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# Effect of Solvent Differences on Flavonoid Levels and Antioxidant Activity of Hass Avocado Peel (Persea Americana Hass)

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## Abstract

*The Hass avocado fruit (Persea Americana Hass) comes from Australia. The color of the Hass avocado skin when it is still raw is green, and when it is ripe, it is black. This study aims to see the effect of different solvents in determining the levels of flavonoids and the antioxidant activity of Hass avocado peel extract. Determination of flavonoid levels using UV-Vis spectrophotometry and for antioxidant activity using 2,2-diphenyl-1-pikrihydrazyl (DPPH) as a source of free radicals, and vitamin C as a positive control, and UV-Vis spectrophotometry to measure the absorbance of Hass avocado peel extract. The results showed that the flavonoid content in the ethanol extract of avocado peel was 2.078 mg/100 mg extract, and for the methanol extract of Hass avocado peel was 1.995 mg/100 mg extract. For the antioxidant activity test, the $IC_{50}$ value of the ethanol extract of avocado peel was 134.181 mg/L, while the $IC_{50}$ value of vitamin C was 19.815 mg/L. For the methanol extract of Hass avocado rind, the $IC_{50}$ value was 149.611 mg/L, while for vitamin C, the $IC_{50}$ value was 37.093 mg/L. Ethanol extract and methanol extract of Hass avocado peel (Persea Americana hass) are classified as moderately strong antioxidants based on $IC_{50}$ values.*

**Keywords:** Flavonoids, antioxidants, hass avocado peel, DPPH, UV-Vis spectrophotometry

## Introduction

The avocado (Persea Americana Hass), particularly the Hass cultivar, has become one of the most widely consumed fruits globally due to its creamy texture, rich flavor, and superior nutritional profile. Hass avocados are frequently consumed as functional foods, both fresh and in nutraceutical products, owing to their high content of monounsaturated fats, dietary fiber, vitamins (K, E), phytosterols, and carotenoids such as lutein and zeaxanthin (Ford et al., 2023).

In the past five years, growing research has focused on the bioactive compound dynamics in avocados during ripening stages. A recent study identified and quantified the phytochemical profiles of Hass avocados across ripening phases using multiple analytical methods. Beyond the edible pulp, industrial byproducts such as peels and seeds have been recognized as a rich source of phenolics and flavonoids with significant antioxidant and antimicrobial activities. For instance, peel extract of Hass avocado demonstrated antimicrobial activities against plant pathogens such as Verticillium and Colletotrichum through DPPH and ORAC analysis. Meanwhile, the seeds contain valuable phytochemicals, including tannin, catechins, chlorogenic acid, and ferulic acid, with promising neuroprotective and antimicrobial potential (Ford et al., 2023).

The flavonoid content in avocado peel (Persea Americana) is higher than in avocado leaves. According to a study by Prasetyo et al. (2022), secondary metabolites such as flavonoids in plants have various important biological activities, including antioxidant, antibacterial, and anticancer properties. Furthermore, other studies have also shown that flavonoids can inhibit the growth of Candida Albicans, a type of opportunistic pathogen in humans (Purwanto et al., 2024).

An antioxidant's primary function is to reduce oxidation in oils or fats and neutralise free radicals (Sari & Sari, 2023). It is known that antioxidants can be found in foods such as vegetables, fruits, and spices, allowing humans to consume these foods as natural sources of antioxidants (Septian et al., 2022).

The efficiency of flavonoid extraction is highly influenced by the type of solvent used. Polar organic solvents such as ethanol and methanol are commonly employed due to their ability to solubilize a broad range of phenolics and flavonoid compounds. Nevertheless, these two solvents differ significantly in polarity, toxicity, and extraction capacity. Methanol with higher polarity is often reported to extract a greater quality of flavonoids. However, ethanol polarity in concentration 70-90 % is generally preferred for food and pharmaceutical

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applications due to its lower toxicity and better regulatory approval (Febriyanti et al., 2024).

Experimental evidence from recent studies highlights the varying effects of ethanol and methanol on extraction outcomes. Febriyanti et al. (2024) report that the use of 96 % ethanol yielded a significantly higher total flavonoid content (13.468%) compared to methanol (10.515%) in clove pod extract ($p<0.001$) (Febriyanti et al., 2024).

Moreover, the extract's antioxidant activity, typically assessed using assays such as DPPH, also varies with solvent choice. Xiang et al. (2024) in the study of Camellia polyodonta flowers showed that extraction with 70 % methanol and 70% ethanol yielded the highest total flavonoid content (421.62 mg Re/g DW). However, the strongest antioxidant activity (DPPH, ABTS, FRAP assays) was observed with 70% methanol and 100% solvents.

In extracting flavonoids, the solvent used greatly affects the quality of the flavonoids, affecting the antioxidant activity. The chromatogram pattern and yield of a study are influenced by the level of solvent polarity in the extraction process (Rizki & Ferdinan, 2020).

Flavonoids are a group of polyphenolic compounds widely found in plants known for their strong antioxidant properties due to their ability to scavenge free radicals. To extract flavonoids effectively. The choice of solvent is crucial, particularly between ethanol and methanol. Two polar solvents are commonly used in modern phytochemical research. Studies comparing ethanol and methanol as extraction solvents across various plant materials have shown mixed results. In red Union peel, 96% ethanol produced the highest flavonoid content (958,84 QE/g) and the strongest antioxidant activity with an IC50 of 34,74 ppm, outperforming methanol (Wahyuni et al., 2022).

This study aims to further investigate the effect of solvent variation, ethanol and methanol, on the total flavonoid content and antioxidant activity of HASS Avocado Peel (Persea Americana Hass) using quantitative analysis (UV-Vis spectrophotometry) and antioxidant assays (DPPH). The results are expected to help identify the most effective solvent for extracting bioactive flavonoids for food or pharmaceutical applications.

## Methods

### *Research Tools and Materials*

The equipment to be used consists: UV-Vis spectrophotometry, dropper pipette, 5 mL measuring pipette (pyrex), test tube, digital scale, analytical scale, spatula, 250 mL erlemeyer flask (pyrex), 10 mL and 50 mL measuring flask (pyrex), shaker, funnel, filter paper, stirring rod, 100 mL measuring cylinder (pyrex), stopwatch, test tube rack, container, cuvette, watch glass, spoon, scissors, 70 mesh sieve, label paper, knife, rotary evaporator, tissue and alumunium foil.

The materials used in this study were samples of hass avocado peel extract, distilled water, absolute ethanol, absolute methanol, 10% aluminum chloride, vitamin C, 1 M potassium acetate, quercetin solution, and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

### *Sample Preparation*

The hass avocado is washed thoroughly using water. After that, the avocado is peeled, and the skin is cut into small pieces. The skin is dried in an aerated manner without exposure to direct sunlight. Then, the dried hass avocado skin is mashed using a blender and sieved into powder for further analysis (Gutiérrez, 2023).

### *Extraction*

Hass avocado peel powder was weighed as much as 5 grams each using a digital balance, then put into erlemeyer one and erlemeyer 2. Ethanol solvent was added to erlemeyer one, methanol solvent was added to erlemeyer two, up to 50 mL, and covered with aluminum foil. After that, it was shaken using a shaker machine for 2 hours at a speed of 200 rpm, then the solution was left for 1 x 24 hours. Then the solution is filtered using filter paper to separate the filtrate and residue. The residue that was formed was macerated again with the same procedure, then the filtrate formed was collected and concentrated using a rotary evaporator until no more liquid dripped, so that a thick extract was obtained from Hass avocado skin (Gutiérrez, 2023).

### *Analysis of Flavonoid Levels*

In the initial treatment, a quercetin standard solution was prepared. Weighed as much as 4 mg of standard quercetin, put it into a 10 mL volumetric flask, and added ethanol up to the mark (200 ppm quercetin mother liquor). Then a series of standard solutions of 20, 40, 60, and 80 ppm was prepared. Pipette 1 mL of each standard solution, and put it in a test tube, then add 1.5 mL of ethanol solution, 0.5 mL of 10 % aluminum chloride, 0.5 mL of 1 M potassium acetate, and 2.8 mL of distilled water. After that, the mixed solution was shaken and allowed to stand for 30 minutes. The absorbance was measured at a wavelength of 432 nm using UV-Vis spectrophotometry (Firlia & Hastuti, 2020).

The next step is to determine the level of flavonoids in the sample. Take 1 mL of the ethanol extract test solution, then add 1.5 mL of 95% ethanol, 0.5 mL of 10% aluminum chloride, 0.5 mL of 1 M potassium acetate, and 2.8 mL of distilled water. After that, it was incubated for 30 minutes. Then, the absorption at the maximum wavelength is measured using UV-vis spectrophotometry (Firlia & Hastuti, 2020). The same steps were repeated for the methanol extract sample of Hass avocado skin using methanol as the solvent.

Data analysis techniques to determine the levels of flavonoids using the formula:

$$F = \frac{C \; x \; V \; x \; 100}{m}$$

Information:
F = Flavonoid Levels (mg/100mg)
c = quercetin equivalent (mg/mL)
V = Volume (mL)
m = Sample Weight (mg)
(Theansungnoen et al., 2022).

***Antioxidant Activity Test***

For the ethanol extract of Hass avocado skin, 10 mg of DPPH was taken and dissolved using ethanol in a 10 mL volumetric flask, then the volume was made up to the mark with ethanol. 1 mL of DPPH solution was taken, then put into a 50 mL volumetric flask, and then the volume was made up with ethanol up to the mark (20 ppm blank solution). Then it is closed and homogenized, and then left for 30 minutes. Then the absorbance was measured using UV-Vis spectrophotometry at a wavelength of 517 nm. The methanol extract of Hass avocado skin, the same treatment was carried out using methanol as a solvent (Nursafitri, 2020).

Hass avocado peel extract of 10 mg each was dissolved in a 10 mL volumetric flask labeled 1 and 2, with the extract in volumetric flask 1 being an ethanol extract, and volumetric flask 2 being a methanol extract. The volume was sufficient with ethanol in the volumetric flask one and methanol in the volumetric flask two up to the mark. For the ethanol extract of Hass avocado peel: Take 10 mg of vitamin C and put it in a 10 mL volumetric flask, and then make up the volume with ethanol up to the mark. The methanol extract of Hass avocado skin, the same treatment was carried out using methanol as a solvent (Nursafitri, 2020).

A total of 0.2 mL, 0.4 mL, 0.6 mL, and 0.8 mL mother liquor from the ethanol extract of Hass avocado peel were put into a 10 mL volumetric flask. Then add ethanol up to the mark. After that, 1 mL of Hass avocado skin extract test solution was taken, then 3 mL of DPPH blank solution was added. The same treatment was carried out using methanol as a solvent for the main solution of the methanol extract of Hass avocado skin. Take as much as 0.2 mL, 0.4 mL, 0.6 mL, and 0.8 mL of the mother liquor of vitamin C. Then, put it into a 10 mL volumetric flask, and add ethanol to the mark. After that, 1 mL of vitamin C comparison solution was taken, and 3 mL of DPPH blank solution was added (Nursafitri, 2020). The methanol extract of hass avocado skin, the same treatment was carried out using methanol as a solvent.

The formula calculates the amount of antioxidant power:
abs Control = DPPH Absorbance
abs Sample = Absorbance of Test Sample
(Baliyan et al., 2022).

## Results and Discussion

The results of the study on the determination of the flavonoid content of Hass avocado peel (Persea Americana Hass) with ethanol solvent produced a flavonoid level of 2.078 mg/100 mg sample, and for the methanol extract produced a flavonoid level of 1.955 mg/100 mg sample. For antioxidant activity in Hass avocado skin (Persea Americana Hass), the $IC_{50}$ value for the ethanol extract of Hass avocado skin was 134.181 ppm. For the methanol extract of Hass avocado skin (Persea Americana Hass), the $IC_{50}$ value was 149.611 ppm. The results showed that the hass avocado peel extract with ethanol solvent produced higher levels of flavonoids and antioxidant activity than the methanol extract.

The same thing was also reported by Padmawati et al. (2020) in their research with rice hyacinth samples, namely, the levels of flavonoids and antioxidant activity with ethanol solvents were higher, 8.26 mg QE/g and 0.49 mg/mL, compared to methanol solvents, which only had a flavonoid content of 6.91 mg QE/g and an $IC_{50}$ value of 0.96 mg/mL. This shows that using ethanol as a solvent makes compounds such as phenolics in plants extracted better, because polar compounds will dissolve compounds that are also polar.

Based on the results above, antioxidant activity is affected by increasing levels of flavonoids in the ingredients, so the higher the levels of flavonoids, the higher the antioxidant activity.

***Analysis of Flavonoid Levels***

Various methods can determine flavonoid levels in herbal samples. The method recognized by the Ministry of Health of the Republic of Indonesia is UV spectrophotometry, which is based on the calorimetry principle. UV-Vis spectrophotometry measured the absorbance of the color formed.

In this research, to determine the levels of flavonoids in avocado peel samples, the study began by preparing a standard quercetin solution with concentrations of 20 ppm, 40 ppm, 60 ppm, and 80 ppm.

The concentration series is used because the method used to determine the levels of flavonoids is a method that uses the standard curve equation. To make a standard curve, several concentration series are first made to obtain a linear equation that can be used to calculate the percent content.

The total flavonoid content from Hass avocado peel (Persea Americana Hass) was determined using complementary colorimetric analysis. This is based on the principle of colour formation, which is the principle of flavonoids using the $AlCl_3$ colorimetric method, which involves the formation of a complex between aluminium chloride ($AlCl_3$) and the keto group at the C-4 position. In addition to the hydroxyl group at the adjacent C-3 or C-5 positions of flavones and flavonols, this complication reaction produces a yellow color whose intensity can be measured spectrophotometrically. It is proportional to the flavonoid concentration in the sample (Mursiany et al., 2023).

The selection of quercetin as a standard is based on the fact that it is a flavonoid belonging to the flavonol class. Due to these structural characteristics, quercetin contains a keto group at

the C-4 position and hydroxyl groups at the adjacent C-3 or C-5 position. The wavelength absorption measurement for avocado peel extract was carried out at 432 nm using UV-Vis spectrophotometry (Mursiany et al., 2023).

The standard quercetin solution curve assumes that the x-axis is the concentration of the quercetin solution and the y-axis is its absorbance. This curve will determine the total flavonoid content in Hass avocado peel samples.

Based on the results of measurements of Quercetin with ethanol solvent at a concentration of 20 ppm of 0.489, 40 ppm of 0.653, 60 ppm of 1.034, and 80 ppm of 1.578. From the measurement results, the greater the concentration of the quercetin standard solution, the greater the absorbance value obtained. The results of the standard solution obtained from the calibration curve are $y = 0.0181x + 0.0355$ with an $R^2$ value of 0.9452. The standard series of quercetin solutions with methanol solvent can be seen in the results of measurements of quercetin with methanol solvent at a concentration of 20 ppm of 0.497, 40 ppm of 0.990, 60 ppm of 1.484, and 80 ppm of 1.876. From the measurement data, the greater the concentration of the quercetin standard solution, the greater the absorbance value obtained. The results of the standard solution obtained from the calibration curve are $y = 0.0232x + 0.054$ with an $R^2$ value of 0.9971.

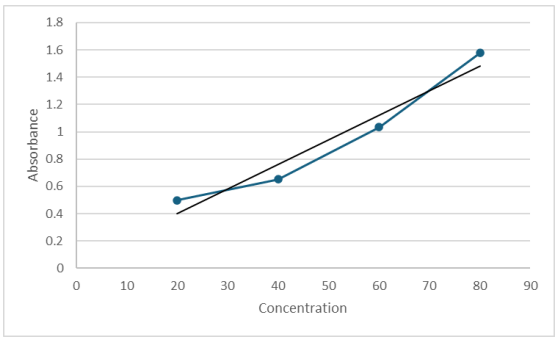


**Figure 1.** Quercetin calibration curve with ethanol solvent

Testing the analysis of flavonoid levels using UV-Vis spectrophotometry at a wavelength of 432 nm. Determining flavonoid content in a plant sample using UV-Vis Spectrophotometry is a method that utilizes the interaction of light with atoms and molecules. The incoming light that strikes the surface of a substance and the light that passes through the sample are in a ratio between the intensity of the incoming light after it passes through the sample (Purnamasari et al., 2022). So, from the results, the levels of flavonoids for the ethanol extract of Hass avocado peel can be seen based on Table 4.1. The calculation results obtained an average of 2.078 mg/100 mg of extract, and an average of 1.955 mg/100 mg of extract for the methanol extract of Hass avocado skin.

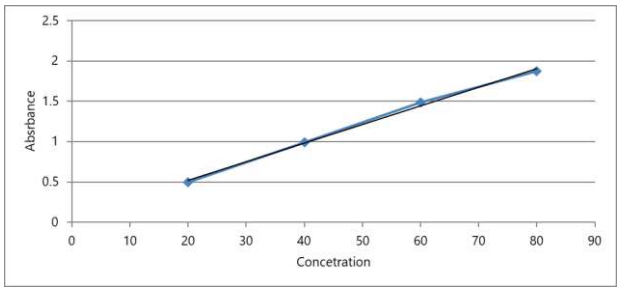


**Figure 2.** Querctin calibration curve with methanol solvent

The use of ethanol and methanol as solvents in this study is based on flavonoids being polyphenolic compounds with polar characteristics, similar to ethanol and methanol, which enable these solvents to dissolve flavonoid compounds in the sample effectively. Ethanol offers a balance between polar and non-polar properties, making it selective for flavonoids and other bioactive compounds, and it is also relatively safer. Meanwhile, with its high polarity, methanol is sometimes more effective in extracting certain Flavonoids but is considerably more toxic than the ethanol solvent (Wibisono, 2023).

The most effective concentration in this research is the solvent, 80 ppm. This is consistent with the study conducted by Dewi et al. (2024) on the research about chayote (Serhium edule), which uses various concentrations (20, 40, 60, 80 ppm) with quercetin as the standard. The result showed increased total flavonoid content as the ethanol concentration increased from 18,23% at 20 ppm to 22,40% at 80 ppm. This indicates that higher concentration yields more optimal flavonoid extraction results.

Based on the results of total flavonoid levels obtained from samples of ethanol extract and methanol extract of Hass avocado skin, there was a slight difference between the flavonoid levels of ethanol and methanol extracts. The highest level of flavonoids between the two is in the ethanol extract of Hass avocado skin. Ethanol solvent is the best for producing total phenolic content and flavonoids. It has a similar polarity level and is more effective in dissolving flavonoid compounds in Hass avocado skin.

### *Antioxidant Activity Test*

This study conducted the quantitative antioxidant activity test using the DPPH (1,1—diphenyl-2-picrylhydrazyl) method. The working principle of the DPPH method involves the presence of a hydrogen atom in antioxidant compounds that binds with the unpaired electron of free radical compounds, transforming free radicals into non-radical compounds. This is indicated by a colour change from purple to yellow (Nawawi, 2023).

The measurement of antioxidant capacity was carried out using UV-Vis Spectropotometry at 517 nm, representing the maximum absorbance wavelength for DPPH. The level of antioxidant activity is indicated by the $IC_{50}$ value, which is the concentration of the sample solution required to inhibit 50% of DPPH free radicals. The concentration variations of the sample solution used in this study were 20 ppm, 40 ppm, 60 ppm, and 80 ppm (Nawawi, 2023).

The results of measuring the absorbance of Hass avocado peel extract can be seen in the following figure:

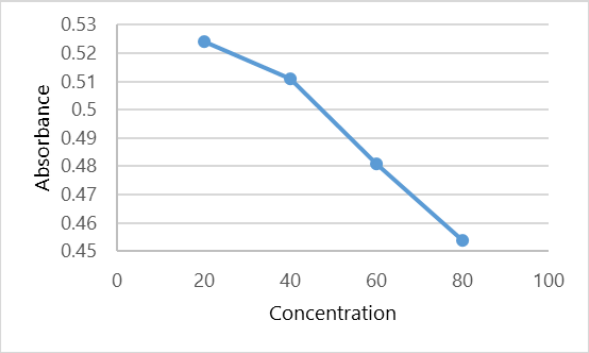


**Figure 3.** Correlation Curve of Concentration of Hass Avocado Peel Ethanol Extract with Absorbance Value

Based on the picture above, the absorbance value decreases with increasing concentration of hass avocado peel extract. This happens because antioxidants reduce DPPH radicals. The higher the concentration, the more antioxidant compound particles are contained, so the greater the antioxidant activity and the decrease in absorbance (Nawawi, 2023).

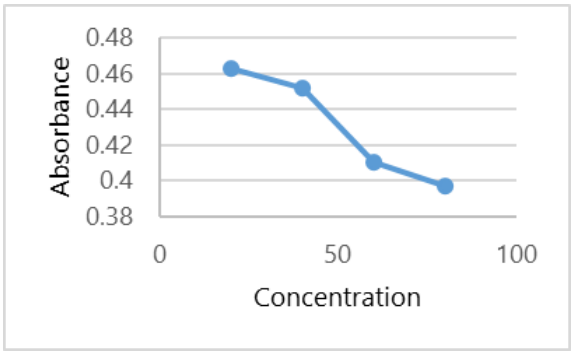


**Figure 4.** Correlation Curve of Concentration of Hass Avocado Peel Methanol Extract with Absorbance Value

Based on the picture above, the absorbance value decreases with increasing Hass avocado peel extract concentration. This happens because antioxidants reduce DPPH radicals. The higher the concentration, the more antioxidant compound particles are contained, so the greater the antioxidant activity and the decrease in absorbance (Nawawi, 2023).

Based on the test that has been carried out, it can be seen that samples that use ethanol as a solvent produce higher levels of flavonoids and antioxidants compared to samples that use methanol as a solvent. This can happen because ethanol is similar in terms of chemical compound structure and polarity level, which resembles and is more effective in dissolving the chemical compounds found in the skin of Hass avocados. Ethanol (especially in hydroethanolic solution at around 70%-80%) has an appropriate polarity for extracting antioxidant compounds such as flavonoids and phenolics. These compounds are semi-polar, too polar for methanol and too non-polar for water. Making ethanol highly efficient for extraction (Mayaringtyas & Susanti, 2023). This aligns with the research conducted by Mayaringtyas & Susanti (2023), where the ethanolic fraction of Gambir leaves showed antioxidant activity of 11,52 ± 0,05, while the metabolic fraction showed 15,25 ± 0,25. The smaller the $IC_{50}$ value, the higher the antioxidant activity.

$IC_{50}$ value in each ethanol extract and methanol extract of Hass avocado skin was determined using the linear regression equation of the relationship curve of sample concentration to percent inhibition, which is presented in the following figure:

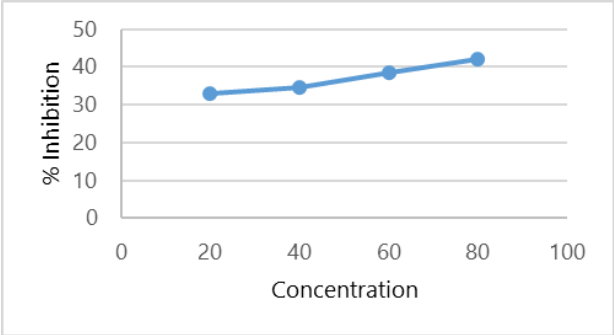


**Figure 5.** Correlation Curve of Concentration (ppm) of Hass Avocado Peel Ethanol Extract with Inhibition Percentage

The curve in Figure 5 obtained the value for linear regression y = 0.1533x + 29.43 for the ethanol extract of Hass avocado peel, and Figure 6 obtained a linear regression value of y = 0.1929x + 21.14 for the methanol extract of Hass avocado peel. It can be seen from the data contained above that the greater the concentration, the greater the percentage of inhibitors produced to inhibit DPPH radicals. The highest inhibitory percentage of each extract was found at a concentration of 80 ppm, with a value of 42.01% for the ethanol extract of Hass avocado peel and 36.17% for the methanol extract of Hass avocado peel. The $IC_{50}$ value for the ethanol extract of Hass avocado peel was 134.181 ppm, and for the methanol extract of Hass avocado peel, the $IC_{50}$ value was 149.611 ppm.

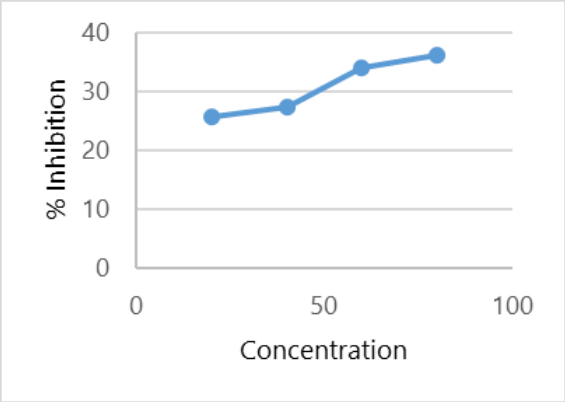


**Figure 6.** Correlation Curve of Concentration (ppm) of HASS Avocado Peel Methanol Extract with Inhibition Percentage

## Conclusions

Based on the results of the research conducted, it can be concluded that the total flavonoid content of the ethanol extract of hass avocado peel was 2.078 mg/100 mg of extract, while for the methanol extract of hass avocado peel was 1.955 mg/100 mg of extract, for the antioxidant power of the ethanol extract of hass avocado skin based on the $IC_{50}$ value obtained of 134.181 ppm (strong enough) and for the antioxidant power of the methanol extract of hass avocado skin based on the $IC_{50}$ value obtained of 149.611 ppm (strong enough). So it can be concluded that ethanol solvent is better at extracting hass avocado skin than methanol solvent.

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