SPIN 7 (1) (2025) 22-28

SPIN

JURNAL KIMIA & PENDIDIKAN KIMIA

https://journal.uinmataram.ac.id/index.php/spin

EFFECT RATIO OF RAW MATERIALS AND SOLVENTS EXTRACTION OF AVOCADO SEED (*PERSEA AMERICANA*) FOR NATURAL PESTICIDES

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DOI: 10.20414/spin.v7i1.12685

History Article
Accepted:
Jan 11, 2025
Reviewed:
May 23, 2025
Published:
July 10, 2025

Kata Kunci:
Esktrak biji alpukat, pestisida alami, rasio bahan baku dan pelarut

Keywords:
Avocado seed extract, natural pesticides, ratio of raw materials and solvents

ABSTRAK

Hingga saat ini serangga pengganggu tanaman masih menjadi tantangan dalam semua jenis pertanian. Pestisida alami adalah bahan alam yang dapat digunakan untuk mengendalikan hama tersebut. Salah satu bahan alam yang bisa dimanfaatkan adalah ekstrak biji alpukat. Biji alpukat mengandung senyawa metabolit sekunder seperti flavonoid, fenolik, tanin, dan saponin yang dapat berfungsi sebagai racun pada sistem pencernaan serangga. Penelitian ini bertujuan untuk mengetahui pengaruh rasio volume pelarut tehadap masa bahan baku yang digunakan dalam proses ekstraksi biji alpukat. Pada penelitian ini bahan baku yang digunakan adalah 30 gram dengan variasi rasio volume pelarut dan massa bahan baku sebesar (1:25), (1:30), (1:35), (1:40), dan (1:45). Pestisida nabati yang dihasilkan diuji efektivitasnya menggunakan LD_{50} terhadap kematian jangkrik. Pada penelitian ini diperoleh hasil uji skrining fitokimia yang dilakukan pada setiap variasi volume massa bahan baku mengidentifikasi adanya senyawa alkaloid, fenolik tanin, dan saponin. Hasil analisa toksisitas LD_{50} terendah pada rasio volume pelarut dan bahan baku (1:40) dan (1:45) yaitu 707,9 mg/kgBB dengan tingkat toksisitas kategori sedang.

ABSTRACT

Until now, plant pests have been a challenge in all types of agriculture. Natural pesticides are natural ingredients that can be used to control these pests. One natural ingredient that can be used is avocado seed extract. Avocado seeds contain secondary metabolite compounds such as flavonoids, phenolics, tannins, and saponins, which can function as toxins in the digestive system of insects. This research aims to determine the effect of the solvent volume ratio on the mass of the raw material used in the avocado seed extraction process. In this study, the raw material used was 30 grams with varying ratios of solvent volume and raw material mass, specifically (1:25), (1:30), (1:35), (1:40), and (1:45). The resulting botanical pesticide was tested for effectiveness using the LD_{50} against cricket mortality. In this study, the results of phytochemical screening tests carried out on each variation in mass volume of raw materials identified the presence of alkaloids, phenolic tannins, and saponins. The lowest LD_{50} toxicity analysis results were in the solvent and raw material volume ratio (1:40) and (1:45), namely 707.9 mg/kgBW with a medium category toxicity level.

How to Cite

Rozy, W. F., Amborowati, C., & Syamsiah, S. (2025). Effect Ratio of Raw Materials and Solvents Extraction of Avocado Seed (Persea Americana) for Natural Pesticides. *SPIN-Jurnal Kimia & Pendidikan Kimia*. 7(1). 22-28.

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p-ISSN: 2580-2623
e-ISSN: 2745-6854

INTRODUCTION

Pesticides are substances used to control various types of plant pests. Previously, humans used natural pesticides to control these pests, but after the discovery of dichloro diphenyl trichloroethane (DDT) in 1939, the use of natural pesticides began to decrease gradually and switched to synthetic pesticides. The use of chemical-based pesticides is considered more effective in controlling pests but has a negative impact on the environment and humans. Therefore, other safer options are needed that do not cause negative effects such as the use of pesticides derived from plants that are environmentally friendly.

Botanical pesticides are pesticides derived from plants. Botanical pesticides are more environmentally friendly because they are more easily decomposed in nature, so they do not cause pollution and are safer for humans and livestock. Natural pesticides function as poisons in the digestive system of insects. One of the plants that can be used in making botanical pesticides is avocado seeds. Based on BPS data in 2022, the amount of avocado production in East Kalimantan is quite large, namely 638,000 tons. Avocados consist of 65% fruit flesh (mesocarp), 20% seeds (endocarp), and 15% fruit skin (pericarp) (Risyad et al., 2016). So that in 2022, 127,000 tons of avocado seeds were produced. The avocado seeds produced have not been utilized properly and have only become waste.

Avocado seeds contain secondary metabolite compounds that can eradicate plant pests. The results of phytochemical screening conducted by Azzahra et al. (2022) showed that ethanol extract from avocado seeds contains various compounds such as polyphenols, alkaloids, flavonoids, saponins, and tannins (Azzahra et al., 2022). Another study conducted by Tarigan (2019) revealed that avocado seed extract had a significant effect on larval mortality, repellency, and the success of pupae becoming imago P. *xylostella* at a concentration of 8,000 ppm (Tarigan, 2019). At a concentration of 8,000 ppm, avocado seed extract can cause larval mortality of 87.84%.

During the process of making botanical pesticides, there are several factors that influence it, one of which is the ratio of solvent volume to raw material mass in the extraction process. The ratio of solvent volume to raw material mass will affect the amount of secondary metabolites that will be taken so that it will affect the bioactivity of the resulting extract. This was revealed through research conducted by Jafar (2018) on the manufacture of natural pesticides using tuba plants (*Derris elliptica*) using ultrasonic extraction. In this study, ultrasonic extraction was used to accelerate the process of extracting compounds that function as natural pesticides (Jafar et al., 2020). Furthermore, the purification process was carried out using a vacuum filter for one hour. The solvents used during extraction were 96% ethanol and methanol with variations in the ratio of solvent volume to raw material mass (1:10), (1:15), and (1:20). The time variations used were 30, 60, and 90 minutes at a temperature of 60°C. The LD_{50} value of the tuba plant root extract tested on crickets with the best results was using ethanol solvent at a solvent volume-raw material mass ratio (1:20) at 90 minutes, with an LD_{50} of 4922.34 mg/kgBW. Based on this, this study carried out avocado seed extraction using variations in the solvent volume ratio to the raw material mass using the maceration method, which was associated with the active compound content of the extract and the LD_{50} value obtained from testing on cricket pests as experimental subjects.

METHODS

Materials

The materials used in this study were avocado seeds, 96% ethanol, crickets, distilled water, dragendrof solution, Liberman-Burchard solution, 10% $FeCl_3$ solution, magnesium powder, concentrated HCl, glacial acetic acid, and H_2SO_4.

Tools

The tools used were a 1000 mL measuring flask, digital scale, spray bottle, blender, stirring rod, filter paper, 500 mL Erlenmeyer flask, 500 mL beaker, dropper pipette, condenser, thermometer, distillation flask, elbow, and flask lid.

Procedure

Avocado seeds are washed using clean water, then drained for 5 minutes and cut into pieces. Then the avocado seeds are dried in the sun for 5 days. After that, the avocado seeds are ground using a blender. Then the avocado seed powder is weighed as much as 30 grams. Then, 96% ethanol is added with a solvent volume-raw material mass ratio of (1:25), (1:30), (1:35), (1:40), and (1:45). Then, maceration is carried out for 3 days. Then filtered using filter paper. After that, it is evaporated at a temperature of 65-70˚C until a thick extract is obtained. The thick extract obtained is then tested for phytochemistry to determine the content of alkaloids, triterpenoids and steroids, phenolics, flavonoids, saponins, and tannins. The thick extract is tested for toxicity using crickets as test animals. Avocado seed extract samples were made with various concentrations, namely 0 ppm, 500 ppm, 1,000 ppm, 2,000 ppm, and 4,000 ppm, then put into a spray bottle. Crickets were put into the container and given a little food. The prepared sample was sprayed onto the cricket food with a frequency of 20 sprays. Then the number of dead crickets was counted to determine the LD_{50} value, which is associated with its toxicity.

RESULT AND DISCUSSION

Effect of Solvent Volume-Raw Material Mass Ratio

The method used for extraction is maceration. This method was chosen because the process is quite easy to do, does not require special treatment, can use a large number of samples, and does not go through a heating process. Ethanol is used as a solvent in the extraction process because it is a universal solvent and can dissolve polar, semi-polar, and non-polar compounds. There are several factors that make ethanol very commonly used as a solvent, namely, it has a lower toxicity level when compared to acetone and methanol, is more affordable, can be applied to various types of extraction methods, and is safe for extracts that will be processed into medicines (Karepu et al., 2020).

The yield obtained for each variation of solvent volume-raw material mass can be seen in Table 1 below.

Table 1. Avocado seed extract yield value

Sample	Concentrated extract mass (grams)	Yield (%)
1:25	10,23	34,11
1:30	12,04	40,13
1:35	13,83	46,08
1:40	12,24	40,18
1:45	13,99	46,64

The highest yield at a ratio of (1:45) is 46.64% and the lowest yield at a ratio of (1:25) is 34.11%. The addition of solvent volume affects the amount of interaction between the solid and the solvent; the more solvent is added, the greater the interaction, which causes the solvent to distribute more to the solid. An even distribution of the solvent will increase the resulting yield. An increase in the amount of solvent will reduce the level of solvent saturation, so that the metabolite compound can be extracted properly. The extraction process will continue until equilibrium is reached in concentration. The active substance will dissolve due to the concentration difference between the active substance solution inside and outside the cell, causing the solution with the higher concentration to be pushed out. The addition of solvent can increase the ability of solvent diffusion into cells and increase the release of active compounds from within cells.

Phytochemical Test

Phytochemical tests aim to determine the group of secondary metabolite compounds contained in avocado seed extract. Phytochemical tests were carried out on each variation of the volume of the avocado seed extract solvent. Phytochemical tests carried out included tests for alkaloids, flavonoids, steroids, triterpenoids, phenolics, saponins, and tannins. The results of phytochemical tests on each variation of the solvent-mass ratio of avocado seed extract raw materials showed positive results in the alkaloid, phenolic, saponin, and tannin tests. In contrast, negative results were shown in the triterpenoid, steroid, and flavonoid tests. The results of the phytochemical test are presented in Table 2 below.

Table 2. Phytochemical test of avocado seed extract

Test	Result	details
Alkaloid	+	Orange precipitate formed
Terpenoids and Steroids	-	No color change occurs
Phenolic	+	A green solution is formed
Flavonoid	-	No color change occurs
Saponin	+	Foam is formed
Tannin	+	There is a greenish color change

Details:
(+) = There are secondary metabolite compounds
(-) = There are no secondary metabolite compounds

Alkaloid testing on avocado seed extract can be done with Dragendorff's solvent. The results of this test show the formation of orange precipitate, indicating the presence of alkaloid compounds, with the reaction mechanism in Figure 1 below:

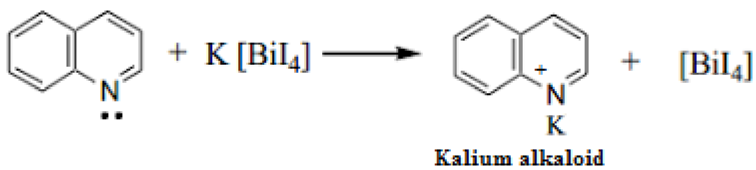

Figure 1. Dragendorff reagent reaction with alkaloid compounds (Nugrahani et al., 2016)

Figure 1 shows the result of the reaction between alkaloid compounds and Dragendorff's reagent. The results of testing alkaloids on all solvents showed positive results for Mayer's reagent, Wagner's reagent, and Dragendorff's reagent. The principle of the precipitation reaction that occurs in alkaloid testing is due to ligand precipitation. Mayer's reagent in the alkaloid test contains K^+ metal ions from potassium tetraiodomercurate (II), which forms a potassium-alkaloid complex, resulting in a precipitate. Testing with Wagner's reagent forms a brown precipitate because the nitrogen atom, which has a free electron pair in the alkaloid compound, can replace the iodide ion (I^-) in the reagent. Testing with Dragendorff's reagent is characterized by the formation of an orange precipitate. The precipitate is potassium alkaloid. Alkaloid compounds using Dragendorff's reagent will form a tetraiodobismuth salt, which is orange to reddish brown (Azzahra et al., 2022).

In the triterpenoid and steroid tests, negative results were shown. This can be seen in the extract samples that did not experience a color change. This shows that there is no reaction in the avocado seed extract to the Liberman-Burchard solution. The reaction did not occur or did not experience a color change when using the Liberman-Burchard solution, indicating that the avocado seed extract does not contain triterpenoid and steroid compounds.

Phenolic testing of avocado seed extract using 10% $FeCl_3$ solution produces a green solution, indicating the presence of phenolic compounds, with the reaction mechanism shown in Figure 2 below:

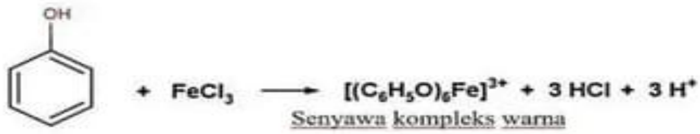

Figure 2. Reaction of $FeCl_3$ with phenolic compounds (Xia et al., 2010)

The green color is caused by the presence of $FeCl_3$ reacting with the hydroxyl group in the phenol compound. The presence of phenol compounds in avocado seed extract causes a color change. Based on these results, it can be indicated that avocado seed extract contains phenolic compounds.

The flavonoid test showed negative results; this can be seen in the extract sample, which did not experience a color change. This indicates that there is no reaction between the avocado seed extract and magnesium metal. Based on these results, it can be indicated that avocado seed extract does not contain flavonoid compounds.

In the saponin test, the method used is shaking, so that after shaking, the avocado seed extract produces foam on its surface. When HCl is added, the foam formed appears to remain stable, indicating the presence of saponin compounds, as shown in Figure 3 below.

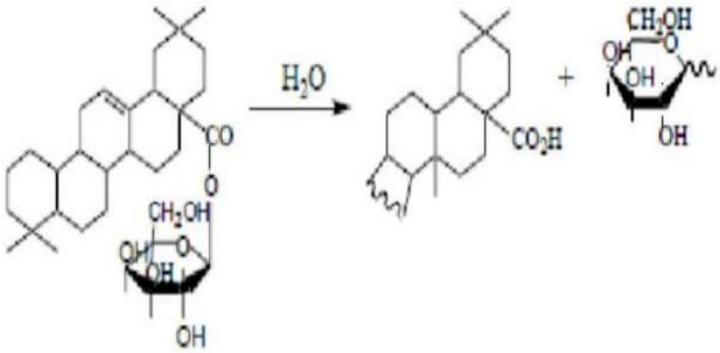

Figure 3. HCl reaction to saponin compounds (Marliana et al., 2005)

In the tannin test using $FeCl_3$, avocado seed extract is reacted with $FeCl_3$ to produce a blackish green or dark blue solution (Jafar, 2020). This indicates the tannin compound in avocado seed extract based on the reaction shown in Figure 4 below:

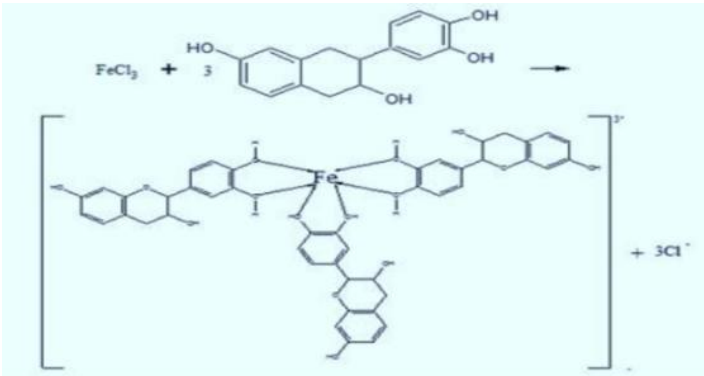

Figure 4. Reaction of $FeCl_3$ to tannin compounds (Taminggu & Tahril, 2022)

In the tannin test using $FeCl_3$ reagent, a positive result is indicated by a color change after being dripped with the reagent to a greenish brown color. This is due to the presence of oxidized phenolic compounds. Phenolic compounds in the sample are estimated to be tannin compounds. The change to a blackish-green or ink-blue color in the sample, when dripped with $FeCl_3$ reagent, is due to the formation of a complex compound between tannin and Fe^{3+} ions.

Toxicity Test

Toxicity test using the LD_{50} method with crickets, an avocado seed extract solution was prepared at concentrations of 0, 500, 1,000, 2,000, and 4,000 ppm for each variation of the solvent ratio to the mass of raw materials at the time of extraction. The solution was tested with ten crickets for one day. After one day, the number of crickets that died in each sample was observed, and the percentage of deaths obtained was calculated. The rate of fatalities obtained was related to the LD_{50} value of the Pharmacopoeia equation, each of which can be classified as toxic.

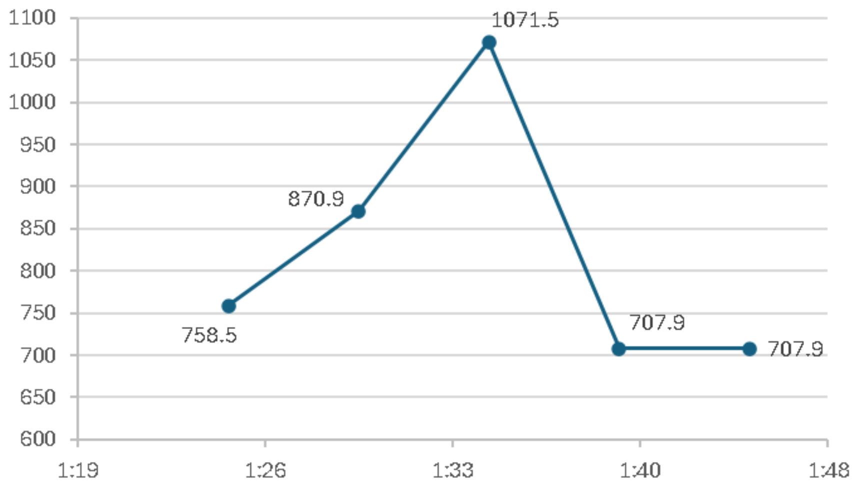

Figure 5. Graph of the relationship between solvent volume and toxicity

In Figure 5, it can be seen that the greater the ratio of solvent volume-raw material mass used, the greater the solvent's ability to absorb active compounds from avocado seeds. The effect of LD_{50} toxicity (lethal dose 50%) shows the lower level of toxicity of a substance, where the lower LD_{50} value indicates a higher level of toxicity. In this study, the ratio of solvent volume to raw material mass with the lowest LD_{50} value was (1:40) and (1:45). This shows that the ratio of solvent volume to raw material mass (1:40) and (1:45) has the highest toxicity. The greater the volume of solvent used, the more secondary metabolite compounds are extracted, which will increase their toxicity. The results of avocado seed extract in all variations of solvent volume-raw material mass showed moderate toxicity. Based on this, it shows the toxicity contained in avocado seed extract, which can be used as a natural pesticide. The LD_{50} value for all variations of solvent-raw material mass ratio used during extraction also includes moderate toxicity.

CONCLUSION

From the results of the study on the use of avocado seed waste as a raw material for making natural pesticides, it can be concluded that the highest yield was obtained at the ratio of solvent volume and raw materials (1:45), with a yield of 46.64%. The results of the phytochemical screening test carried out on each variation of the mass volume of raw materials identified the presence of alkaloids, phenolics, tannins, and saponins. The results of the lowest LD_{50} toxicity analysis at the ratio of solvent volume and raw materials (1:40) and (1:45) were 707.9 mg/kgBW, in the results of the study that had been conducted using avocado seed raw materials, these results showed the presence of toxicity properties with a moderate level of toxicity that can be used as a natural pesticide.

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