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Analgesic Activity Test of Ethyl Acetate, n-Hexane, Water Fractions of Cemcem Leaves Extract (*Spondias pinnata* (Linn.f.) Kurz.) as Part of Usadha Bali

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***Abstract*---*Pain is the most common reason why patients come to seek medical help. Pain is an unpleasant sensory and emotional feeling and is associated with tissue damage. Indonesia is one of the countries that still uses traditional medicine in addition to formal treatment. Spondias pinnata plant (Linn.f.) Kurz or Cemcem Plant is one that is used empirically as a pain reliever during menstrual pain by Balinese people. This study aims to prove the analgesic activity of the leaves of Cemcem (Spondias pinnata (Linn.f.) Kurz) that grow in Bali by making Cemcem leaves in 3 (three) fractions including ethyl acetate fraction, n-hexane fraction, and water fraction, so there is a comparison of analgesic activity of the three fractions. The three types of fractions were subjected to phytochemical screening to determine the class of secondary metabolite compounds present in Cemcem leaves extract. Analgesic activity test uses heat induction method with a temperature of 55^{o}C in 20 mice divided into four groups, namely the negative control group was given tween 80, the positive control group was given mefenamic acid 100 mg / kg BW, the ethyl acetate fraction group, the fraction group n-Hexane, and water fraction of Cemcem leaves extract. Pain response in mice is characterized by jumping or licking his feet after being placed on a hot plate. Observations were made at 30 minutes and 60 minutes after treatment. The results of this study were then performed statistical tests using one way ANOVA and continued with post hoc Tukey test. The results of phytochemical screening test and analgesic activity test can be concluded that the three fractions of extract of Cemcem leaves (Spondias pinnata (Linn.f.) Kurz) contain secondary metabolite compounds namely alkaloids, flavonoids, tannins, steroids,and terpenoids. The three fractions of Cemcem leaves extract had analgesic activity at doses of 250 mg / kgBB with a marked difference in negative control (p <0.05) at the 30th and 60 minutess.*
Keywords*---*analgesics, Cemcem Leaves extract, fraction, phytochemical screening, Spondias pinnata.

1 Introduction

Pain is an unpleasant sensory and emotional feeling and is associated with tissue damage. Drugs that are commonly used to reduce or eliminate pain, one of which is a group of NSAIDs (Non Steroidal Anti Inflamatory Drugs). NSAIDs are drugs that reduce pain, fever, and inflammation. If used long term it will give side effects such as headache, nausea, vomiting until liver and kidney damage (Mita & Patihul, 2017).

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Seeing the side effects caused by the painkillers, we need a safer alternative, especially from natural ingredients. Treatment that comes from nature generally has side effects that are lower in danger than synthetic drugs (Lesiasel, 2013). One of the natural materials that can be used is the leaves of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Which belongs to the Anacardiaceae family and is widely known as a traditional plant that is widely used in the community, especially in Penglipuran Village in Bangli Bali (Ariati, 2012). All parts of the Cemcem plant including its leaves have been used as herbal medicines for coughing, dysentery, diarrhea, abdominal pain, rheumatism, diabetes, and fever (Bora *et al.*, 2014).

The results of Setiawati (2018), showed that the ethanol extract of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Leaves had an analgesic effect on mice. The results of this research screening revealed that the ethanol extract of Cemcem leaves (*Spondias pinnata* (Linn.f.) Kurz.) Contained phytochemical compounds, namely alkaloids, flavonoids, terpenoids / steroids, saponins and tannins (Setiawati, 2018). The active ingredients which are responsible for analgesic activity are not yet known. Flavonoids act as analgesics by the mechanism of action inhibits the action of the enzyme cyclooxygenase (Suryanto, 2012). Inhibition of the cyclooxygenase enzyme will reduce prostaglandin production thereby reducing pain. Prostaglandins are mediators of pain and inflammation (Prasetya, 2015).

Based on the background above, the leaves of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Have the potential to have analgesic activity as well as the potential to be used as raw materials for traditional medicine as pain relievers or analgesics. However scientific research on phytochemical content and analgesic activity of ethyl acetate fraction, n-hexane, water from the leaves of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Has never been done. From the description above, a study was conducted on analgesic activity in three types of fractions, including the ethyl acetate fraction, the n-hexane fraction, and the Cemcem leaves extract fraction (*Spondias pinnata* (Linn.f.) Kurz.) And phytochemical content (secondary metabolites) which contained in it, so there is a comparison of analgesic activity of the three factions (Satpathy *et al.*, 2011; Attanayake *et al.*, 2014; Sameh *et al.*, 2019).

2 Materials and Methods

Research design

This type of research is Posttest Only Control Group Design. Mice were divided into 5 groups namely negative control treatment groups (Tween 80), positive controls (mefenamic acid), ethyl acetate fraction group (dose 250 mg / kg / BW), n-hexane fraction group (250 mg / kg / BW dose) and water fraction group (dose 250 mg / kg / BW). This sampling technique uses a purposive sampling technique that is done by taking subjects based on the existence of certain objectives, researchers determine where to take the sample (Tata, 1958; Suzuki & Okamuranoji, 1995).

Tools and Materials

The tools used in this study are general tools in the chemical laboratory, trays, blenders, glass jars, Buchner funnels, filter paper, ovens, experimental animal containers, and hot plates. The materials used in this study were Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Leaves extract, ethyl acetate solvent, n-hexane solvent, water solvent, 70% ethanol technical solution, concentrated HCl solution, dragendorff reagent, Pb-acetate solution, NaOH solution, FeCl3 solution, distilled water, chloroform solution, concentrated H2SO4 solution, Liebermann-Burchard reagent, Tween 80, and mefenamic acid.

Method

1) *Making of Cemcem Leaf Extract (Spondias pinnata (Linn.f.) Kurz.)*
   Extraction of simplex powder of Cemcem Leaves (*Spondias pinnata* (Linn.f.) Kurz.) Was carried out using maceration method. The simplicia powder was put into a vessel then soaked with 70% ethanol with a ratio of simplicia and solvent 1: 5, allowed to stand for 3 days with occasional stirring. Then the filtrate is filtered to obtain a liquid extract. The liquid extract is concentrated using an oven at 100oC until a concentrated extract is obtained. This concentrated extract was weighed to get the yield (Setiawati, 2018).
2) *Making of Cemcem Leaf Extract (Spondias pinnata (Linn.f.) Kurz.) (Ethyl Acetate Faction, n-Hexane Faction, Water Faction)*
   A 10 g thick extract was fractionated using various levels of polarity, by first dissolving it with 50 ml of distilled water, then fractionation in a separating funnel using a low polarity solvent that is 100 ml of n-hexane, liquid fraction of n-hexane was obtained and then fractionation was carried out in a separating funnel using a low

polarity solvent of 100 ml n-hexane, liquid fraction of n-hexane was obtained and liquid fraction of water. The water fraction was fractionated again using a semipolar solvent, 100 ml of ethyl acetate, a liquid ethyl-acetate fraction and a water fraction were obtained. Liquid water fraction, liquid ethyl-acetate fraction and n-hexane liquid fraction, respectively separated from the solvent and concentrated with the aid of an oven into water, ethyl acetate, and n-hexane fractions.

3) *Phytochemical Screening of Cemcem Leaf Extract (Spondias pinnata (Linn.f.) Kurz.) (Ethyl Acetate Faction, n-Hexane Faction, Water Faction)*
Phytochemical screening is carried out through test tube tests, using samples in the form of test solutions. Preparation of the test solution was carried out by dissolving 500 mg of viscous fraction into 50 ml of 70% ethanol solvent. Identification is carried out for compounds of alkaloids, flavonoids, saponins, tannins, anthraquinones, and steroids / terpenoids (Farnsworth, 1966; Sparg *et al.*, 2002).

4) *Testing analgesic activity*
In analgesic testing, healthy male white mice were tested ± 2 months old at the time of the test treatment with mice weights 20-28 grams as pain inducers used hot plates, as a control used aquadest and test materials namely the three types of Cemcem leaves extract fraction (*Spondias pinnata* (*Spondias pinnata*) Linn.f.) Kurz.)
Mice were adapted to the research environment for 6 days. Twenty-five mice were further grouped into 5 groups, each group consisting of 5 mice. Before being treated with mice it had been fasted for 6 hours but was still given a drink. Mice group division included negative control group (Tween 80), positive control group (mefenamic acid), ethyl acetate fraction group (dose 250 mg / kg / BW), n-hexane fraction group (250 mg / kg / BW dose), and water fraction group (dose 250 mg / kg / BW). Analgesic activity testing was carried out at 30 and 60 minutes after administration of treatment. Tests carried out using the method of induction of the hot method using a hot plate with a temperature of 55 ° C. The stopwatch is turned on when the mouse touches the surface of the hot plate and is observed until the mouse shows a pain response that is characterized by jumping or licking its feet. The time when the pain response arises in mice is recorded when the mouse first jumps or licks its feet.

5) *Processing and data analysis*
The data obtained were statistically tested using the SPSS 22 computer program, using the One Way Anova test with a 95% confidence level followed by a post hoc test using the Tukey test.

3 Results

Table 1
Phytochemical Screening Results of the Three Cemcem Leaves Extract Fractions (*Spondias pinnata* (Linn.f.) Kurz.)

No.	Phytochemical Compounds	Reactor	Ethyl Acetate Fraction	N-Hexane fraction	Water Fraction
1.	Alkaloid	*Dragendroff*	+	+	+
		Mayer	+	+	+
2.	Flavonoid	Pb-Asetat 10%	+	+	+
		NaOH 20%	+	+	+
3.	Tanin	$FeCl_3$ 5%	+	+	+
4.	Steroid /	*Lieberman*	+	-	+
	Terpenoid	*Burchard*	+	+	-
5.	Saponin	Aquadest and HCl 2N	-	-	-
6.	Antrakuinon	NaOH 1N	-	+	+

Table 2
Test Results of Analgesic Activity of Cemcem Leaves (*Spondias pinnata* (Linn.f.) Kurz.) At the 30 minutes for the Three Fractions

Minutes to	Treatment Group Pain Response Time (Average ± SEM, units of seconds)				
	Positive Control	Negative Control	Fraction Ethyl acetate	Fraction n-hexane	Fraction Water
30	20,96±0,58	11,27±0,93	18,09±0,93	22,86±0,83	18,96±0,78
60	19,55±0,94	11,01 ± 0,84	21,59 ± 1,08	28,97±1,08	20,65±1,04

Information:
SEM : Standard Error Mean

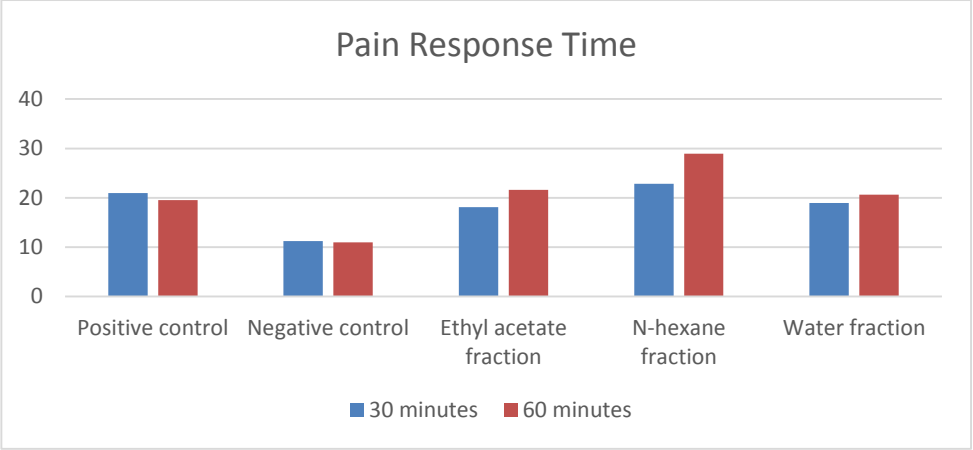

Figure 1. Mouse Pain response time

The results of data analysis of the three fractions of Cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) Had analgesic activity at a dose of 250 mg / kg body weight with a significant difference in negative control ($p < 0.05$) at 30 minutes and into -60.

4 Discussion

In this study phytochemical screening and analgesic activity tests of the water extracts of the Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Extract on Wistar strain male mice (Mus musculus) were conducted. Leaves Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) From the Anacardiaaceae family obtained in Kubu Village, Penglipuran Bangli, Bangli Regency has been determined by the Indonesian Institute of Sciences UPT Plant Conservation Center Eka Karya Bali. Phytochemical testing is one of the important steps in efforts to uncover the potential of medicinal plant resources (Cahyaningsih *et al.*, 2019). Secondary metabolites that are commonly found in plants are: alkaloids, flavonoids, steroids, saponins, terpenoids and tannins (Harborne, 1987). In phytochemical testing, all three fractions contained positive alkaloids, flavonoidair extract of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Positive leaves containing alkaloids, flavonoids, tannins, and terpenoids.

Testing the effectiveness of analgesics in this study uses the heat induction method (hot plate method). Stimulation given to test animals is in the form of heat stimulation with a temperature of 55°C (Rodriguez *et al.*, 2018; France *et al.*, 1984; Fujimoto & Wang, 1970). The measured parameter is the time taken when the pain response first arises in mice (the first time a mouse licks its leg). After the mice are placed on a hot plate then the reaction time is required. The reaction time to consider is after administration at the 30 minutes and 60 minutes. Reaction time is the time interval between the placement of mice on a hot plate and the appearance of the first response to mice in the form of jumping or licking his feet as a reaction to reduce pain.

From the test results of the Test of Normality and Test of Homogenity of Variances, the results show that the data are normally distributed and homogeneous with a value of $P > 0.05$. After analyzing the data with the One Way ANOVA test, P value <0.05 so that it can be said that there are at least 2 groups of pain response time that are significantly different. To find out which groups were significantly different, a post hoc test was performed with the Tukey test.

After testing for analgesic activity, the results of pain response time (reaction time) on the 30 minutes for the negative control group were 11.27 seconds, positive control 20.96 seconds, at the 60 minutes for the negative group 11, 01 seconds, positive control 19.55 seconds. This shows a significant difference between positive control and negative control ($P < 0.05$). Positive control data indicate an extension of the pain response time due to mefenamic acid administration.

The results of the pain response time of the ethyl acetate fraction of Cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) Dose of 250 mg / kg BW at the 30 minutes were 18.09 seconds and the 60 minutes was 21.59 seconds. The results showed that there was an extension of the pain response time in the ethyl acetate fraction of

Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Leaves extract dose of 250 mg / kg body weight 30 minutes and 60 minutes compared with negative controls. When compared to the positive control in the 30 minutes is 20.9 seconds. Longer positive control provides extended pain time. In the positive control the 60 minutes showed that the pain response time was 19.55 seconds, so the pain response time was longer than the positive control. So in the ethyl acetate fraction of Cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) Dose 250mg / kg BW with positive control at 60 minutes (T60) longer to provide a pain response than in the 30 minutes (T30).

The pain response time of the n-hexane fraction 250 mg / kg BW at 30 minutes (T30) was 22.86 seconds and at 60 minutes was 28.97 seconds. These results were compared with positive and negative controls at 30th (T30) and 60th (T60). The results obtained that there was an extension of the pain response time in the n-hexane fraction of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Leaves extract 250 mg / kg BW at 30 (T30) and 60 (T60) compared to controls negative. The results of the n-hexane fraction 250 mg / kg body weight of the pain response time is longer than the positive control. This indicates that at the 30 minutes the fraction of n-hexane 250 mg / kgBW was more effective than mefenamic acid.

The pain response time of the water fraction of Cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) 250 mg/kg BW at 30 minutes (T30) was 18.96 seconds and at 60 minutes was 20.65 seconds. The results of the pain response of the water fraction of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Leaves extracts were compared to positive and negative controls at the 30th (T30) and 60th (T60), comparison results were obtained. 30th (T30) of the water fraction of Cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) 250 mg / kgBB with positive control obtained a value of p> 0.05 and on the comparison between the water fraction of Cemcem leaves extract (*Spondias pinnata* (*Spondias pinnata*) Linn.f.) Kurz.) 250 mg / kg body weight with negative control p value <0.05. Whereas at the 60th (T60) results of comparison of the water fraction of Cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) 250 mg / kgBB with positive control obtained a p value> 0.05, and the comparison of the water fraction of Cemcem leaves extract *Spondias pinnata* (Linn.f.) Kurz.) 250 mg / kg body weight with negative control get p value <0.05. From these results it is known that the water fraction of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Leaves extract 250 mg / kgBB has anti-pain (analgesic) activity which is similar to positive control or mefenamic acid.

The resulting analgesic activity can be caused by the presence of flavonoid compounds from the leaves of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.). Flavonoids act as analgesics by the mechanism of action inhibits the action of the enzyme cyclooxygenase (Suryanto, 2012). Inhibition of the cyclooxygenase enzyme will reduce prostaglandin production thereby reducing pain (Harborne, 1987). Prostaglandins are mediators of pain and inflammation (Prasetya, 2015). According to Purnomo (2002), alkaloids and terpenoids function as antibiotics and anti-inflammatory which can reduce pain. This is consistent with the study of Panda *et al.* (2012), states that the ethanol extract of Cemcem bark with flavonoid content can provide analgesic effects at a dose of 50-100mg / KgBB. Setiawati (2018) mentioned that the ethanol extract of Cemcem leaves (*Spondias pinnata* (Linn.f.) Kurz.) Especially those grown in the stronghold village, bipling with a dose of 280 mg / Kg BB-560 mg / Kg BB can provide analgesic effect on mice (Setiawati, 2018). It can be said from research that has been carried out that the three types of ethyl fraction of Cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) At a dose of 250 mg / kg BW can still provide analgesic effects on mice that have the potential to develop as an alternative raw material for manufacturing traditional medicine for analgesics.

5 Conclusions

The results of phytochemical screening tests and analgesic activity tests can be concluded that the three fractions of Cemcem leaves extract (*Spondias pinnata* (Linn.f.) Kurz.) Contain secondary metabolite compounds namely alkaloids, flavonoids, tannins, steroids, and terpenoids. All three fractions of Cemcem (*Spondias pinnata* (Linn.f.) Kurz.) Leaves extract had analgesic activity at a dose of 250 mg / kg body weight with a marked difference in negative control (p <0.05) at 30 and 60 minutes.

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