS AND DISCUSSION

Results of Etlingera elatior (Jack) R.M. Smith Leaf Processing

The dried Etlingera elatior (Jack) R.M. Smith leaves were then pulverized using a blender after which they were sieved with sieve number 60 to obtain a fine powder fineness degree. Because the degree of fineness of simplisia powder for making extracts is fine simplisia powder (Depkes RI, 2017). Processing Results of Etlingera elatior (Jack) R.M. Smith Leaf, Etlingera elatior (Jack) R.M. Smith leaves can be seen in Table 1.

Table 1. Results of Etlingera elatior (Jack) R.M. Smith Leaf Processing

Wet Leaves	Dried Simplisia	Powdered Symplisia
3 kg	900 gr	850 gr

Phytochemical Screening Results of 96% Ethanol Extract of Etlingera elatior (Jack) R.M. Smith Leaf

Phytochemical screening in this study was conducted to determine the chemical compounds contained in Etlingera elatior (Jack) R.M. Smith leaves using the test tube method. Based on the results of phytochemical screening, it can be seen that 96% of ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves positively contains alkaloid compounds, flavonoids, tannins, and saponins. The result of chemical content identification in 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves can be seen in Table 2.

Table 2. Chemical Content Identification Results of 96% Ethanol Extract of Etlingera elatior (Jack) R.M. Smith Leaf

Compound Groups	Reactions	Result	Colors
Alkaloids	HCl 2N + *Meyer*	-	Brown sediment
	HCl 2N + *Bouchardat*	+	Dark brown sediment
	HCl 2N + *Dragendrof*	+	Brick-red sediment
Flavonoids	Mg + HCl powder	+	Red
Tannin	FeCl3 1%	+	Blackish green
Saponin	HCl 2N	+	Permanent foam formation
Terpenoid	*Lieberman-Burchard*	-	Brown

Description:
Positive (+): Contains chemical compounds
Negative (-): Does not contain chemical compounds

Analgesic Effectiveness Test Results

a. Number of Writhing Mice

The average number of mice writhing for 30 minutes in the treatment group was the least at a dose of 24 mg/20 gBB mice with a result of 26.80 writhing times. The data can be seen in Table 3.

Table 3. Mean number of mice writhing for 30 minutes ± SD

Group Treatment	Mean±SD
Negative Controls	$99,60 \pm 7,503^{b}$
Positive Controls	$22,20 \pm 2,387^{a}$
Dosage 6 mg	$35,20 \pm 4,324^{ab}$
Dosage 12 mg	$32,20 \pm 4,494^{ab}$
Dosage 24 mg	$26,80 \pm 1,924^{a}$

Description:
(a): There is a significant difference with the negative control group ($p<0.05$)
(b): There is a significant difference with the positive control group ($p<0.05$)

Based on the results of statistical analysis, it is known that in the K (-) group, there is a significant difference with the K (+) group and 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves doses of 6 mg, 12 mg, and 24 mg with a value of ($p <0.05$) this is because K (-) is given 1% CMC Na which has no efficacy as an analgesic and only serves as a solvent.

In the K (+) group there is a significant difference with 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves in doses of 6 mg and 12 mg, this is because the content of compounds in Etlingera elatior (Jack) R.M. Smith leaves that act as analgesics, namely flavonoids, alkaloids, tannins and saponins at doses of 6 mg and 12 mg has not been able to equalize the analgesic effect on K (+).

The K(+) group was given mefenamic acid which has an analgesic effect by blocking the effect of an enzyme called cyclooxygenase (COX), this enzyme helps the body to produce chemicals called prostaglandins, by blocking the effect of the COX enzyme, fewer prostaglandins are produced, so that

pain and inflammation will subside or improve (Katzung, 2011).

In the K (+) group there was no significant difference with the 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves at a dose of 24 mg with a value of (p>0.05) this is because the compounds contained in the 24 mg dose almost equalize the analgesic effect on K (+) and there are flavonoids, alkaloids, tannins and saponin compounds that are more numerous than in doses of 6 mg and 12 mg. The content of compounds contained in Etlingera elatior (Jack) R.M. Smith leaves, namely flavonoids, can have potential as analgesics. This study is in line with the research of Amri & Mamboya (2012) on papain enzymes that flavonoid compounds can inhibit the enzyme cyclooxygenase I which plays a role in prostaglandin biosynthesis as a mediator of pain formation so that inhibition of COX-1 will cause inhibition of the onset of pain.

Alkaloid compounds can also act as analgesics with the mechanism of action of inhibiting prostaglandin pathway dehydrogenase which will inhibit the activation of prostaglandins (Robinson, 1995). This research is in line with the research of Tamimi, Queljoe & Siampa (2020) on the analgetic effect test of moringa leaf extract that alkaloid compounds have a function as inhibitors of an important phase in prostaglandin biosynthesis, namely in the cyclooxygenase pathway in the arachidonic acid metabolism pathway which can inhibit the onset of pain. Tannin compounds can be analgetic, according to research conducted by Hesturini, Herowati & Widodo (2017) on the analgetic test of gandarusa leaf extract that the mechanism of action of tannins as analgetics by stimulating the biosynthesis of lipomodulin proteins which can inhibit the enzymatic work of phospholipase, which is the enzyme responsible for the release of arachidonic acid and blocking the cyclooxygenase and lipooxygenase pathways so that its metabolites, namely prostaglandins, leukotrienes, prostacyclin, and thromboxane, cannot be formed.

In the results of homogeneous subsets, it is known that the K (+) group is in the same column as the 96% ethanol extract of kecombrang leaves at a dose of 24 mg. It can be seen that K (+) and a dose of 24 mg have an almost equivalent effect as analgesic effectiveness, which means that a dose of 24 mg is the most optimal dose among doses of 6 mg and 12 mg, This is reinforced by research conducted by Safitri, Andre & Irsan (2013) on the analgesic test of cocor bebek leaves that the higher the dose used, the more the content of compounds that act as analgesics, namely flavonoids, alkaloids, tannins, and saponins. The results of homogeneous subsets can be seen in Table 4.

Table 4. Result Homogeneous subsets

Groups	Subset		
	1	2	3
Positive Controls	22,20		
Dosage 24 mg	26,80	26,80	
Dosage 12 mg		32,20	
Dosage 6 mg		35,20	
Negative Controls			99,60

b. % Writhing Protection

% Writhing Protection is done to determine the amount of inhibition of the number of writhing mice in the analgesic test. Based on the results of % writhing protection, it can be seen that the largest % writhing protection is shown in the K (+) mefenamic acid group which is 77.71% and for the 96% ethanol extract group of the highest Etlingera elatior (Jack) R.M. Smith leaves in a row is a dose of 24 mg with a percentage of writhing protection of 73.09%, a dose of 12 mg with a percentage of writhing protection of 67.67% and a dose of 6 mg with a percentage of writhing protection of 64.66%.

c. % Analgesic Effectiveness

% Analgesic Effectiveness is done to show how much the drug's ability to cause effects or

benefits. Based on the results of % analgetic effectiveness, it can be seen that the % analgetic effectiveness of mefenamic acid as K (+) is 100% and for the highest 96% ethanol extract group of Etlingera elatior (Jack) R.M. Smith leaves in a row is a dose of 24 mg with a percentage of analgetic effectiveness of 94.05%, a dose of 12 mg with a percentage of analgetic effectiveness of 87.08% and a dose of 6 mg with a percentage of analgetic effectiveness of 83.21%.

Based on the percentage of analgesic effectiveness, all doses of 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves have analgesic effectiveness. This research is in line with the research of Sianturi et al. (2018) on the analgetic potential of beginning ganang leaf extract that the effective requirement as an analgetic is with a percent effectiveness of >50%.

The data results of % writhing protection and % analgetic effectiveness obtained indicate that the greater the % writhing protection and % analgetic effectiveness, the greater the analgetic effect, and vice versa if the smaller the % writhing protection and % analgetic effectiveness, the smaller the analgetic effect, It can be seen that all doses of 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith leaves can provide analgesic effects and have almost equal flavonoid, alkaloid, tannin, and saponin content between the three doses, but the most optimal is at the largest dose, namely the dose of 24 mg/20 gBB mice.

CONCLUSION

a. 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith) leaves can provide analgesic effectiveness in male Swiss Webster mice induced by 1% acetic acid.

b. The optimal dose of 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith) leaves which provides analgesic effectiveness in male Swiss Webster mice induced by 1% acetic acid is a dose of 24 mg/20 gBB mice.

c. The highest percentage of analgesic effectiveness of 96% ethanol extract of Etlingera elatior (Jack) R.M. Smith) leaves which provide analgesic effectiveness in male Swiss Webster mice induced by 1% acetic acid is 94.05%.

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