

Comparison Between The Suspension and Capsule Preparation from Waste of Avocado Seeds As Antidiarrhea in Induced Mouse

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Abstract

Diarrhea is one type of disease with the most sufferers every year. Thus, it is considered an endemic disease in Indonesia and the potential disease of Extraordinary Events accompanied by mortality. One of the plants that can be used as a traditional medicine for diarrhea is the avocado seed, as it contains tannins, alkaloids, flavonoids, steroids and glycosides, which act as antidiarrheals. This study aims to determine the antidiarrheal effect of avocado seed extract suspension on mice induced by *Oleum ricini* and the optimum concentration of avocado seed extract suspension and capsules with an antidiarrheal effect in white male mice induced by *Oleum ricini*. The dosage forms chosen were suspension and capsules. This study used an experimental method with a test sample of the avocado seed. Avocado seed *Simplicia* was extracted by the percolation method, then an oral suspension and capsule formulation were made from the avocado seed methanol extract. It was evaluated and tested for its effectiveness with mice to cure diarrhea. Normal data were analyzed by One Way ANOVA and Post Hoc Tuckey Method. The results of this study showed that the administration of a suspension of avocado seed methanol extract at a dose of 800 mg/KgBB had the most optimum effect as an antidiarrheal against white male mice with a stool weight of 0.39 grams and a duration of diarrhea for 74 minutes. Furthermore, the administration of avocado seed extract capsules at a dose of 75 mg/KgBB had the most optimum effect as antidiarrheal against white male mice with Loperamide as a positive control. Therefore, it can be concluded that all suspension formulations and capsules of avocado seed methanol extract met the requirements for preparation evaluation. Suspension and avocado seed methanol extract capsules can cure diarrhea in white male mice.

Keywords: Antidiarrheal; Avocado Seeds; *Oleum ricini*; Suspension; Capsule

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INTRODUCTION

Diarrhea in Indonesia has become an endemic disease and is a potential

extraordinary event (KLB) accompanied by death.¹ There are various kinds of treatment to treat this diarrheal disease, ranging from traditional medicine to

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modern medicine. People prefer traditional medicine that uses plants as this type of treatment has fewer side effects than conventional preparations. One of the traditional treatments is using avocado (*Persea Americana Mill.*) Avocado seeds contain alkaloids, tannins, triterpenes, and quinones.² Avocado seed waste can be used for traditional medicine by drying and then mashing. Empirically, avocado seeds are used as medicine to treat diarrhea, diabetes medicine, cholesterol medicine and treat toothache.

There are various pharmaceutical preparations, such as capsules and suspensions. Researchers chose capsules and suspension preparations to investigate the antidiarrheal effect of methanol extract of avocado seeds (*Persea Americana Mill.*). Capsules that mask unpleasant tastes and odors are easy to consume and prepare. The medicinal ingredients are protected from external influences (light, humidity). Powder formulations often require the addition of fillers, lubricants, and glides to the active ingredients to facilitate the capsule filling process. Capsules are solid preparations consisting of a soluble hard or soft shell drug; the shell is generally made of gelatin, starch, or other suitable materials. The extracts were made using the percolation method.³ Meanwhile, the suspension is widely used as it is easy to use for children, infants, and adults who have difficulty swallowing tablets or capsules. Suspensions can also be added with additives to mask the unpleasant taste of the active substance. In general, the liquid form is preferred over the tablet or capsule form as it is easy to swallow and adjust the dose for children.⁴ The advantages of these two preparations are that they are practical to provide comfort for drug consumers and can cover unpleasant tastes and odors in drugs.⁵

Based on the description of the background, this study aims to identify whether there is a difference between the suspension and capsules of avocado seed methanol extract that meets the physical quality requirements of the preparation and to determine whether the suspension and capsules of avocado seed methanol extract (*Persea Americana Mill.*) had an antidiarrheal effect. Furthermore, this study also aims to identify the optimum concentration of avocado seed methanol extract that can have an antidiarrheal effect on mice.

METHOD

This research is an experimental study with a sample of avocado seeds (*Persea Americana Mill.*). The chemicals used in this study were Avocado Seed, Na CMC, Simple Syrup, Loperamide HCl, Oleum ricini, distilled water, HCl, Bouchardat reagent, Dragendroff reagent, Fehling's reagent A, Fehling's reagent B, HNO₃, Mayer's reagent, Molish's reagent, NaOH, Pb(C₂H₃O₂), Lieberman-Bouchardat reagent and HNO₃.FeCl₃

Making Simplicia and Extract

Simplicia was made by cleaning avocado seeds from dirt, then dried in a drying cabinet at a temperature of $\pm 40^{\circ}\text{C}$. It was then mashed and weighed of the dry powder obtained. The next step was to make methanol extract of avocado seeds by the percolation method. The procedure for making extracts in the percolation process was that the dry powder of avocado seeds was weighed as much as 500 grams and first soaked for 24 hours with 1200 ml of methanol in a closed glass vessel and dark in color. The extraction process was continued in a percolator, and the solvent used was 2.305 L to the liquid that dripped from the clear percolator. The percolate liquid was allowed to drip while the filtered

fluid was added repeatedly; thus, the filtered fluid limit remained 10 cm above the simplicia powder. The percolate yield obtained was 2.100 L. The percolation process was carried out until the percolate liquid no longer gave a cloudy color. The percolation results were evaporated in a vaporizer cup covered with aluminum foil and given a small hole. It was left in an open room for a week until the solvent evaporated and obtained a thick extract. The extract was later weighed.

Phytochemical Screening

Phytochemical screening was carried out qualitatively on avocado seed Simplicia powder (*Persea Americana Mill.*), including examination of alkaloids, flavonoids, tannins, saponins, glycosides, cyanogenic glycosides, anthraquinone glycosides, steroids/triterpenoids.

Preparation Evaluation

Organoleptic Examination

Organoleptic examination of the suspension and capsules of avocado seed methanol extract included color, odor, taste and texture.

An avocado seed methanol extract suspension was an organoleptic examination that included a light brown color, characteristic aromatic odor, sweet taste, and sandy texture.

Organoleptic examination of avocado seed methanol extract capsules included dark brown color, aromatic odor, bitter taste and gritty texture.

Stability Test

The stability evaluation for the suspension was carried out by the cycling test method. It was accelerated storage under forced conditions, carried out by storing at 40°C. Furthermore, it was put in the refrigerator and stored at 4°C in the oven alternately for 24 hours for 6 cycles with organoleptic

test parameters (color), odor, dosage form, and the pH of the preparation.

Viscosity Test

The suspension viscosity measurement was carried out after reconstituted using a Brookfield viscometer spindle number 1 at a speed of 30 RPM. Before and after accelerated storage conditions, the shear stress was calculated.

Sedimentation Test

The measurement of the sedimentation volume of the suspension has been made before and after accelerated storage conditions. It was carried out by comparing the sediment's final volume (V_u) with the original (V_o) before deposition. The redispersion ability would be good if the suspension was completely dispersed when shaken by hand for a maximum of 30 seconds.

Specific Weight Test

Specific gravity is the ratio of the weight of the substance to water in the same volume, which is weighed at room temperature before and after being given accelerated storage conditions at 5°C and 35°C for 12 hours each for 5 cycles.

- a. Use a clean and empty pycnometer, then fill it with distilled water; the outside of the pycnometer is dried and weighed
- b. Discard the distilled water, dry the pycnometer and then fill it with liquid syrup at the same temperature and at the time of measurement of distilled water and weigh it.

$$\text{Formula: } \rho = \frac{m}{V}$$

Information :

ρ = Density (g/cm³) or (g/ml)

M = Mass of object (g)

V = Volume of object (cm³) or (ml)

pH Test preparation

Evaluation of the preparation's pH was carried out using a calibrated pH meter. The suspension was put in a beaker glass, and then the pH meter was dipped into the suspension. The pH value of the suspension was identified by looking at the numbers listed on the pH meter.

Weight Uniformity Test

Thoroughly, 10 capsules were weighed one at a time and identified. The contents of each capsule in an appropriate manner were then removed.

Destroyed Time Test

6 capsules were inserted in each tube in the basket under the 10 mesh steel plates. The medium water temperature of 37°C was then used. In the observation of the capsules, all capsules must be crushed, except for part of the capsule shell.

Hygroscopicity Test

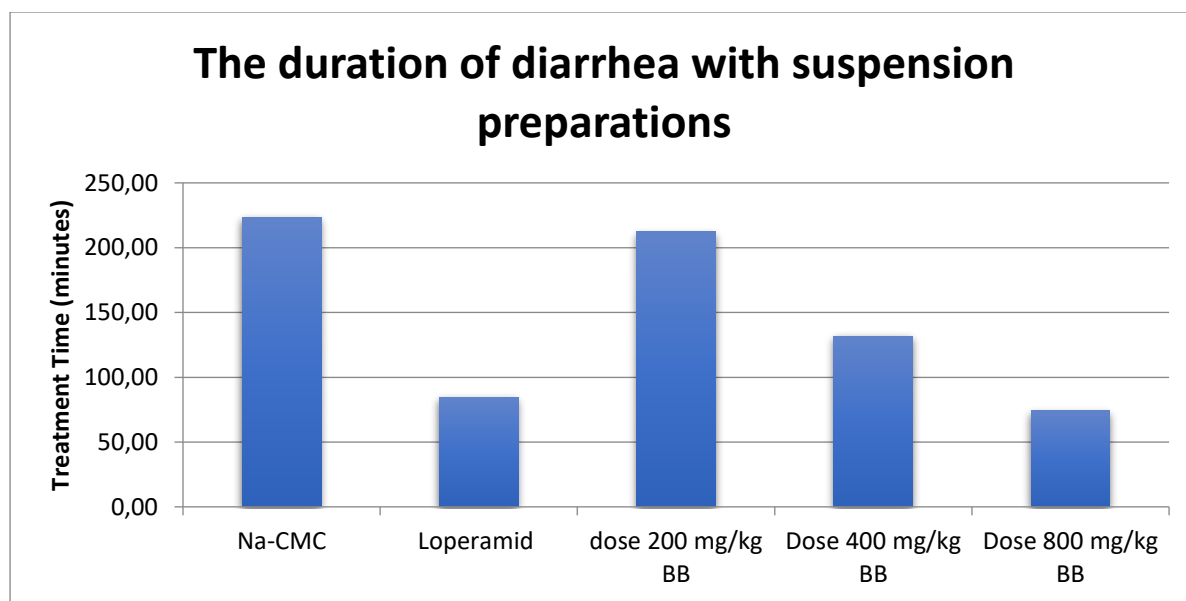
A total of 3 capsules was placed in a brown bottle and stored in a desiccator. Each treatment was observed every day for seven days and every week for a month. Observations were made on changes in capsule weight, capsule shape, and capsule contents.

Experimental Animal Preparation

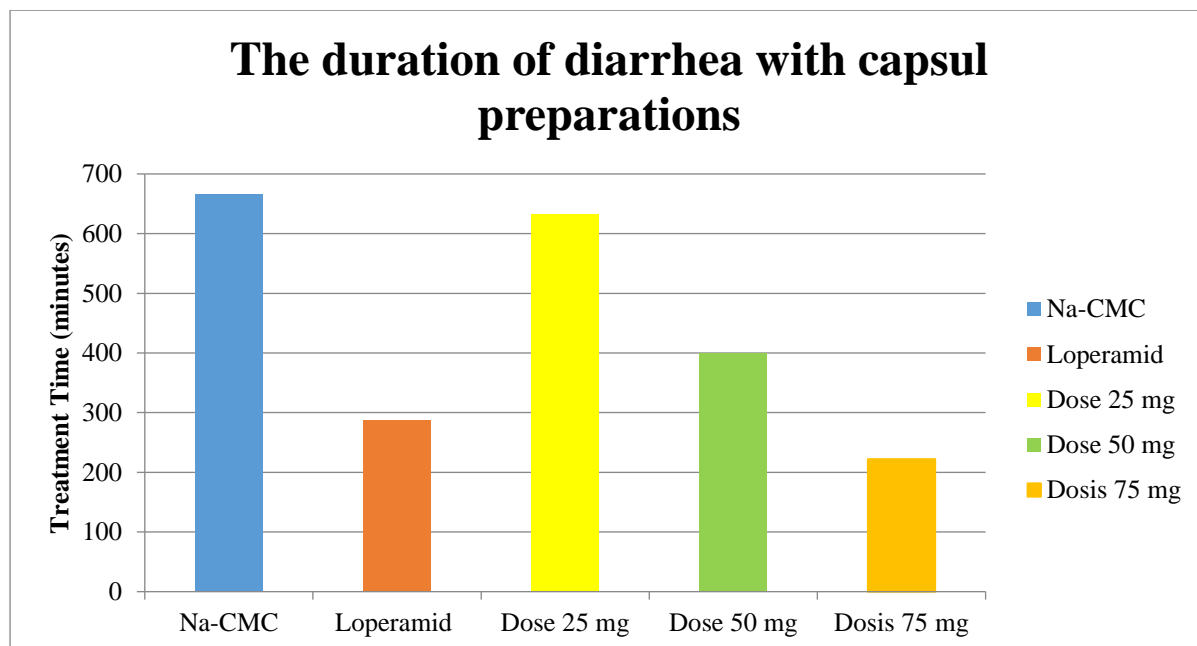
The test animals used in this study were adult mice (*Mus musculus*) weighing 20-30 grams. The total sample was 25 mice divided into 5 groups. Each treatment group consisted of 5 mice. Group 1 was a negative control, group 2 was a positive control, and groups 3, 4, and 5 were treatment groups. Two weeks before the experiment, the mice were adapted to the experimental environment.

Antidiarrheal Effectiveness Test

The dose of avocado seed methanol extract given to experimental animals used a dose ratio with loperamide. In a positive control group, loperamide HCl was used. Oleum ricini was used as induction, and four test groups were given suspension and capsules of avocado seed methanol extract with three dose ratios. It was given to 25 mice adapted to the research environment for 1 week. Before the study was conducted, the mice fasted for 18 hours while still being given water before testing. They were grouped into 5 groups; each group consisted of 5 mice. All mice were given Oleum ricini as a diarrhea induction of 0.75 ml per mouse orally except for the normal group.



Grafic 1. Grafic of duration of diarrhea in suspensions preparations



Grafic 2. Grafic of duration of diarrhea in capsule preparations

After being given *Oleum ricini* as much as 0.75 ml per head as an inducer, the capsule and suspension were left for 30 minutes. It was then given the test material orally according to each group of animals. The antidiarrheal effect was determined by

observing the onset of diarrhea, the frequency of feces, the weight of the feces in grams, and the duration of diarrhea. In this experiment, the antidiarrheal effect was observed for 5 hours.

RESULTS AND DISCUSSION

Phytochemical Screening Results

Table 1. Phytochemical Screening Results

Compound Group	Content of Dried Avocado Seed <i>Simplicia</i>	Phytochemical Screening Results
Alkaloids	+	Mayer: Dark Chocolate Draendroff: Muddy Chocolate Bouchardat: Cloudy Yellow
Saponins	+	White Foam
Flavonoids	+	Red Orange
Tannins	+	Blackish Green
Glycoside	+	Glycon: Purplish brown/purple in the middle Aglycone: Reddish Purple
Anthraquinone glycosides	-	Pink/Red
Cyanogenic Glycoside	-	Blood red on filter paper
Steroids	+	Green

Test Results of Avocado Seed Methanol Extract Suspension on Mice with Post Tukey

Table 2. Test Results of Fecal Weight when Given Avocado Seed Methanol Extract Sustenance

Treatment	Fecal Weight (Gram)			Amount	Average (Gram)	Statistical Result RAL
	Repetition					
	1	2	3			
Na-CMC	0.62	0.6	0.41	1.63	0.543333333	0.526
Loperamid HCl	0.31	0.31	0.24	0.86	0.286666667	
Dosage of 200 mg/kgBB	0.29	0.4	0.61	1.3	0.433333333	
Dosage of 400 mg/kg BB	0.27	0.38	0.73	1.38	0.46	
Dosage of 800 mg/kg BB	0.25	0.26	0.67	1.18	0.393333333	

Table 3. Results of the Post Tukey Fecal Frequency Test when Suspended with Avocado Seed Methanol Extract

Treatment	N	Subset for alpha=0.05
		1
Dosage of 400 mg	3	9.6667
Dosage of 800 mg	3	10.0000
Loperamide HCl	3	10.3333
Dosage of 200mg	3	10.3333
Na_CMC	3	12.3333
Sig.		0.299

Table 4. Post Tukey Test Results in Fecal Weight when Given Avocado Seed Methanol Extract Sustenance

Treatment	N	Subset for alpha=0.05
		1
Loperamide HCl	3	0.2867
Dosage of 800 mg	3	0.3933
Dosage of 200mg	3	0.4333
Dosage of 400 mg	3	0.4600
Na_CMC	3	0.5433
Sig.		0.436

Test Results of Avocado Seed Methanol Extract Suspension on Mice with Post Tukey

Table 5. Diarrhea Results When Given Avocado Seed Methanol Extract Capsules

Treatment	Fecal Weight (Gram)			Amount	Average (Gram)
	Repetition				
	1	2	3		
Na-CMC	0.75	0.72	0.42	1.89	0.63
Loperamid	0.23	0.23	0.17	0.63	0.21
Dosage of 25 mg	0.22	0.25	0.61	1.08	0.36
Dosage of 50 mg	0.27	0.32	0.61	1.2	0.4
Dosage of 75 mg	0.27	0.39	0.36	1.02	0.34

Table 6. Results of the Post Tukey Test of Feces Frequency when Given Avocado Seed Methanol Extract Capsules

Treatment	N	Subset for alpha=0.05	
		1	
Loperamide HCl	3	8.0000	
Dosage of 800 mg	3	8.0000	
Dosage of 200mg	3	9.0000	
Dosage of 400 mg	3	10.0000	
Na_CMC	3	12.0000	
Sig.		0.211	

Table 7. Post Tukey Test Results in Fecal Weight when Given Avocado Seed Methanol Extract Capsules

Treatment	N	Subset for alpha=0.05	
		1	2
Loperamide HCl	3	0.2100	
Dosage of 800 mg	3	0.3400	
Dosage of 200mg	3		0.3600
Dosage of 400 mg	3		0.4000
Na_CMC	3		0.6300
Sig.		0.581	0.221

Based on the results of phytochemical screening of avocado seed methanol extract, it is known that avocado seeds contain a group of compounds such as alkaloids, saponins, flavonoids and tannins. Alkaloid compounds work by inhibiting the growth of *Salmonella typhimurium* bacteria which have been known to have the potential to cause diarrhea.⁶ Meanwhile, tannin compounds are astringent, which can help stop diarrhea.⁷ Flavonoids are antidiarrhea with the mechanism of inhibiting intestinal motility by reducing fluid and electrolytes. Another flavonoid activity (quercetin) is by

inhibiting the release of acetylcholine in channel.⁸

Furthermore, the results of organoleptic observations included odor, color, and taste which were observed every 14 days. In each suspension formula of Avocado seed methanol extract, organoleptic changes occurred in the suspension. There is a change in taste in the table due to a fermentation reaction. Fermentation is the main energy-producing process of various microorganisms. The following table measuring pH Meter.

Table 8. Table Measuring pH Meter

Replication	pH Meter	
	Before the suspension was made (pH)	After the suspension was made (pH)
F1	4.8	4
F2	4.7	3.7
F3	4.5	3.8
Average	4.75	3.83

The pH test results were tested by shelf-life. It revealed the suspension formulation with the suspending agent CMC Na. The pH decreased from 4.75 to 3.83 during the storage cycle, and the optimum suspension pH was 5-6.⁹ The pH of the preparation was acidic due to the presence of additives (preservatives). It was used in the form of benzoic acid with

a pH <4.5 to affect the pH of the preparation. Meanwhile, the specific gravity measurement results showed a change in the specific gravity of the suspension before and after the accelerated storage conditions from 1.03 to 1.02, which met the suspension-specific gravity requirements, namely >1.00 g/ml.¹⁰

Table 9. Table measuring pH Meter

Replication	pH Meter	
	Before the suspension was made	After Accelerated Storage Conditions
	(pH)	(pH)
F1	4.8	4
F2	4.7	3.7
F3	4.5	3.8
Average	4.75	3.83

Based on the results of statistical tests on observing the initial diarrhea time with ANOVA testing, a significance value was 0.366 ($p > 0.05$).¹¹ Furthermore, H_0 was accepted, indicating that the five treatments had unequal variances (not homogeneous), which could be concluded to accept H_0 . It meant that the average value of diarrhea in each of these treatments was not significantly different.¹²

The decision-making of the Tukey method can be carried out by looking at the placement of the statistical test results in the column. The test results for the duration of diarrhea at a dose of 800 mg were in the same column as loperamide which concluded that the diarrhea value in each treatment was not significantly different. Meanwhile, a dose of 200 mg, 400 mg and Na-CMC are in different columns, indicating that the diarrhea value in each treatment was significantly different from the positive control group.¹³

Furthermore, in the statistical test results in the column, the results of the frequency

of diarrhea at a dose of 400 mg, a dose of 800 mg, Loperamide, a dose of 200 mg and Na-CMC were in the same column, concluding that the value of diarrhea in each of these treatments was not significantly different. The results of the loperamide stool weight at a dose of 800 mg, 200 mg, 400 mg and Na-CMC were in the same column, concluding that the value of diarrhea in each of these treatments was not significantly different. Meanwhile, the results of the frequency of diarrhea at a dose of 50 mg, loperamide, a dose of 75 mg, 25 mg and Na-CMC were in the same column, concluding that the diarrhea value in each treatment was not significantly different.¹⁴

The results of the 75 mg dose of feces and loperamide were in the same column, indicating that, in each treatment, it was not significantly different (Significant). However, it was significantly different at 25 mg, 50 mg and Na-CMC. Meanwhile, the 50 mg dose, 25 mg dose and Na-CMC were in the same column, indicating that the diarrhea value at 50 mg dose, 25 mg dose and Na-CMC was not significantly

different. The results of the frequency of diarrhea at a dose of 50 mg, loperamide, a dose of 75 mg, a dose of 25 mg and Na-CMC were in the same column, indicating that the diarrhea value in each treatment was not significantly different.¹⁵

CONCLUSION

Based on the result of this study, it can be concluded that the results of the study indicated that avocado seeds contained alkaloids, flavonoids, glycosides, and saponins compounds. The extract yield obtained was 17.23%, and the water content was 3.7. The results of the preparation evaluation tests, such as pH testing on formulas I, II, and III, respectively, were 4.8; 4.7; 4.5. The average formula-specific gravity measurement was 1.03 before accelerated storage. After accelerated storage, it was 1.02. Furthermore, the result of the viscosity measurement was 199.8, while the result of the sedimentation volume measurement was 0.76; 0.8; 0.8. %. The administration of avocado seed methanol extract suspension at a dose of 800 mg/kg BW had the most optimum effect as an antidiarrheal against white male mice with a stool weight of 0.39 grams and a duration of diarrhea of 74 minutes. The test results were slightly different between the dose of 800 mg/kg bb and Loperamide as a positive control. In addition, the results of preparation evaluation tests such as weight uniformity testing in formulas 1, 2 and 3 were 120 mg. The results of the average disintegration time of 3 minutes showed that the formula was destroyed, and the hygroscopicity test results on the three formulas were relatively stable. Furthermore, the administration of avocado seed extract capsules at a 75 mg/kg BW dose had the most optimum effect against white male mice as an antidiarrheal agent. The results of the average disintegration time were 3

minutes. The formula was then destroyed, and the hygroscopicity test results on the three formulas were relatively stable.

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