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The Analgesic Activity Study of Ethanol Extract of *Plantago Major* L. in Mice (*Mus Musculus* L.) using *Writhing Test* Method

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Abstract: *Plantago major* L. are known as weeds on plantations. *Plantago major* L. is effective to overcome pain because they contain secondary flavonoid metabolites that have the potential as pain relievers. The purpose of the experiment was to determine the analgesic effect of ethanol extract on *Plantago major* L. plant on male white mice. The experiment, phytochemical screening of ethanol and powder extracts from *Plantago major* L. plant, powder of simplicia characterization examination and analgesic effect using writhing test were carried out. As a pain inducer, 0.5% acetic acid is used intraperitoneally injected the lower abdomen of the mice and the amount of stretching is calculated with an interval of 5 minutes for 1 hour. The suspension of ethanol extract of *Plantago major* L. was given at a dose of 500, 1000 and 2000 mg / kg BW, 2% methampyron suspension as a positive control and 0.5% CMC suspension as a negative control. Pharmacological test results based on ANOVA test found that the amount of stretching between treatment groups was significantly different ($p < 0.05$). The results of the analgesic effectiveness percentage obtained that the EEPM (Ethanol Extract of *Plantago Major* L.) suspension dose 500, 1000 and 2000 mg / kg BW had the highest analgesic power at a dose of 2000 mg / kg BW of 85.84%, EEPM suspension group 1000 mg / kg BW of 74.96 % and in the EETDS suspension group 500 mg / kg BW 62.37%.

Keywords: Analgesic, *Plantago major* L., *writhing test*.

Introduction

Plants are widely used by people as medicine in modern times based on hereditary factors. Medicines containing chemicals are still widely used because they believe that medicines containing chemicals can cure diseases quickly. However, chemical drugs have many side effects, one of which is damaging internal organs if the drug is consumed for a long period of time. The price of chemical drugs is also relatively more expensive for the lower middle class and the side effects of chemical drugs are greater. In addition, natural medicines are considered to have almost no dangerous side effects (Permadi, 2015). Many plants around us have not been utilized properly, some plants are even considered useless. This happens because of limited information to the public. For this reason, it is necessary to develop scientific research on medicinal plants so that they can be utilized as much as possible for public health. There are various traditional medicines from plants and their chemical content and properties have been widely studied. However, there are still many plants whose analgesic content is not known so that further research is needed (Afrianti, 2014).

One of the plants that has medicinal properties is the *Plantago major* L. *Plantago major* L. is a weed in tea and rubber plantations

or grows wild in forests, fields and slightly damp grassy yards, sometimes planted in pots as a medicinal plant (Satya, 2013). *Plantago major* L. contains plantagin, aucubin, ursolic acid, bethasitosterol, hentria-contane, plantaglucide, vitamin B1, vitamin C, vitamin A, potassium, rhinantin, planterolic acid and plantasan which have benefits for treating urinary tract disorders, curing vaginal discharge, treating dysentery, treating diarrhea, treating digestive disorders, treating nosebleeds, treating whooping cough, treating wounds, healing boils, treating liver disease, curing dengue fever, treating pain and treating eye inflammation (Suparni and Ari, 2012). Specifically, *Plantago major* L. contains plantagin, aukubin, flavonoids (apigenin), ursolic acid, beta-sitosterol. N-hentriacontane and plantagluside. Flavonoids (Apigenin) are effective as anti-inflammatories that can reduce swelling and pain (analgesic) (Utami, 2003). Flavonoid compounds from the flavone group, namely apigenin, have also been known to provide good effects on human health. Apigenin is an aglycone of apiin, this compound is solid and yellow in color. Apigenin compounds have the ability, among others, as anti-inflammatory, antibacterial, to overcome stomach problems and reduce pain (Cadenas and Packer, 2002).

According to The International Association for the Study of Pain (IASP), pain is an unpleasant sensory and emotional experience due to damage or threat of tissue damage. Based on this definition, pain is a combination of objective components (sensory physiological aspects of pain) and subjective components (emotional and psychological aspects). Administration of analgesic medication is the first stage of treatment in almost all cases of pain. Analgesic drugs are drugs that reduce pain without worsening sensory modalities. NSAIDs are an example of a group of drugs used as pain medication (Christiana, et al. 2012). Based on the description above, researchers are interested in testing the analgesic effect of ethanol extract of *Plantago major* L. on male white mice (*Mus musculus* L.) using the acetic acid-induced writhing method with metampirone as a comparator because this plant is only considered a weed or nuisance plant in the researcher's home area.

Materials and Method

Types of research

The research conducted was an experimental research, namely in the form of taking and drying *Plantago major* L., characterization of *Plantago major* L. simplicia, extraction of *Plantago major* L. simplicia, phytochemical screening of *Plantago major* L. extract and testing the analgesic effect using the writhing test method.

Research tools and materials

The research tools consist of a drying cabinet, blender, container or jar, animal scales (Ohaus), analytical scales (Metter Toledo), rotary evaporator (Eyela), filter paper, glassware (Pyrex), water bath, 1 ml syringe (One Med), oral sonde, aluminum foil, mortar and stamper. The research materials consisted of Plantago major L., 96% ethanol, distilled water, CMC (Carboxy Methyl Cellulose), acetic acid, 500 mg antalgin tablets, iron (III) chloride, potassium iodide. Bismuth (II) nitrate, magnesium powder, hydrochloric acid (p), amyl alcohol, acetic anhydride, sulfuric acid (p), chloroform, mercury (II) chloride, lead (II) acetate, methanol, iodine, alpha naphthol, nitric acid, toluene.

Sample determination

Plant determination was carried out at the Medanense Herbarium Laboratory (MEDA), University of North Sumatra.

Sample collection

The sample used was *Plantago major* L. taken from Rembele Village, Wih Pesam District, Bener Meriah Regency, Aceh Province. The sampling was carried out purposively, namely without comparing plants in other areas.

Sample preparation

The collected *Plantago major* L. are wet sorted to separate the samples from dirt or other foreign materials involved in the sample collection. The samples are washed with running water to remove soil and other foreign objects that are attached and drained and then finely chopped.(Sahputra et al., 2021). The sample is then placed in a drying cabinet at a temperature of 40-50°C. The drying process is carried out until the raw material is easily broken. Then dry

sorting to separate the simplicia from foreign objects that are involved in the drying process. Then the simplicia is powdered using a blender, sieved and weighed. The obtained simplicia powder is stored in a clean, tightly closed container and stored at room temperature and protected from light.(Pulungan et al., 2022).

Characterization of Simplicia

Examination of the characterization of the simplex includes: macroscopic examination of the simplex, microscopic examination of the simplex powder, determination of water content, determination of water-soluble essence content, determination of ethanol-soluble essence content, determination of total ash content, determination of acid-insoluble ash content.

Macroscopic examination

Macroscopic examination is carried out by observing the shape, color, taste and smell of *Plantago major* L. simplicia (Rafita et al., 2022).

Microscopic examination

The powder of Plantago major L. was examined under a microscope after being dusting it on a glass item that had been covered with a glass deck and dripped with one drop of chloral hydrate (Nasution et al., 2022).

Determination of water content

200 milliliters of toluene and two milliliters of distilled water are added to a round-bottom flask, which is then allowed to distill for two hours, cooled for thirty minutes, and the volume of water in the collection tube is measured. Subsequently, 5 g of precisely weighed powdered simplicia is added to the flask and gently cooked for 15 minutes. The drip rate is set at two drops per second until part of the water is distilled, at which point it is increased to four drops per second once the toluene begins to boil. Toluene is used to clean the cooler's interior once all of the water has been distilled. Following five minutes of continuous distillation, the receiving tube is allowed to cool to room temperature. At this point, the water and toluene have completely separated, and the volume is measured with a precision of 0.05 ml. The water content of the material under examination is determined by calculating the difference between the two volumes of water. The percentage of water content is computed (Robiatun *et al*., 2022).

Determination of water-soluble essence content

Weigh 5 grams of the powdered simplicia, and macerate it in 100 milliliters of chloroform water (2.5 milliliters of chloroform in distilled water to make 1 liter) in a stoppered flask for 24 hours, shaking it once every 6 hours. After 18 hours, filter it, and evaporate 20 milliliters of the first filtrate in a flat-bottomed evaporator cup that has been heated to 105°C until constant weight. The percentage of extract that is soluble in water is computed in relation to the air-dried material (Rani et al., 2023).

Determination of essence content soluble in ethanol

Weighed 5 g of powdered simplicia that had been air-dried and macerated for 24 hours in 100 ml of 96% ethanol in a stoppered flask. The mixture was shaken every 6 hours for the first 18 hours of the maceration. After filtering, the filtrate was heated to 105°C until it reached constant weight, and 20 ml was evaporated in a shallow cup until it was dry based on the tared average. In comparison to the air-dried material, the ethanol-soluble extract content is computed.

Determination of total ash content

Up to 2 g of the precisely weighed powdered simplicia are added to a tared, heated porcelain flask. The flask is heated to 600°C until all of the charcoal is utilized, then it is cooled and weighed until a steady weight is achieved. The amount of ash in the flask is determined by comparing it to the material that has been air-dried (Septiana et al., 2024).

Determination of acid insoluble ash content

After adding 25 milliliters of dilute hydrochloric acid to the ash obtained from the calculation of the total ash content, the mixture was allowed to boil for five minutes. The insoluble part of the acid was then discarded, filtered through ash-free filter paper, and washed with hot water. After cooling and weighing, the residue and filter paper were heated at 600°C until their weight remained constant. The amount of ash insoluble in acid was determined by

comparing it with the air-dried material (Ridwanto, Trizaldi, et al., 2023).

Extract preparation

The maceration method was used for the extraction process. 500 grams of powdered prickly amaranth leaf were weighed, placed in a vessel, and 75 parts (3750 ml) of ethanol solvent liquid were added. The mixture was then periodically agitated. The combination was filtered and the leftovers were squeezed after five days. The leftovers were rinsed with enough ethanol solvent liquid to yield 100 parts, or 5000 milliliters, of macerate. then put into a sealed container, filtered, and kept in a cool, dark area for two days. A thick extract was produced after the macerate was concentrated using a rotary evaporator at a temperature of no more than 70°C (Nasution et al., 2024).

Phytochemical screening

Phytochemical screening conducted in this study included examination of alkaloids, flavonoids, saponins, tannins, and steroids/triterpenoids.

Alkaloid examination

Weighing 0.5 g of powdered simplicia and extract, adding 1 ml of 2 N HCl, and 9 ml of distilled water. Then, heated for 2 minutes on a water bath, cooled and then filtered, the filtrate is used for alkaloid examination. 3 drops of filtrate are added with 2 drops of Mayer's reagent, a white or yellow lumpy precipitate will form. 3 drops of filtrate are added with 2 drops of Bouchardat's reagent, a brown to black precipitate will form. 3 drops of filtrate are added with 2 drops of Dragendroff's reagent, a brown or orange precipitate will form. If there is sediment or turbidity in at least 2 test tubes in the experiment above, then the alkaloid is positive (Suryani et al., 2024).

Flavonoid examination

10 grams of simplisia and prickly spinach leaf extract were weighed, and 100 milliliters of hot water were added. The mixture was then cooked for five minutes and strained while still hot. After that, 5 ml of the filtrate was removed, to which 0.1 g of magnesium powder, 1 ml of strong hydrochloric acid, and 2 ml of amyl alcohol were added. The mixture was then agitated and allowed to separate. If the alcohol layer becomes colored in red, yellow, or orange, flavonoids are present (Nasri et al., 2024).

Tannin examination

Add 10 ml of distilled water to a test tube containing 0.5 grams of simplicia and prickly amaranth leaf extract. The tube is then stirred and filtered. Distilled water is added to the filter until it is colorless. One or two drops of iron (III) chloride reagent are added to two milliliters of the solution. Tannin is present if a blackish blue or blackish green color appears (Nasri et al., 2023).

Saponin examination

Prickly amaranth leaf extract and up to 0.5 g of medication were added to the test tube. After that, 10 cc of boiling water was added, allowed to cool, and given a 10-second shake. A stable foam that was 1 to 10 cm high, did not diminish after 10 minutes, and did not vanish when one drop of 2 N hydrochloric acid was introduced was indicative of the presence of saponins (Rambe et al., 2021).

Steroid/triterpenoid examination

1 gram of simplicia and prickly amaranth leaf extract were macerated in 20 milliliters of ether for two hours before being filtered. 5 drops of anhydrous acetic acid and 5 drops of concentrated sulfuric acid (Liberman-Burchardad reagent) were added after the filtrate evaporated in the evaporator cup. The presence of triterpenoids is indicated by the formation of a purple to purplish red color, while the presence of steroids is indicated by the formation of a bluish green color (Kaban et al., 2022).

Glycoside examination

Weighed up to 3 g, samples of simplex and prickly amaranth leaf extract were added, along with 10 ml of a 7:3 ethanol and distilled water mixture and 10 ml of 2 N hydrochloric acid. The mixture was refluxed for 10 minutes, cooled, and filtered. After adding 25 ml of distilled water and 25 ml of 0.4 M lead (II) acetate to 20 ml of filtrate, shake well, and allow to stand for five minutes before filtering. Three times, the filtrate was passed through a separating funnel containing 20 milliliters of a mixture of isopropanol and chloroform (2:3). Anhydrous

sodium sulfate was added to the water layer and the isopropanol-chloroform mixture layer after they had been separated. Both layers were then filtered and allowed to evaporate at a temperature of no more than 50ºC. The residue was utilized in the subsequent experiment after being dissolved in two milliliters of methanol: After evaporating the filtrate from the water layer (sugar component) in the test tube to a volume of 0.1 ml above the water bath, 2 ml of water and 5 drops of Molish reagent were added. After adding concentrated sulfuric acid gradually through the tube wall, a purple ring formed at the two liquids' border, signifying the existence of glycone, a sugar component. A blue or green hue was obtained by adding up to 0.1 ml of the filtrate from the chloroform-isopropanol mixture layer (non-sugar component) to 5 ml of anhydrous acetic acid and 10 drops of concentrated sulfuric acid (Liberman-Bourchard reaction) indicating the presence of a non-sugar component (aglycone)(Syahputra et al., 2023).

Analgesic effects using the writhing test method

Preparation of 0.5% (v/v) Acetic Acid Solution

Glacial acetic acid contains not less than 99.5% and not more than 100.5% w/w acetic acid. From glacial acetic acid, 0.5% acetic acid is made by the dilution method using physiological NaCl (0.9% NaCl) as a solvent (Sentat and Pangestu, 2016).

Preparation of 0.5% CMC Suspension

Weigh 500 mg of CMC and then sprinkle it in a porcelain cup containing 1/3 of the water volume of hot distilled water, leave it for 30 minutes, stir it until a transparent mass is obtained, then add distilled water little by little and put it into a 100 ml volumetric flask, the volume is made up with distilled water to 100 ml.

Preparation of 2% Methampyrone Suspension

Weighed 500 mg of CMC and then sprinkled in a porcelain cup containing 1/3 of the water of hot distilled water, left for 30 minutes, stirred until a transparent mass was obtained. Take 4 metampiron tablets (2000 mg metampiron) and then grind them in a mortar, then add 0.5% CMC suspension little by little while grinding homogeneously, poured into a container, then add 100 ml of distilled water, grind homogeneously and put in a container (Anief, 1998).

Preparation of Ethanol Extract Suspension of Plantago major L.

The dose of ethanol extract of *Plantago major* L. given to experimental animals, namely 500, 1000, 2000 mg/kg BW. The extract was weighed as much as 10 g then put into a mortar containing a little 0.5% CMC suspension, ground homogeneously and then added with 0.5% CMC suspension up to 100 ml in a 100 ml measuring flask.

Analgesic Effect Test

Mice were randomly divided into 5 groups, each group consisting of 6 mice. Mice that had been fasted for 18-24 hours were then induced with 0.5% acetic acid (0.5 ml) intraperitoneally and then their wriggling was observed and the number of wriggling was counted for 5 minutes. After 5 minutes of observation, each group was given the following oral treatment (Sentat and Pangestu, 2016).

Group I : Negative control in the form of 0.5% CMC suspension (Dose 0.5 ml)
Group II : Positive control or comparison in the form of 2% Methampyron suspension (Dose 0.1 ml)
Group III : Ethanol Extract Suspension of *Plantago major* L. (EEPM) with a dose of 500 mg/kg BW
Group IV : Ethanol Extract Suspension of *Plantago major* L. (EEPM) with a dose of 1000 mg/kg BW
Group V : Ethanol Extract Suspension of *Plantago major* L. (EEPM) with a dose of 2000 mg/kg BW

Then the wriggling was observed and the number of wriggles was counted every 5 minutes for 1 hour. Wriggling was marked by the mouse deflating its stomach and pulling its two hind legs back so that its body looked elongated. This characteristic wriggling was used as a reference.

Data analysis

The acquired data were subjected to analysis using the Levene test to determine data homogeneity and the Shapiro-Wilk test to determine data distribution. With SPSS version

23.0 for Windows, the one-way analysis of variance (ANOVA) test is carried out with a 95% confidence level if the collected data is homogeneous and regularly distributed. The ANOVA test is used to ascertain whether or not there is a significant difference between the treatment groups, and the LSD (Least Significant Difference) test is subsequently performed. The purpose of the LSD test is to ascertain if the two treatment groups under comparison differ significantly (significantly) or not.

The research data is in the form of the cumulative number of wriggles in each treatment group. From these data, it is then used to calculate the percentage of the analgesic power of the test material, namely the ability of the test material to reduce the response of mice wriggling caused by acetic acid induction. This percentage describes the analgesic power of the test material. The percentage of analgesic power is obtained by comparing the average number of wriggles of the test material group to the negative control group. The percentage of analgesic power against acetic acid induction with the formula (Turner, 1965).

$$\% \text{ Analgesic power } = 100 - \left\{\frac{\text{experiment}}{\text{negative control}} \text{x } 100\%\right\} \quad \textbf{(1)}$$

To see the percentage of analgesic effectiveness of the test material, this was done by comparing the percentage of analgesic effect of the test material group to the percentage of analgesic power of the positive control group (Metampyrone) which was calculated using the formula below (Wahyuni, et al., 2003).

$$\% \text{ Effectiveness } = \frac{\% \text{ Analgesic power of samples}}{\% \text{ Analgesic power of positive control}} \text{ x } 100\% \quad \textbf{(2)}$$

Result and Discussion

Characterization of simplicia

The results of macroscopic examination of the *Plantago major* L. simplicia are in powder form, brownish green in color, have a distinctive odor and bitter taste. The results of microscopic examination of the *Plantago major* L. simplicia powder show the presence of epidermis, vascular bundles, guard cells, stomata and glandular hairs. The results of the characterization of *Plantago major* L. simplicia can be seen in Table 1.

Table 1. Characterization of *Plantago major* L.

No	Parameter	Result
1	Water content	8.6%
2	Water Soluble Essence Level	32.7%
3	Ethanol Soluble Essence Level	21%
4	Ash Content	12.4%
5	Acid Soluble Ash Content	0.4%

In determining the water content, it is done to provide a minimum level or range of the amount of water content in the material. The results of determining the water content of the *Plantago major* L. simplicia obtained a percentage content of 8.6%. The water content in the sample (simplicia) should not be more than 10% because excess water in the simplicia will encourage the growth of microorganisms and mold (fungus), decay reactions, enzymatic reactions, which are ultimately followed by hydrolysis reactions of chemical compounds in the simplicia. The method used in determining the water content is the azeotropic distillation method.

The principle of this distillation method is the combination of two solvents that have different boiling points and different polarities. Distillation is used to separate mixtures consisting of two or more components that are difficult to separate. The solvent used in determining this water content is toluene. Toluene has a lower specific gravity than water, the specific gravity of toluene is 0.866 g / ml. The use of a solvent that has a lighter specific gravity than water aims to make the water at the bottom of the container glass so that volume measurement is easier. Water and solvent (toluene), condensed so that condensation occurs and falls on a graduated tube, marked by the formation of two-phase layers, namely water and toluene. The water phase is below while toluene is above. Water is below because the specific gravity of water is greater than toluene (Yuza et al., 2023).

Plantago major L. simplicia powder, the percentage content of water-soluble essence was measured and found to be 32.7%, whilst the percentage content of ethanol-soluble essence was found to be 21%. The obtained content results meet the requirements where the content of water-soluble essence is not less than 30% and the content of ethanol-soluble essence is not less than 4%. The content of water-soluble and

ethanol-soluble essence is a test to determine the amount of compound content that can be dissolved in water (water-soluble essence content) and the content of compounds that can be dissolved in ethanol (ethanol-soluble essence content) (Ningtias & Rani, 2023)

The herbal medication is macerated with water and 96% ethanol for ± 24 hours to determine the levels of water-soluble and ethanol extracts. When determining the levels of water-soluble extracts, the herbal medicine is added with chloroform first, the addition of chloroform is intended as an antimicrobial agent. Because during maceration only water is used, the extract may be damaged because water is a good medium for microbial growth and it is feared that a hydrolysis process will occur which will damage the extract, thereby reducing the quality and quality of the extract. Meanwhile, in determining the levels of ethanol-soluble extracts, chloroform is not added, because ethanol already has antibacterial properties so there is no need to add chloroform. Data on the levels of extracts in certain solvents are usually needed to determine the solvent to be used to extract certain compounds so that more extracted substances are extracted from the herbal medicine to be extracted(HM Nasution et al., 2022).

Plantago major L. simplicia powder was tested for total ash content, and the results showed that the percentage content was 12.4%, while the percentage level for acid-insoluble ash content was 0.4%.The ash content determination findings continue to fulfill the standards, which state that the total ash content cannot exceed 12.4% and the amount of ash that is acid-insoluble cannot exceed 0.4%. An overview of the internal and external mineral content starting from the first step and ending with the creation of the extract is intended to be provided by the determination of ash content. Generally speaking, the extract is heated to a temperature that destroys organic components and their derivatives, causing them to evaporate and leaving behind only mineral and inorganic elements. The presence of several minerals in the extract obtained from the maceration process is shown by the high total ash level in each extract. On the other hand, the existence of ash content that is insoluble in acid suggests the continued presence of sand or other contaminants.

Phytochemical Screening of Simplicia and *Plantago major* L. Extract

Phytochemical screening was conducted to test for the presence of secondary metabolite compounds including alkaloids, flavonoids, saponins, steroids/triterpenoids, tannins and glycosides. The results of phytochemical screening of simplicia and ethanol extract of *Plantago major* L. can be seen in Table 2. Based on the table above, it shows that in the alkaloid examination there are 3 tests, namely testing with Mayer, Bouchardat and Dragendorf reagents. In the first test with Mayer's reagent showed a positive result because a white or yellow lumpy precipitate was formed. Then the second test with Dragendorf's reagent showed a positive result marked by the formation of an orange color. In the third test with Bouchardat's reagent also showed a positive result because it formed a brown precipitate. From the results obtained above, it can be concluded that the simplicia and extract of *Plantago major* L. positively contain alkaloids.

Table 2. Phytochemical Screening of *Plantago major* L. Extract

No.	Inspection	Powder Result	Extract Results
1	Alkaloid	+	+
2	Flavonoid	+	+
3	Saponins	+	+
4	Tannin	+	+
5	Steroid	+	+
6	Triterpenoid	+	+
7	Glycosides	+	+

Description: (+) : contains secondary metabolite compounds

This is in accordance with the literature that alkaloids are considered positive if there is a precipitate or turbidity in at least 2 reactions out of 3 experiments. Basically, the principle of testing alkaloid compounds in precipitation reactions with heavy metal ions. Nitrogen atoms (basic) in the alkaloid structure have the ability to interact with metal ions. The addition of 2 N HCl is intended to attract alkaloid compounds in the extract because alkaloids are basic, so by adding an acid such as HCl, salt will be formed, so that the alkaloids will be separated from other components of the plant cells that are also

extracted by distributing them to the acid phase (Rachmawati & Suriawati, 2019).

Flavonoid testing showed positive results in the simplicia and extract of *Plantago major* L. because of the formation of orange color in the amyl alcohol layer. Flavonoid compounds containing hydroxyl groups will interact with Mg2+ ions to form colored complex compounds. The reaction of flavonoid compounds with Mg and HCl metals will form red or orange flavylium salts (Yanti & Vera, 2019). In the tannin examination showed positive results for the simplicia and extract of *Plantago major* L. This is due to the formation of a complex compound between phenolic compounds containing hydroxyl groups (OH) with iron ions (III). Oxygen atoms have free electron pairs so they are reactive to positive ions forming complex compounds (Djindadi et al., 2020).

Saponin examination showed positive results for the simplex and extract of *Plantago major* L. because the foam formed after shaking lasted a long time and did not disappear with the addition of 1 drop of 2N HCl. Foam is formed because saponin has properties that can reduce the surface tension of water. Like soap, saponin has large molecules that contain hydrophilic and lipophilic groups. In water, saponin molecules align themselves vertically on its surface, with the lipophilic groups facing away from the water.(Sahputra et al., 2019). In the triterpenoid test, it is based on the ability of triterpenoid and steroid compounds to form color by concentrated H2SO4 in glacial acetate solvent which forms a purple to red purple color indicating the presence of triterpenoids and the formation of a blue green color indicating the presence of steroids. Based on the results of the phytochemical screening that has been carried out, it is known that the simplicia and extract of *Plantago major* L. positively contain Triterpenoid and steroids (Djindadi et al., 2020).

In the glycoside examination, positive results were shown for the simplicia and extract *Plantago major* L. because no purple ring was formed at the boundary of the remaining solution liquid after the addition of Molisch reagent and concentrated sulfuric acid. The mechanism of the formation of the purple ring comes from carbohydrates that are hydrolyzed by sulfuric acid into monosaccharides, then both are condensed to form furtural which reacts to form a purple ring (Nurbaity, 2020).

Analgesic Effect Ethanol Extract of *Plantago major* L.

The test animals were first acclimatized for 1-2 weeks, during acclimatization the test animals were fed and watered as usual. Test animals were declared healthy if during observation they did not show any deviation in body weight (>10%) and visually did not show any unhealthy symptoms. Having approximately the same body weight and divided into 5 groups (more homogeneous). The day before testing the test animals were fasted for ±18 hours, but were still given water, this was done to avoid the possibility of analgesic effects from the food given to the test animals.

Before the EEPM analgesic effect test was conducted, a preliminary test was conducted on the administration of acetic acid as a pain inducer. Each test group was fasted for ±18 hours then induced with 1% and 0.5% acetic acid with the same volume of 0.5 ml intraperitoneally (IP). Acetic acid has a duration of about 1 hour as a pain inducer so this observation lasted for 1 hour after being induced by acetic acid. The pain response was marked by writhing legs pulled back, stretching and the abdomen touching the floor. This is because the induction of acetic acid causes pain due to severe irritation of the mucosal membrane of the abdominal cavity. The number of mice writhing was observed every 5 minutes for one hour produced by each group of preliminary test treatments attached in Table 3.

Table 3. Average Number of Mice Writhing Induced by Acetic Acid

Acetic acid	Time (Minutes)												Number of
0.5 ml	5'	10'	15'	20'	25'	30'	35'	40'	45'	50'	55'	60'	movements
0.5%	11.5	27.8	25.1	23.3	21	18.1	15.5	13.5	10.8	9.1	7.5	5.3	188.5
1%	7.8	22.1	30.1	26	24	21.1	18.3	16.1	14.3	11.6	9.3	7.6	208.3

Based on Table 3 above, it shows that after administration of 1% acetic acid concentration, it gave a pain response in the form of an average writhing of 7.8 in the 5th minute with an average writhing peak of 30.1 in the 15th minute, several days later several test animals died. This is different from administration of 0.5% acetic acid concentration which gave a pain response with an average writhing of 11.5 in the 5th minute and an average writhing peak of 27.8 in the 10th minute and no test animals died. It can be concluded that 0.5% acetic acid concentration can provide good pain stimulation as indicated by the resulting writhing response. The writhing produced at this dose is not too much and not too little and no test animals died. Therefore, a dose of 0.5% acetic acid concentration of 0.5 ml was chosen as a pain stimulus for the next experiment, then a test preparation was given, namely a suspension of ethanol extract of the *Plantago major* L. with a dose of 500, 1000 and 2000 mg/kg BW and the administration of 2% methampyrone suspension as a comparison.

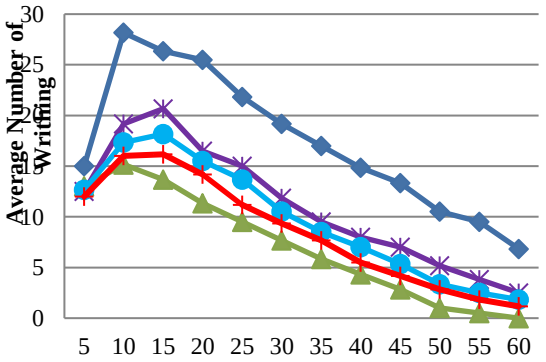

Figure 1. Graph of Average Number of Mice Writhing in Each Group

Description :
- CMC 0.5% administration
- Methampyrone Suspension 2%
- EETDS Suspension 500 mg/kg BW
- EETDS Suspension 1000 mg/kg BW
- EETDS Suspension 2000 mg/kg BW

In this test, there were 5 treatment groups, namely the negative control group given 0.5% CMC suspension, the positive control group given 2% metampiron suspension and the group of ethanol extract suspension of *Plantago major* L. with doses of 500, 1000, and 2000 mg/kg BW which had been induced 5 minutes earlier with 0.5% acetic acid and then the number of wriggles was counted every 5 minutes for 1 hour. The average number of wriggles of mice in each test group after being given the test preparation can be seen in Figure 1. Figure 1 shows that the administration of 0.5% CMC suspension is the highest number of wriggles of 192.7 while the administration of 2% metampiron suspension is the lowest number of wriggles of 71.7. In the administration of EEPM suspension with doses of 500, 1000 and 2000 mg/kg BW, the dose of 2000 mg/kg BW was the lowest number of wriggles of 90 while the EEPM dose of 500 mg/kg BW was the highest number of wriggles of 119.1, the administration of EEPM dose of 1000 mg/kg BW obtained the number of wriggles of 103.6.

Based on Figure 1, an increase in the number of squirming mice was seen at the 10-minute mark, suggesting that 0.5 ml of 0.5% acetic acid caused pain in mice as evidenced by a wriggling reaction. The fifth minute was when the treatment started. When compared to the administration of 0.5% CMC suspension as a negative control, the test results on the average number of wriggling mice showed that there was a decrease in the number of wriggling at the 15th minute, which continued to decrease until the 60th minute, and the ethanol extract suspension of the *Plantago major* L. gave a decrease in the number of wriggling at the 20th minute to the 60th minute. The peak wriggling occurred at the 10th minute with an average of 28.1 and started to experience a gradual decrease at the 15th to the 60th minute. This shows that in the negative control group, the administration of 0.5% CMC suspension did not have any inhibitory power against wriggling or did not have analgesic power, the results of this negative control test were the same as the previous 0.5% acetic acid orientation.

The 2% metampyrone suspension provided a faster decrease in the number of rat wriggles compared to the EEPM suspension. Meanwhile, the administration of EEPM suspension at a dose of 2000 mg/kg BW provided a faster decrease in the number of rat wriggles compared to the administration of EEPM suspension at doses of 500 mg/kg BW and 1000

mg/kg BW, so that the most effective *Plantago major* L. ethanol extract suspension in reducing the number of rat wriggles was the EEPM suspension at a dose of 2000 mg/kg BW, the effect of which was almost close to the effect of the 2% metampyrone suspension.

The results of the data normality test using the Shapiro-Wilk test showed that the data was normally distributed because the significance value was greater ($p>0.05$). Furthermore, it was tested using the homogeneity of variance test, the results of the significance value were greater ($p>0.05$) so that the data was homogeneous. The results of the one-way ANOVA test showed that the number of wriggles between treatment groups was significantly different ($p<0.05$). The results of the Post Hoc Test using the LSD (Least Significant Difference) test showed that the positive control group (2% metampirone suspension) EEPM suspension group with doses of 500, 1000 and 2000 mg/kg BW showed significantly different analgesic effects ($p<0.05$) against the negative control group of 0.5% CMC suspension so that it can be stated that the group has an analgesic effect.

The positive control group with doses of 500, 1000 and 2000 mg/kg BW EEPM showed significantly different results. The EEPM group showed significantly different results at each dose. Referring to the statistical results, it can be concluded that EEPM doses of 500, 1000 and 2000 mg/kg BW have significantly different analgesic power in mice induced by acetic acid. The analgesic effect of EEPM is because EEPM contains flavonoid compounds that have analgesic effects. This is supported by many previous studies that have examined that medicinal plants containing flavonoids have analgesic effects (Hastuti, 2015).

Percent Analgesic Power

From Figure 2, the percentage graph of analgesic power of the 0.5% CMC suspension group, the 2% metampirone suspension group, the ethanol extract suspension group of the *Plantago major* L. at a dose of 500 mg/kg BW, 1000 mg/kg BW and 2000 mg/kg BW can be depicted as follows:

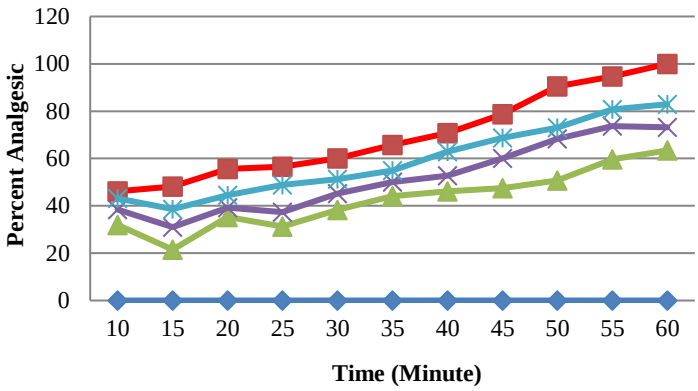

Figure 2. Percentage Graph of Analgesic Power of Each Group

Description :
- CMC Suspension 0.5%
- Methampyrone Suspension 2%
- EEPM Suspension 500 mg/kg BW
- EEPM Suspension 1000 mg/kg BW
- EEPM Suspension 2000 mg/kg BW

Based on Figure 2, it can be seen that the 0.5% CMC suspension is at position 0, which means that the 0.5% CMC suspension has no analgesic effect. The 2% methampyrone suspension provides an analgesic effect at the 20th minute with the percentage of analgesic power reaching >50%, which each minute provides an increasing percentage of analgesic power until the 60th minute and has the highest percentage of analgesic power, which is 65.00% when compared to the percentage of analgesic power of other treatment groups. EEPM suspension with a dose of 500 mg/kgBW provides analgesic power from the 50th minute with an average percentage of analgesic power of 40.54%. EEPM suspension with a dose of 1000 mg/kgBW provides an analgesic effect at the 35th minute with an average percentage of analgesic power of 48.73%.

EEPM suspension with a dose of 2000 mg/kgBW provides an analgesic effect at the 30th minute with an average percentage of analgesic power of 55.80%. The higher the dose, the less pain felt by mice. It was found that the analgesic effect of EEPM suspension at a dose of 2000 mg/kg BW was close to the analgesic power percentage of 2% metampirone suspension which provided analgesic effects starting from

the 30th minute and the average analgesic power percentage of EEPM suspension 2000 mg/kg BW which was close to the average analgesic power percentage of 2% metampirone suspension. Furthermore, the percentage of effectiveness was calculated, namely the price of the effectiveness of the test material as an analgesic by comparing the analgesic power percentage of the EEPM suspension group with the positive control group (2% methampyrone suspension). The results can be seen in Table 4.

Table 4. Average Number of Mice Wriggling Induced by Acetic Acid

No.	Treatment group	X (%)	Y (%)	Percentage of effectiveness (%)
1	EEPM 500 mg/kg BW	40.54	65.00	62.37
2	EEPM 1000 mg/kg BW	48.73	65.00	74.96
3	EEPM 2000 mg/kg BW	55.80	65.00	85.84

Description: X = Percentage of analgesic power of the EEPM suspension group
Y = Percentage of analgesic power of the 2% methampyrone suspension group

Based on Table 4, the group with the highest percentage of effectiveness is the EEPM 2000 mg/kg BB suspension group of 85.84% which is close to the percentage of effectiveness of the metampiron 2% suspension, meaning that the EEPM 2000 mg/kg BB suspension provides almost the same effectiveness as the metampiron 2% suspension. Results of the research that has been done and statistical data analysis, the ethanol extract suspension of the *Plantago major* L. provides analgesic activity through its ability to inhibit and reduce the amount of writhing in mice. This is because the ethanol extract of the *Plantago major* L. contains flavonoids which are known to be able to inhibit the formation of inflammation that causes pain. Flavonoids inhibit the cyclooxygenase II enzyme which plays a role in the biosynthesis of prostaglandins as a mediator of pain formation so that inhibition of cyclooxygenase will reduce the production of prostaglandins by arachidonic acid, thereby reducing pain.

Conclusion

The powder of the simplicia of the *Plantago major* L. and the ethanol extract of the *Plantago major* L. showed the presence of secondary metabolite compounds of the alkaloid, tannin, saponin, flavonoid, steroid and glycoside groups. Administration of EEPM 500, 1000 and 2000 mg/kg BW gave an analgesic effect which could reduce pain in mice that had been induced by 0.5% acetic acid as much as 0.5 ml. EEPM 500, 1000 and 2000 mg/kg BW had a significantly different analgesic effect with 2% methampyrone.

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