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ETHANOL EXTRACT ANALGESICS ACTIVITIES OF MAHKOTA DEWA LEAVES (*Phaleria macrocarpa* (Scheff.) Boerl) FOR *IN VIVO*

AKTIVITAS ANALGESIK EKSTRAK ETANOL DAUN MAHKOTA DEWA (*Phaleria macrocarpa* (Scheff.) Boerl) SECARA *IN VIVO*

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ABSTRACT
Pain is a feeling of discomfort caused by intense or destructive stimuli, which, if left untreated, can affect your daily routine. Pain can be treated with analgesics. One of the plants that are thought to have analgesic effects is the leaves of the mahkota dewa. The purpose of this study was to determine the analgesic effect and dosage of the ethanol extract of mahkota dewa leaves and to determine the effectiveness of the ethanol extract of the mahkota dewa leaves as an analgesic using the stretching method.
The study began with the collection and processing of the leaves of the mahkota dewa into ethanol extract using the maceration method; then, the research was continued within in vivo analgesic activity testing of 25 mice that had been induced by pain using 1% acetic acid. The induced mice were divided into five treatment groups, where the mice in the first group served as a negative control group; in this group, they were given CMC at a dose of 0.5%. The second group of mice, which were positive controls, were given mefenamic acid at a dose of 500 mg. In contrast, in mice, the third, fourth, and fifth groups, which were the treatment group, were given ethanol extract of the leaves of mahkota dewa with consecutive doses of 0.25 g/kg BW, 0.50 g/kg BW, 0.75 g/kg BW. Parameters measuring the effectiveness of the extracts used in this study included the amount of stretching, the percentage of analgesic power, and analgesic effectiveness.
The results showed that the positive control group had the highest percentage of analgesic power at 59.9%. At EEDMD 0.75 g/kg BW gained 55.5%. EEDMD at a dose of 0.25 g/kg BW obtained 46.33%. Furthermore, EEDMD at a dose of 0.50 g/kg BW amounted to 55.06%. Furthermore, the percent effectiveness showed that EEDMD 0.75 g/kg BW had the highest percentage effectiveness, namely 92.65%. Whereas at EEDMD 0.25 g/kg BW is 77.29% and in EEDMD 0.50 g/kg BW is 91.91%. Based on the results of statistical analysis using ANOVA, it was found that the ethanol extract of the leaves of the mahkota dewa dosage of 0.75 g / kg BW had analgesic activity close to 500 mg of mefenamic acid.

Keywords : Mahkota dewa leaves, acetic acid, mice, stretching, mefenamic acid

ABSTRAK
Nyeri merupakan perasaan tidak nyaman yang disebabkan oleh rangsangan yang kuat atau merusak, jika dibiarkan dapat mempengaruhi rutinitas sehari-hari. Nyeri dapat ditangani dengan analgetika. Salah satu tumbuhan yang diduga mempunyai efek analgetik adalah daun mahkota dewa. Tujuan dari penelitian ini adalah untuk mengetahui efek analgesik dan dosis dari ekstrak etanol daun mahkota dewa, serta untuk

mengetahui keefektifan Ekstrak Etanol Daun Mahkota Dewa sebagai analgesik dengan metode geliat. Penelitian dimulai dengan pengumpulan dan pengolahan daun mahkota dewa menjadi ekstrak etanol menggunakan metode maserasi, kemudian penelitian dilanjutkan dengan pengujian aktivitas analgesik secara *in vivo* pada 25 ekor mencit yang telah di induksi nyeri menggunakan asam asetat 1%. Mencit yang telah diinduksi tersebut dibagi menjadi lima kelompok perlakuan, dimana kelompok pertama sebagai kontrol negatif diberikan CMC 0,5%, pada kelompok kedua sebagai kontrol positif diberikan asam mefenamat 500 mg, sedangkan pada kelompok ketiga, keempat dan kelima yang merupakan kelompok perlakuan ekstrak, diberikan ektrak etanol daun mahkota dewa dengan dosis berturut-turut sebesar 0,25 g/kgBB; 0,50 g/kgBB; 0,75 g/kgBB. Parameter pengukuran efektivitas dari ekstrak yang digunakan dalam penelitian ini meliputi jumlah geliat, persentase daya analgesik dan persentase efektivitas analgesik.
Hasil penelitian menunjukkan kelompok kontrol positif memiliki persentase daya analgesik yang tertinggi dengan jumlah 59,9%. Pada EEDMD 0,75 g/kg BB memperoleh 55,5%. EEDMD dosis 0,25 g/kg BB memperoleh 46,33%. dan EEDMD dosis 0,50 g/kg BB jumlah 55,06%. Hasil persen efektivitas menunjukkan pada EEDMD 0,75 g/kgBB mempunyai persen efektivitas tertinggi yaitu 92,65%, sedangkan pada EEDMD 0,25 g/kg BB yaitu 77,29% dan pada EEDMD 0,50 g/kg BB yaitu 91,91%. Berdasarkan hasil analisis statistik menggunakan ANOVA didapatkan hasil ekstrak etanol daun mahkota dewa dosis 0,75 g/kg BB mempunyai aktivitas analgesik mendekati asam mefenamat 500 mg.

Kata kunci : Daun mahkota dewa, asam asetat, mencit, geliat, asam mefenamat

INTRODUCTION

Indonesia is rich in natural resources that can potentially become medicine precursors. More than 20.000 types of medicinal plants are scattered all around Indonesia. About 1.000 types of plants have been identified, and only 300 of them have been used as traditional medicine. The use of the plant as traditional medicine ingredients needs more scientific research to understand the efficacy and use as a guiding compound precursor for newly synthesized medicine (Akbar, 2010).

Pain is one of the general problems that occur in society and one reason people come to see a doctor; pain disturbs sufferers' social function and life quality. Pain is defined as sensory and emotional experience connected with tissue dysfunction (Merskey and Bogduk, 1997). Compounds in therapeutic dosage slow down or suppress pain without having general anesthesia, which is called analgesic (Mutschler, 1991). Analgesic that is commonly used is *non-steroidal anti-inflammatory drugs* (NSAIDs), opioid, and antidepressant. *Non-steroidal anti-inflammatory drugs* (NSAIDs) is a medicine that is commonly used in therapy because of analgesic effect and anti-inflammatory. *Non-steroidal anti-inflammatory drugs* (NSAIDs) can affect many complexions like hypertension, edema, kidney disorders, and gastrointestinal bleeding. Analgesics for tempering or suppressing the pain not only come from chemical precursors but also medicinal plants.

In recent years, study on herbal plants has been done, concerning efficiency and side effects of chemical medicine (Ripa *et al.*, 2015). On the other hand, medicine from natural resources has advantages like practical efficacy, good tolerance effect, fewer side effects, and allergy(Hasan *et al.*, 2015).

One of the traditional plants that can be used as medicine is mahkota dewa (*Phaleria macrocarpa*). Mahkota dewa contains flavonoids, polyphenols, saponins, resins, tannins, and steroids with antimicrobial activity (Wahab *et al*., 2020). Phalerin is an active substance inside mahkota dewa and anti-inflammatory (Wahyuningsih *et al*., 2005). Mahkota Dewa leaf extract is reported to have antihyperglycaemic through inhibition of the *α-glucosidase* enzyme, which is carbohydrates digesting enzyme. The guess on analgesic effect happened because saponins and polyphenols mahkota dewa leaf also contain alkaloids and flavonoids (Anonim, 1999; Harmanto, 2001), flavonoids can take apart as an analgesic by inhibiting cyclooxygenase enzyme by reducing the production of prostaglandins by arachidonic acid. Other than that, flavonoids also

block out cytokines, free radicals, and enzymes in inflammation (Sasongko *et al*., 2016). Based on the guessing of analgesic effects, the author is interested in testing analgesic effects from mahkota dewa leaf ethanol extract against male mice.

The purpose of this research is to see if there is an Analgesic activity of mahkota dewa leaf (*Phaleria macrocarpa*) ethanol extract against mice (*Mus musculus*). The benefit of this research is that we hope that it could elevate the knowledge of utilizing mahkota dewa leaf (Phaleria macrocarpa) ethanol extract with analgesic effects. In addition, for an educational institution, as a reference and reading material for bachelor of pharmacy program towards utilizing mahkota dewa (*Phaleria macrocarpa*) as analgesic effects. We hope to continue this discovery to benefit as an analgesic effect for the following researchers.

RESEARCH METHODS

Tools used

Tools used include analytical balance, knife, sieve, steam bowl, mortar, pestle, blender, water bath, stopwatch, filter paper, observing cage, injection syringes, oral probes, glasses, and *rotary evaporator*.

Ingredients used

Ingredients used include mahkota dewa leaf, ethanol 70% (technical), Na-CMC, aquades, acetic acid 1%, and mefenamic acid 500 mg. This research used 25 male mice with 2-3 months of age and weighed 20-30 gram.

Plant Sample

Five kilogrammes of mahkota dewa leaf is collected at Tempua street, Sei Sikambing B Village, Medan Sunggal District, Medan. Determination is done at Herbarium Medanense (MEDA), Universitas Sumatera Utara, Medan.

Sample Processing

The sample that is used in this research is mahkota dewa leaf. ±5 kg of collected mahkota dewa leaf is cleaned from dirt, cut the stalk, then air-dried at room temperature. Dried simplicia then is mashed with a blender to create powder simplicia and weight in the dried powder. Simplicia powder is then covered in a closed container to avoid sunlight and moisture.

In this research, the extraction method used is maceration with tincture making an Indonesian herbal pharmacopeia with repetitive maceration (re-maceration) using ethanol solvent 70%.

Two hundred grams of dried powder simplicia is put into Erlenmeyer, added ethanol 70%, soaked for first 6 hours while stirring occasionally, then let it sit for 18 hours. Macerate is separated by filtration. The extraction process is repeated at least twice with the same amount of solvent. Macerate ethanol 70% is collected and concentrated by using a *rotary evaporator* until achieving thick extract. After achieving the thick extract, weigh-in, and place in a closed glass container, it is called Ethanol Extract of Mahkota Dewa Leaf (EEDMD).

Macroscopic Examination

Macroscopic Examination is done towards mahkota dewa leaf that includes shape, smell, color, and flavor.

Water Content Determination

Water Content Determination is done by using the azeotropy method (toluene distillation), using toluene that has been saturated with the following steps: 200 mL toluene is put into a round bottom glass then adding 2 mL aquades, then set up and distilled for 2 hours. Distillation is then stopped and cooled down for ± 30 minutes, then water volume at the receiving tube is read with 0,001 mL precision.

Putting 10 g of simplicia powder that has been precisely weighed, then carefully heated for 15 minutes, after toluene is boiled, drop speed is set into two drops for each second until more water is distilled, inside the cooler is washed with toluene. Continue distillation for 5 minutes, then let the receiving tube cool down at room temperature. After water and toluene are entirely separated, water volume is read with 0,001 mL precision. Check the difference between both water volumes inside the ingredients. Water content is calculated in a percent:

$$\% \text{ water content} = \frac{final\ Volume - Initial\ Volume}{Sample\ weight} \times 100\%$$

(Depkes RI, 1995).

Alkaloids Examination

Weight 0,5 g of simplicia powder of mahkota dewa leaf, then add 1 mL of HCL 2 N and

9 mL of distilled water, heated at water bath for 2 minutes, let that cool down, and filter. The filtrate obtained is used for alkaloids test: take 3 test tubes, then add 0,5 mL of filtrate.

At respective test tube :

1. Tube 1 added two drops of Mayer's reagent
2. Tube 2 added two drops of Bouchardat's reagent
3. Tube 3 added two drops of Dragendorff's reagent

Alkaloids are positive if at least 2 of 3 test tubes are sedimented and occurred turbidity (Depkes, RI).

Triterpenoids and steroids Examination

Macerate 1 g of sample with 20 mL *n*-hexane for 2 hours, the filter. The filtrate is evaporated at the steam bowl. At rest, it is added two drops of acetic anhydride acid and one drop of concentrated sulfuric acid; when purple or red color occurs, then change into green-blue, it shows that there are steroids Triterpenoids (Harbone, 1987).

Saponins Examination

Put 0,5 g of simplicia powder of mahkota dewa leaf inside a test tube, then add 10 mL hot water, cool it down, and then shake for 10 seconds. If formed stable foam 1-10 cm under 10 minutes and not disappeared with an additional one drop of chloride acid 2 N shows that there are saponins (Depkes, RI).

Flavonoids Examination

10 g of simplicia powder of mahkota dewa leaf is added to 100 mL of hot water, boiled for 5 minutes, and filtered while hot. Then, take 5 mL of filtrate, add 0,1 g of Mg powder and 1 mL of concentrated HCL, and 2 mL of amyl alcohol, shook and separated. If red, yellow, and orange color, positive flavonoids occur at the layer of amyl alcohol (Farnsworth, 1966).

Tannins Examination

0,5 g of sample is extracted with 10 mL of distilled water, filtered then the filtrate is diluted in distilled water until no color. Take 2 mL of liquid, then added 1 to 2 drops of iron (lll) chloride reagent. Blackened green or blue occur-show that there are tannins (Farnsworth, 1966).

Making of 1 % of acetic acid

Put 1 mL of concentrated acetic acid into beaker glass. Add aquades little by little until 100 mL. Put into a vial then covered with aluminum foil dan sterilized in autoclave with 121 ^{0}C for 15 minutes.

0,3 mL acetic acid is inducted into mice with intraperitoneal (Tatiya *et al.*, 2017).

Making of Na-CMC 0,5 %

They were making Na-CMC by using the following dosage, which is 0,5%. First, weigh 0,5 g of Na-CMC, then sprinkled at 100 mL of hot water, stirred vigorously inside mortar for homogeneous until the concentration of Na-CMC was 0,5%.

Data Analysis

Obtained data is tested with the *Shapiro-Wilk* test to see the distribution data and the *Levene* test to see the homogeneity of the data. If obtained data is usually distributed, then continued with the analysis of variance test (ANOVA) in one way with 95% confidence level using *SPSS version 20 for windows*. This ANOVA test is to know if significant differences between testing groups then continued into the Tukey HSD test. *Tukey HSD* (*Honestly Significant Difference*) test is a test for knowing if there are significant differences or not between two comparatively experimental groups.

RESULTS AND DISCUSSION

Five kg of the fresh leaf of mahkota dewa is cleaned and washed from any dirt and then dried until having 950 grams of simplicia. 200-gram simplicia powder of mahkota dewa leaf is macerated in ethanol 70% and obtained 61 grams of the extract with 30% yield. According to the Indonesian Ministry of Health (2008), yield is compared in terms of the amount (quantitative) of extract which is resulted from plant extraction yield using percent (%). Extract quality that is produced is usually inversely proportional to the amount of resulting yield. The higher the yield result signified that, the more extraction is produced. The fewer yield results, the higher the obtained quality. Yield standard parameter is 17% - 33% (Rosida, 2017).

Macroscopic test towards mahkota dewa leaf is a single leaf, elongated oval and pointed tip with side by side face, short stem and pointed base, flat edge, 7-10 cm long, 2-5 cm wide, pinnated bone, smooth surface, dark green color,

no flavor, distinctive smell and not stinging (Lumbantobing, 2016). Leaf of mahkota dewa simplicia is brownish-green, distinctive flavor and smell. At the same time, the thick extract has a sticky extract, dark green color, distinctive smell, and a bitter taste.

Water content determination of simplicia is done by the azeotropy method. The result is 7,5%. Therefore, the terms on determining water content in simplicia are <10%. So the simplicia itself has fulfilled the terms which are less than 10%.

Determining the water content is done to put the maximum limit or range of how high water content is. The higher water content inside one ingredient can push the enzyme to do its activity to change chemical compounds inside into another product which probably does not have a pharmacology effect as its originals (Zainab, 2016).

Tabel 1. Phytochemistry Screening Result

Screening	Result
Alkaloids	+
Steroids and Triterpenoids	+
Saponins	-
Flavonoids	+
Tannins	+

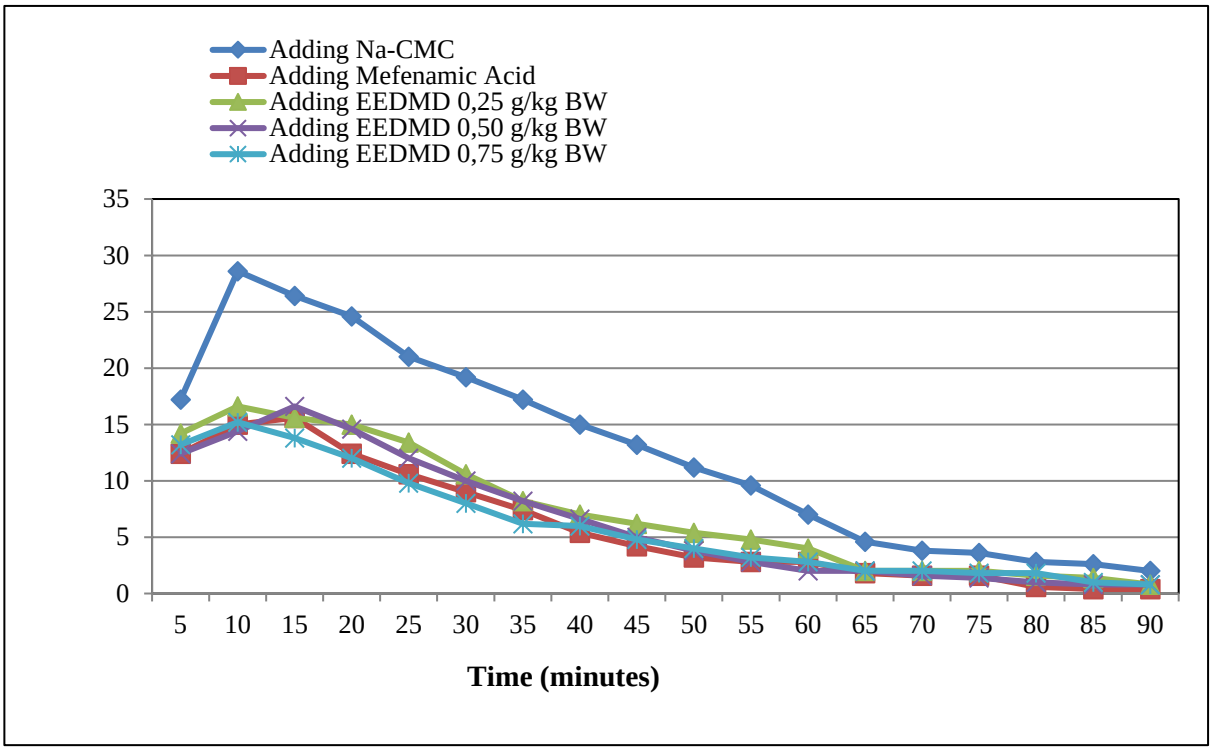
Information : +: contain the examined substance
-: not contain the examined substance

Result of phytochemistry screening of simplicia powder of mahkota dewa leaf contains secondary metabolites compound alkaloids, tannin, flavonoids, steroids, and Triterpenoids. Nevertheless, the result on saponins showed negative results (not forming a foam after being shaken). This happened because of possibly a difference in genetic variation and geographical condition of growing places of examined plants.

According to Christien (2014), the differentiation in secondary metabolite compounds in the same plant often happened because of environmental influence, genetic variation, and individual and geographical conditions of its growing places. However, the research done by Hanif *et al.* (2020) showed that there were saponins in mahkota dewa leaf simplicia.

Analgesic effect test towards mahkota dewa leaf (*Phaleria macrocarpa*) in this research is observed through the squirming method.

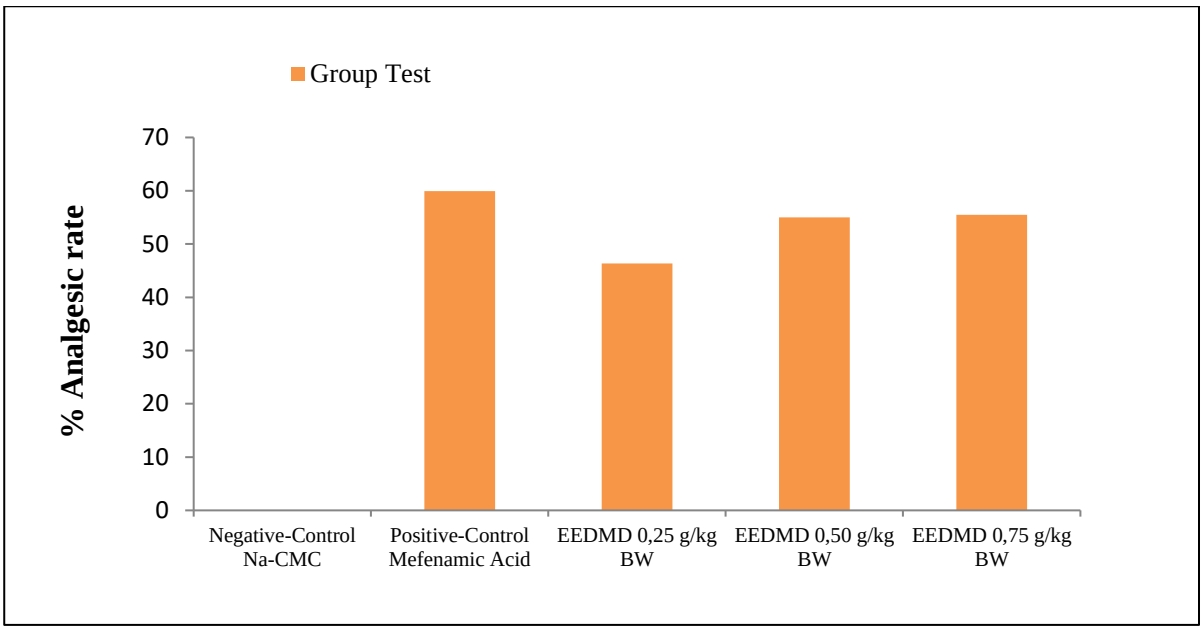
There is five treatment group in this test which are negative control group that is added Na-CMC 0,5%, positive control group that is added 500 mg of mefenamic acid suspension, and mahkota dewa leaf extract group with 0,25 g/kg BB; 0,50 g/kg BB; dan 0,75 g/kg BB dosage where 5 minutes beforehand is inducted with glacial acetic acid 1% with a purpose to give pain effect to mice (*Mus musculus*) then counting the squirming every 5 minutes for one and half hour. Research results that have been done then obtained an average of squirming at each group respectively.



Graphic 1. Average graphic of mice squirming numbers suspense group of Na-CMC 0,5%, suspense group of mefenamic acid 500 mg and EEDMD group with a dosage of 0,25; 0,50 dan 0,75 g/kg BB which beforehand is inducted with glacial acetic acid 1% observed every 5 minutes for one and a half hours.

Based on graphic 1, at 10 minutes mark, there is an increase of squirming on mice that showed that glacial acetic acid 1% 0,3 mL dosage gives a pain effect marked by the squirming response on mice. After the 5 minutes mark, treatment is given. A test result of average squirming on mice shows a decrease in the numbers of squirming at 15 minutes mark, which is still decreasing until 90 minutes mark. Mahkota dewa leaf extract giving the decrease of squirming at 20 minutes until 90 minutes mark if comparing to the suspense given of Na-CMC 0,5% as a negative control of squirming peak that happened at 10 minutes mark with the average of 28,6 and slowly decreasing at 15 minutes until 90 minutes mark. This showed that at negative control of suspense given by Na-CMC 0,5% does not have an inhibition rate towards squirming or analgesic rate. This negative control result is the same as glacial acetic acid orientation 1% before. Mefenamic acid suspense gives the decrease of squirming number faster than mahkota dewa leaf extract with 0,75 g/kg BB dosage, gives many decreasing of squirming faster than mahkota dewa leaf extract 0,25 g/kg BB and 0,50 g/kg BB, so the effective mahkota dewa extract in decreasing the number of squirming is mahkota dewa leaf extract of 0,75 g/kg BB dosage which its effect is almost similar to mefenamic acid suspense of 500 mg.

The data result of squirming each next group is calculated in percent of analgesic rate, which a rate of test ingredients in decreasing number of squirming responses on mice. A test ingredient is said to have an analgesic rate if the test animal has squirming decrease until 50% or more. Results can be seen in graphic 2.



Graphic 2. Percentage graphic of Analgesic rate after giving suspense group of Na-CMC 0,5%, mefenamic acid suspense group 500 mg and EEDMD group with 0,25; 0,50 and 0,75 g/kg BB dosage which is beforehand inducted with glacial acetic acid 1% observed every 5 minutes for one and half hours

Based on graphic two, the highest protection percentage is shown by the positive control group. The highest percentage dosage group is in the third dosage group, which means the third dosage is more effective to give analgesic effect.

Effectivity percentage is several effects of a test ingredient with analgesic by comparing the percent of analgesic rate from the EEDMD group with the positive control group (mefenamic acid suspense). The result can be seen in table 2.

Tabel 2. Effectivity analgesic percentage datA.

No.	Treatment groups	X (%)	Y(%)	Effectivity percentage (%)
1	EEDMD 0,25 g/kg BB	46,3	59,9	77,29 %
2	EEDMD 0,50 g/kg BB	55,06	59,9	91,91 %
3	EEDMD 0,75 g/kg BB	55,5	59,9	92,65 %

Keterangan : X = EEDMD analgesic rate percentage
Y = Mefenamic acid 500 mg analgesic rate percentage

It can be seen from table 2, a group that has higher effectivity percentage is EEDMD 0,75 g/kg BB with 92,65%, which is closer to mefenamic acid suspense 500 mg, which means EEDMD 0,75 g/kg BB is giving almost the same effectivity as mefenamic acid suspense 500 mg.

Data analysis results of male mice squirming are using *One Way* ANOVA statistical analysis with a 95% trust level. This is done to compare average squirming at each mice in each group. Statistical data analysis test is done using the SPSS program application in version 20, the Tukey test.

ANOVA test is done after getting mice squirming results that have been given the preparation test, then normality and homogeneity are done afterward. Based on that result, we know that squirming data is homogeneous and normally distributed. The result of the ANOVA test obtains a sig number of 0,000, which is less than 0,05 (P<0,05). This is said that there are differences between the analysis group

These research results show that mahkota dewa leaf extract can give analgesic activity through its ability to inhibit and decrease the number of squirming on mice. These happen

because mahkota dewa leaf extract contains flavonoids and alkaloids known to inhibit inflammation causing pain. Flavonoids and alkaloids have a function to inhibit the biosynthesis of prostaglandins (Wemay *et al*., 2013). Cyclooxygenase enzyme, which takes part in biosynthesis prostaglandins as a mediator of causing pain, inhibits cyclooxygenase that will reduce prostaglandins production by arachidonic acid it can reduce pain (Siswanto *et al*., 2016).

CONCLUSION

From the research results that have been done, it can be concluded that secondary metabolites compound inside leaf extract of mahkota dewa (*Phaleria macrocarpa* (Scheff.) Boerl) have analgesic effect at 0,75 g/kg BB dosage with effectivity percentage of 92,65%. The yielded result of ethanol extract of mahkota dewa leaf that is obtained is 30%.

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