**COMPARISON OF SUSPENSION AND CAPSULE PREPARATIONS FROM WASTE AVOCOUNE SEEDS (*Persea Americana Mill*.) AS ANTIDIARE IN INDUCED MOUSE *Oleum ricini***

**Muharni Saputri 1\* ; Nilsya Febrika Zebua 2 ; Fivi Nur Asih 3; Ghera Fakhira Putri 4**

1,2,3 Pharmacy, Faculty of Pharmacy, Tjut Nyak Dhien University, Medan, Indonesia

\*email: muharnisaputri@gmail.com

***Abstract***

*Diarrhea is one type of disease with the most sufferers every year so it is designated as an endemic disease in Indonesia and was potential disease of Extraordinary Events accompanied by death. One of the plants that can be used as a traditional medicine for diarrhea is avocado seed because it contains tannins, alkaloids, flavonoids, steroids and glycosides which act as antidiarrheals. Purpose of this study was to determine the antidiarrheal effect of avocado seed extract suspension on mice induced by Oleum ricini, to determine the optimum concentration of avocado seed extract suspension and capsules that had 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 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, then 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. 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. So it can be concluded that all suspension formulations and capsules of avocado seed methanol extract meet the requirements for preparation evaluation. Suspension and capsules of avocado seed methanol extract can cure diarrhea in white male mice.*

**Keywords**: antidiarrheal; avocado seeds; oleum ricini, suspension, capsule

**Introduction**

Diarrhea in Indonesia has become an endemic disease and is a potential extraordinary event (KLB) accompanied by death1. There are various kinds of treatment to treat this diarrheal disease, ranging from traditional medicine to modern medicine. People prefer traditional medicine that uses plants because this type of treatment has fewer side effects than conventional preparations. One of the traditional treatments is using avocado (*Persea amaericana Mill*.) Avocado seeds contain alkaloids, tannins, triterpenes, and quinones2. Avocado seed waste can be used for traditional medicine by drying and then mashing it. 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 can mask unpleasant tastes and odors, are easy to consume, easy to prepare and the medicinal ingredients are protected from external influences (light, humidity). Powder formulations often require the addition of fillers, lubricants, and glidans 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 or starch or other suitable materials. Making extracts in this study using the percolation method,3. While the suspension is widely used because it is easy to use for children, infants, and also for 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 because it is easy to swallow and easy to adjust the dose for children4. 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 drugs5.

Based on the description of the background, the researcher conducted this study with the aim of knowing whether there was a difference between the suspension and capsules of avocado seed methanol extract that met the physical quality requirements of the preparation and to determine whether the suspension and capsules of avocado seed methanol extract (*Persea americana Mill*.) has an antidiarrheal effect and what is the optimum concentration of avocado seed methanol extract that can have an antidiarrheal effect on mice.

**Methods**

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 , and Pb(C2H3O2), , Lieberman-Bouchardat reagent and HNO3.$FeCl\_{3}$

**Making Simplicia and Extract**

Making simplicia is done by cleaning avocado seeds from dirt, then dried in a drying cabinet at a temperature of ± 400C then mashed and weighed the weight of the dry powder obtained. The next step is to make methanol extract of avocado seeds by the percolation method. The procedure for making extracts in the percolation process, 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, then the extraction process was continued in a percolator, the solvent used was 2,305 L to the liquid that drips from the percolator is clear. The percolate liquid was allowed to drip while the filter fluid was added repeatedly so that the filter fluid limit remained 10 cm above the simplicia powder. The percolate yield obtained is 2,100 L. The percolation process is carried out until the percolate liquid no longer gives a cloudy color. The percolation results were evaporated in a vaporizer cup covered with aluminum foil and given a small hole, left for a week in an open room until the solvent evaporated and a thick extract was obtained, then the weight of the extract was weighed.

**Phytochemical Screening**

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

**Preparation Evaluation**

***Organoleptik Examination***

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

***Stability Test***

The stability evaluation for the suspension was carried out by the cyling test method, namely accelerated storage under forced conditions, carried out by storing at 40C which was put in the refrigerator and storage at 400C in the oven alternately for 24 hours for 6 cycles with organoleptic test parameters (color), odor, and dosage form as well as the pH of the preparation.

***Viskosity Test***

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

***Sedimentation Test***

Measurement of the sedimentation volume of the suspension that has been made before and after accelerated storage conditions. The measurement of sedimentation volume is carried out by comparing the final volume (Vu) of sediment with the original volume (V0) before deposition occurs. Good redispersion ability, if the suspension is 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 50C and 350C for 12 hours each for 5 cycles.

1. Use a clean and empty pycnometer, then fill it with distilled water, the outside of the pycnometer is dried and weighed
2. 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.

Rumus ρ = $\frac{m}{V}$

Information :

ρ = Density (g/cm3 ) atau (g/ml)

M = Mass of object (g)

V = Volume of object (cm3 ) atau (ml)

***pH Test preparation***

Evaluation of the pH of the preparation using a calibrated pH meter. The suspension was put in a beaker glass, then the pH meter was dipped into the suspension. The pH value of the suspension is known by looking at the numbers listed on the pH meter.

***Weight Uniformity Test***

Thoroughly weigh 10 capsules, one at a time, identify each capsule, remove the contents of each capsule in an appropriate manner.

***Destroyed Time Test***

A total of 6 capsules, inserted in each tube in the basket, under which there is a 10 mesh steel mesh. Use medium water temperature of 370C. Observation of the capsules, all capsules must be crushed, except for part of the capsule shell.

***Hygroscopicity Test***

A total of 3 capsules were 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.

**Eksperimental Animal Preparation**

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

**Antidiarrheal Effectiveness Test**

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

After being given Oleum ricini as much as 0.75 ml per head as an inducer, left for 30 minutes, then given the test material orally according to each group of animals. Then 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 Simplicia** | **Phytochemical Screening Results** |
| Alkaloids | + | Mayer: Dark ChocolateDraendroff: Muddy ChocolateBouchardat: Cloudy Yellow |
| Saponins | + | White Foam |
| Flavonoids | + | Red Orange |
| Tannins | + | Blackish Green |
| Glycoside | + | Glycon: Purplish brown/purple in the middleAglycone : Reddish Purple |
| Anthraquinone glycosides | - | Pink/Red the |
| Cyanogenic Glycoside | - | Blood red on filter paper |
| Steroids | + | Green |

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

Table 2. Post Tukey Test Results Fecal Weight when Giving Avocado Seed Methanol Extract Sustenance

|  |  |  |
| --- | --- | --- |
| **Treatment** | **N** | **Subset for alpha=0.05** |
| **1** | **2** | **3** |
| Dosage 800 mg | 3 | 74,0000 |  |  |
| Loperamide HCl | 3 | 84.6667 |  |  |
| Dosage 400 mg | 3 |  | 131.6667 |  |
| Dosage 200mg | 3 |  |  | 212.3333 |
| Na\_CMC | 3 |  |  | 223.6667 |
| Sig. |  | .870 | 1,000 | .844 |

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 400 mg | 3 | 9.6667 |
| Dosage 800 mg | 3 | 100000 |
| Loperamide HCl | 3 | 10.3333 |
| Dosage 200mg | 3 | 10.3333 |
| Na\_CMC | 3 | 12.3333 |
| Sig. |  | .299 |

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

|  |  |  |
| --- | --- | --- |
| **Treatment** | **N** | **Subset for alpha=0.05** |
| **1** |
| Loperamide HCl | 3 | .2867 |
| Dosage 800 mg | 3 | .3933 |
| Dosage 200mg | 3 | .4333 |
| Dosage 400 mg | 3 | .4600 |
| Na\_CMC | 3 | .5433 |
| Sig. |  | .436 |

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

Table 5. Results of the Post Tukey Test on Diarrhea when Giving Avocado Seed Methanol Extract Capsules

|  |  |  |
| --- | --- | --- |
| **Treatment** | **N** | **Subset for alpha=0.05** |
| **1** | **2** | **3** |
| Dosage 75 mg | 3 | 74,0000 |  |  |
| Loperamide | 3 | 96.0000 |  |  |
| Dosage 50 mg | 3 |  | 133.0000 |  |
| Dosage 25mg | 3 |  |  | 211.00000 |
| Na\_CMC | 3 |  |  | 222.0000 |
| Sig. |  | .310 | 1,000 | .838 |

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

|  |  |  |
| --- | --- | --- |
| **Treatment** | **N** | **Subset for alpha=0.05** |
| **1** |
| Loperamide HCl | 3 | 8.0000 |
| Dosage 800 mg | 3 | 8.0000 |
| Dosage 200mg | 3 | 90000 |
| Dosage 400 mg | 3 | 100000 |
| Na\_CMC | 3 | 120000 |
| Sig. |  | .211 |

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

|  |  |  |
| --- | --- | --- |
| **Treatment** | **N** | **Subset for alpha=0.05** |
| **1** | **2** |
| Loperamide HCl | 3 | .2100 |  |
| Dosage 800 mg | 3 | .3400 |  |
| Dosage 200mg | 3 |  | .3600 |
| Dosage 400 mg | 3 |  | .4000 |
| Na\_CMC | 3 |  | .6300 |
| Sig. |  | .581 | .221 |

**Discussion**

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 has been known to have the potential to cause diarrhea6. Tannin compounds are an astringent which can help stop diarrhea7. Flavonoids as antidiarrhea with the mechanism of action of inhibiting intestinal motility so as to reduce fluid and electrolytes. Another flavonoid activity (quercetin) is by inhibiting the release of acetylcholine in the channel8.

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. In the table there is a change in taste, this occurs due to a fermentation reaction. Fermentation is the main energy-producing process of various microorganisms.

The pH test results were tested by shelf-life, in the suspension formulation with the suspending agent CMC Na, the pH decreased from 4.75 to 3.83 during the storage cycle, the optimum suspension pH was 5-6, the pH of the preparation was acidic due to the presence of additives (preservatives). Used in the form of benzoic acid with a pH <4.5 so that it can affect the pH of the preparation9. Meanwhile, the results of the specific gravity measurement showed a change in the specific gravity of the suspension before the accelerated storage conditions and after the accelerated storage conditions from 1.03 to 1.02 which met the suspension specific gravity requirements, namely >1.00 g/ml.

Based on the results of statistical tests on the observation of the initial time of diarrhea with ANOVA testing, a significant value was obtained 0.366 (p> 0.05), then Ho was accepted, meaning that the five treatments had unequal variances (not homogeneous) which can be concluded to accept Ho, which means the average the value of diarrhea in each of these treatments was not significantly different10.

The decision making of the Tukey method can be done by looking at the placement of the statistical test results in the test results column, the results of the test for the duration of diarrhea at a dose of 800 mg are in the same column as loperamide which concludes that the diarrhea value in each treatment is not significantly different. (Significant), while at a dose of 200 mg, a dose of 400 mg and Na-CMC are in different columns, this means that the diarrhea value in each treatment is significantly different (Significant) to the positive control group.

The statistical test results in the test results 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 are in the same column, this concludes that the value of diarrhea in each of these treatments is not significantly different. real (Significant). The statistical test results in the test results column, the results of the loperamide stool weight at a dose of 800 mg, a dose of 200 mg, a dose of 400 mg and Na-CMC are in the same column, this concludes that the value of diarrhea in each of these treatments is not significantly different. (Significant). The statistical test results in the test results column, 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 are in the same column, this concludes that the diarrhea value in each of these treatments is not significantly different. real (Significant).

The statistical test results in the test results column, the results of the 75 mg dose of feces and loperamide are in the same column, which means that in each treatment it is not significantly different (Significant), but significantly different to the 25 mg dose, 50 mg and Na-CMC, while the 50 mg dose, 25 mg dose and Na-CMC are in the same column, this concludes that the diarrhea value at 50 mg dose, 25 mg dose and Na-CMC is not significantly different Significant). The statistical test results in the test results column, 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 are in the same column, this concludes that the diarrhea value in each of these treatments is not significantly different. real (Significant).

The statistical test results in the test results column, the results of the 75 mg dose of feces and loperamide are in the same column, which means that in each treatment it is not significantly different (Significant), but significantly different to the 25 mg dose, 50 mg and Na-CMC, while the doses of 25 mg and Na-CMC are in the same column.

**Conclusions**

Based on the description of the results and research, it is known that the results of the study indicate that avocado seeds contain alkaloid compounds, flavonoids, glycosides, saponins, the extract yield obtained is 17.23% and the water content is 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 result of the average formula specific gravity measurement is 1.03 before accelerated storage and after accelerated storage is 1.02. The result of the viscosity measurement is 199.8, while the result of the sedimentation volume measurement is 0.76; 0.8; 0.8. %. The administration of a suspension of avocado seed methanol extract at a dose of 800 mg/KgBW 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. the test results that occurred were slightly different between the dose of 800 mg/kgBB 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 are 120 mg. The results of the average disintegration time of 3 minutes, the formula was destroyed and the results of the hygroscopicity test on the three formulas were relatively stable. Administration of avocado seed extract capsules at a dose of 75 mg/KgBW had the most optimum effect as an antidiarrheal against white male mice. The results of the average disintegration time were 3 minutes, the formula was destroyed and the results of the hygroscopicity test on the three formulas were relatively stable. Administration of avocado seed extract capsules at a dose of 75 mg/KgBB had the most optimum effect as an antidiarrheal against white male mice. The results of the average disintegration time were 3 minutes, the formula was destroyed and the results of the hygroscopicity test on the three formulas were relatively stable. Administration of avocado seed extract capsules at a dose of 75 mg/KgBB had the optimum effect as antidiarrheal against white male mice.

**Acknowledgement**

The researcher would like to thank the Institute for Research and Community Service, Tjut Nyak Dhien University, Medan for all their support in this research.

**References**

1. Artaya P. Uji Anova Maternal Health Book. Surabaya: Naratoma University Press; 209AD.

2. Suena NMDS. Evaluasi Fisik Sediaan Suspensi Dengan Kombinasi Suspending Agent Pga (Pulvis Gummi Arabici) DAN CMC-Na (Carboxymethylcellulosum Natrium). J Ilm Medicam. 2015;1(1):33–8.

3. Departemen Kesehatan RI. Farmakope Indonesia. Edisi III. Jakarta: Departemen Kesehatan RI; 1971.

4. Ansel H. Pengantar Bentuk Sediaan Farmasi, Edisi IV. Jakarta: Universitas Indonesia; 1989.

5. Fajar IR., Cahyo H. Uji Aktivitas Ekstrak Etanol Daun Sawo Manila ( Manilkara Zapota L ) Sebagai Antidiare Terhadap Mencit Putih Jantan ( Mus musculus ). IONTech. 2020;01(01):17–25.

6. Fatmawati U. Formulasi Suspensi Analgesik-Antipiretik Ibuprofen Dengan Suspending Agent Gom Arab Dan Cmc-Na. J Pharm Care Anwar Med. 2018;1(1):35–48.

7. Halimah AD. Pengolahan Limbah Biji Alpukat untuk Pembuatan Dodol Pati Sebagai Alternatif Pengobatan Ginjal. J Ilm Mhs Undip. 2014;4(1):33–4.

8. Malangngi L, Sangi M, Paedong J. Penentuan Kandungan Tanin dan Uji Antioksidan Ekstrak Biji Buah Alpukat (Persea americana Mill.). J MIPA. 2012;1(1):5–10.

9. Wirasti, Ulfah F, Slamet. Karakterisasi Sediaan Suspensi Nanopartikel Ekstrak Etanol Daun Afrika ( Vernonia amygdalina Del .) Cendekia J Pharm. 2020;4(2):138–48.

10. Zulkifli J, Mukhlis R. Test Of Effect Antidiare Extract Leaf Prasman (Eupatorium Triplinerve Vahl.) On Rats Of Wistar Train (Rattus Norvegicus). Maj Farm. 2017;14(1):18.