

THE EFFECT OF COMPARATIVE MATERIALS AND SOLUTIONS ON THE LEVELS OF AVOCADO LEAF EXTRACT FLAVONOIDS (*Persea americana mill*)

Rismayenti¹, Tri Yuni Hendrawati¹, Ummul Habibah Hasyim^{1*}, Wan Azmi bin Wan Hamzah²

¹Department of Chemical Engineering, Department of Chemical Engineering, Faculty Of Engineering, Universitas Muhammadiyah, Jakarta, Indonesia

²Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Kuantan

*ummul.hh@umj.ac.id

Abstract.

Avocado leaves (*Persea americana mill*) contain flavonoid compounds rich in antioxidants and can be used as raw materials for making natural antibacterial hand sanitizer. This study aims to extract the content of flavonoid compounds contained in avocado leaves and get the best yield from 5 comparisons of materials and solvents as well as the highest flavonoid levels. The method used in this study was the extraction of maceration using ethanol solvents 96 % then made a comparison variation of materials and solvents (1:5, 1:10, 1:15, 1:20, 1:25 b/v) with a length of soaking of the material for 24 hours. The obtained maceration results are filtered and glued with rotary vacuum evaporator. The extract is then analyzed flavonoid levels with kuersetin as a standard comparison that has been known with certain flavonoid content. In this study obtained the best yield on the comparison of dried avocado leaves and ethanol 1:25 which is 6,267 %. The highest total flavonoid content obtained in the comparison of dried avocado leaves and ethanol 1:5 is 3.1724 mgQE/gram extract with the equation $y = 0.07173x - 0.00213$, and $R^2 = 0.99998$.

Keywords: ramie, cellulose, nitration, nitrocellulose

Introduction

Indonesia is a tropical country that is rich in various plants that have many benefits. Almost all parts of the plant can be used for various needs. One of the plants that are rich in benefits is the avocado plant. Avocado is a fruit plant that belongs to the Lauraceae family which is easy to find, especially in highland areas with high rainfall. The part of this plant that is often used is avocado fruit or flesh as various preparations that are rich in protein, fat and vitamins. In addition, avocado leaves and seeds also have benefits that are no less far than the fruit. Avocado leaves and seeds are often used as traditional medicine (Duarte et al., 2016).

In this study, the authors used avocado leaves as a raw material for making natural antibacterial hand-sanitizers. Avocado leaves contain bioactive components such as flavonoids, phenols, saponins, tannins and alkaloids. The compounds with the highest content in avocado leaves are flavonoids (Arukwe et al., 2012).^{369 / 5.000} Flavonoids are polar compounds because they have a number of unsubstituted hydroxyl groups. These flavonoid compounds can be used as anti-microbial, wound infection medicine, anti-fungal, anti-viral, anti-cancer, and anti-tumor. In addition, flavonoids can also be used as anti-bacterial, anti-allergic, cytotoxic, and anti-hypertensive (Sriningsih, 2008).

Method

This research was conducted at the Chemical Engineering Laboratory, Faculty of Engineering, University of Muhammadiyah Jakarta for 3 months. The materials used include: avocado leaves, 96% ethanol, HCl, aquades, Mg powder, quercetin standard, AlCl₃, CH₃COONa, whatman filter paper, and aluminum foil. The tools used include: blender, analytical balance, glass bottles 500 and 700 ML, funnel, 500 mL erlenmeyer, rotary evaporator, oven, evaporating dish, desiccator, water bath, test tube, watch glass, measuring cup 50 mL and 500 mL, 250 mL beaker glass, 10 mL measuring pipette, 1, 2, 3, 4 and 5 mL volumetric pipettes, dropper pipettes, 10, 25, 50 mL volumetric flasks, and spray bottles.

The stages of Avocado Leaf Extraction and Yield Obtaining were carried out in several stages, including: the avocado leaf refining process was carried out by cleaning the avocado leaves with water and then drying them by aerating. The dried avocado leaves are mashed using a blender. Avocado leaf extraction was carried out with a mass of 20 grams and added 96% ethanol solvent, then the solution was put into a glass bottle. Repeat 5 times. Add 96% ethanol solvent.

Table 1. Comparison of 96% ethanol solvent

Ratio	Solvent (mL)
1 : 5	100
1 : 10	200
1 : 15	300
1 : 20	400
1 : 25	500

The entire side of the glass bottle is wrapped with aluminum foil to prevent oxidation. Then let stand for 24 hours at room temperature while occasionally shaking. The solution was filtered through Whatman filter paper until a clear solution was obtained. Concentration and drying of avocado leaf extract was carried out by evaporating the filtrate with the solvent using a rotary evaporator. This process is stopped when there is no more condensed solvent. The liquid extract obtained was then evaporated using a water bath to obtain a thick extract. The thick extract was weighed to determine the best yield from each comparison and tested for flavonoid content. The method of analysis of the results was carried out using the Spectrophotometric Analysis method to determine the maximum wavelength and the levels of flavonoid compounds in avocado leaf extract were tested by means of a UV-Vis Spectrophotometer.

The analysis equation is as follows:

Yield Percentage Calculation

$$\text{Yield} = \frac{\text{Extract weight}}{\text{Avocado left weight}} \times 100\%$$

Calculation of Levels of Flavonoid Compound

Measurement of the spectrum of flavonoid compounds using a UV-Vis Spectrophotometer instrument with a wavelength of 435 nm. Then compared with standard quercetin.

$$Y = a + bX$$

Y = Dependent variable (prediction value) X = Independent variable

a = Constant (intercept on graph)

b = Regression coefficient (slope on graph)

$$\text{Total Rate. : } \frac{\text{Concentration } \left(\frac{\text{mg}}{\text{L}}\right) \times \text{Vol spl (L)}}{\text{weight spl (gr)}} \times \text{FP}$$

Qualitative Testing of Flavonoid Compounds

This is done by weighing 50 mg of avocado leaf extract. Add 5 drops of hot water then stir. Add 1 mL of concentrated HCl. Add 10 mg of Mg powder. Avocado leaf extract is positive for flavonoids if there is a change in color from red orange to red purple.

Quantitative Testing of Flavonoid Compounds

Quercetin standard solution 10 mg of quercetin standard was carefully weighed and put into a 100 ml volumetric flask. 96% ethanol solvent was added to the mark, then homogenized (100 ppm). Prepare 5 50 mL volumetric flasks to make standard series with concentrations of 2, 4, 6, 8 and 10 ppm. Pipette solutions of 1, 2, 3, 4, and 5 mL each were added to a 50 mL volumetric flask. 96% ethanol solvent was added to the mark, then homogenized. 1 mL of each concentration was pipetted, then 1 mL of 10% AlCl₃ and 1 mL of 1 M sodium acetate were added. The solution was incubated for 30 minutes at room temperature.

Sample solution

Weighed 15 mg of each avocado leaf extract and dissolved in 10 mL of 96% ethanol. 1 mL of each solution was pipetted and 1 mL of 10% AlCl₃ and 1 mL of 1 M sodium acetate were added. The solution was incubated for 30 minutes at room temperature.

Blank solution

Pipette 1 mL of 96% ethanol then add 10% AlCl₃ and 1 mL of 1 M sodium acetate. Incubate for 30 minutes at room temperature

Assay with UV-Vis Spectrophotometer

Measure the maximum of the standard solution at a wavelength between 300–600 nm. Measure the absorbance of the standard solution and the sample solution at a wavelength of ± 435 nm.

Result and Discussion

Avocado Leaf Extract Yield Results

The results of the extraction of avocado leaves which were carried out by the maceration extraction method were tested for the yield, namely comparing the results of the avocado leaf extract obtained with the initial weight of the avocado that was weighed.

Table 2. Yield results on various comparison of materials and solvents

No.	Ingredient: Solvent	Dried Avocado Leaf Weight (gram)	Extract Weight (gram)	Yield (%)
1.	1 : 5	20.0000	0.5442	2.7210
2.	1 : 10	20.0000	0.8250	4.1250
3.	1 : 15	20.0000	0.9142	4.5710
4.	1 : 20	20.0000	1.1883	5.9415
5.	1 : 25	20.0000	1.2534	6.2670

Qualitative Testing of Flavonoid Compounds

To determine the presence of flavonoid compounds in avocado leaves, a qualitative identification test was carried out. Avocado leaf extract is positive for flavonoid compounds if the color changes to red orange to red purple.

Table 3. Observation results of qualitative testing of flavonoid compounds of avocado leaf extract in various comparison of materials and solvents

Step	Ratio	Observation
Hot water	1 : 5	Fixed color (brown yellow)
	1 : 10	
	1 : 15	
	1 : 20	
	1 : 25	
HCl	1 : 5	Brown yellow
	1 : 10	
	1 : 15	
	1 : 20	
	1 : 25	

Flavonoid Quantitative Test

Quantitative testing is in the form of absorbance measurements to calculate the levels of flavonoids in avocado leaf extract. The wavelength of 435.0 nm is the maximum wavelength of flavonoids. The following is the test data from the avocado leaf extraction research that has been carried out:

Table 4. Measurement results of quercetin standard series

No.	Concentrate	Absorbance
1	0 ppm	0.0000
2	2 ppm	0.3408
3	4 ppm	0.3337
4	6 ppm	0.2725
5	8 ppm	0.1493
6	10 ppm	0.1410

This research was conducted to utilize the flavonoid compounds contained in avocado leaves by extraction with the maceration method. Avocado leaves were soaked in 96% ethanol solvent for 24 hours and then continued with the filtering and concentration process. The viscous extract obtained was then calculated its yield, tested for qualitative identification and determined using the spectrophotometric method. The yield in question is the ratio between the weight of the extraction results and the weight of the raw materials used for the extraction process.

Table 5. Test results for avocado leaf extract flavonoid levels

No	Ingredient: Solvent	Yield of Flavonoid (mgQE/ grain ekstrak)
1	1 : 5	3.1724
2	1 : 10	3.0944
3	1 : 15	2.5339
4	1 : 20	1.4027
5	1 : 25	1.3241

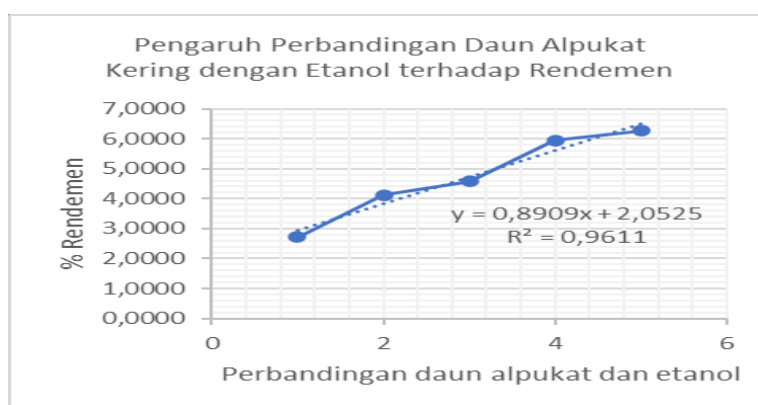


Figure 1. Effect of comparison of dried avocado leaves with ethanol on yield

From Figure 1, the results of the linear regression $Y = 0.0089X + 2.0525$ with $R^2 = 0.9611$. Where X is the ratio of dried avocado leaves with ethanol and Y is the percentage yield. The best yield is found in a ratio of 1: 25 with the amount of ethanol 500 mL, which is 6.267%. From the graph, it can be seen that the more the amount of ethanol used, the greater the yield obtained. This is because the more volume added, the stronger the solvent penetrates the cell wall and enters the cell cavity containing the active substance. To determine the content of avocado leaf extract, quercetin standard was used as a comparison solution whose wavelength measurement was determined first. From the measurement results, the maximum wavelength is obtained at 435.0 nm. Then the standard absorbance measurement was carried out using the maximum wavelength. From Figure 4.2, we get the linear regression equation $Y = 0.07173X - 0.00213$ with $R^2 = 0.99998$.

Conclusion

From this research it can be concluded as follows

1. Flavonoid compounds can be obtained by extracting avocado leaves using the maceration method to produce a thick, brown extract.
2. From the results of the comparison study of dried avocado leaves and ethanol solvent, the best yield was obtained at a ratio of 1: 25, which was 6.267%.
3. Positive avocado leaf extract contains flavonoid compounds because the color changes to red-orange.
4. From quantitative testing, the highest flavonoid content was 3.1724 mgQE/gram extract, namely the ratio of dried avocado leaves to ethanol 1: 5.

References

- [1] Antia, B.S., J. Okokon dan PA Okon. 2005. Hypoglycemic activity of aqueous leaf extract of *Persea Americana* Mill. *Research Letter*, 37 (5): 325-326.
- [2] Arukwe, B.A., M.K. Duru, E.N. Agomuo, E.A. Adindu. 2012. Chemical Composition of *Persea Americana* Leaf, Fruit and Seed. *International Journal of Recent Research and Applied Studies*. 11 (2):346-349.
- [3] Asolu, M.F., S.S. Asaolu, J.B. Fakunle, B.O. Emman, Okon, E.O. Ajayi, R.A Togun.2010. Evaluation of in-vitro Antioxidant Activities of Methanol Extracts of *Persea americana* and *Cnidosculus aconitifolius*. *Pakistan Journal of Nutrition*. 9 (11) : 1074-1077.
- [4] Darwis, D. 2000. *Teknik Dasar Laboratorium Dalam Penelitian Senyawa Bahan Alam Hayati, Workshop Pengembangan Sumber Daya Manusia Dalam Bidang Kimia Organik Bahan Alam Hayati*. Padang : Universitas Andalas.
- [5] Departemen Kesehatan. 2020. *Parameter Standar Umum Ekstrak Tumbuhan Obat*. Cetakan 1,10,17-19. Jakarta : Dirjen POM, Depkes RI.
- [6] Duarte, J., Rodriguez, F., Caley, D., Valdivelso, M.A., Antoranz, J.C., Rubio, M.A., Barreno, P., Canizo, J.F. 1989. Blood Biochemistry Values of Sheep. *Comp. Biochem. Physiol.* Vol 94 A, No. 4:717-71.
- [7] Lopez, V.M.G. 2002. Fruit Characterization of High Oil Content Avocado Varieties. *Scientia Agricola* 59(2):403-406.
- [8] Marjoni, R. 2016. *Dasar-Dasar Fitokimia untuk Diploma III Farmasi*. Jakarta : Trans Info Media.
- [9] Markham, K.R. 1988. *Techniques of Flavonoid Identification*. London: Academic Pr. Markham, K.R. 1988. *Techniques of Flavonoid Identification*. London: Academic Pr.
- [10] Marzouk, M.M. (2016). Flavonoid Constituents And Cytotoxic Activity Of *Erucaria Hipanica* (L.) Druce Growing Wild In Egypt. *Arabian Journal Of Chemistry*, 9, 411– 415.
- [11] Nuramalia, W. (2019). *Evaporator Design Pada Pabrik Sorbitol Menggunakan Bahan Baku Dekstrosa Dengan Proses Hidrogenasi Katalitik*. Semarang: Universitas Negeri Semarang.
- [12] Paramawati, R. 2016. *Khasiat Ajaib Daun Avocad*. Penebar Swadaya : Jakarta.
- [13] Putri, E. T., & Afriliana, C. (2018). *Pra Rencana Pabrik Pembuatan Etil Asetat Dari Etanol Dengan Kapasitas 77.000 Ton/Tahun*. Palembang: Universitas Sriwijaya.
- [14] Qinghu, W., Jinmei, J., Nayintai, D., Narenchaoketu, H., Jingjing, H., Baiyinmuqier, B. (2016). Anti-Inflammatory Effects, Nuclear Magnetic Resonance Identification And High-Performance Liquid Chromatography Isolation Of The Total flavonoids From *Artemisia Frigida*, *Journal Of Food And Drug Analysis*, 24, 385-391.
- [15] Sari, Mei Ambar. 2015. *Aktivitas Antioksidan Teh Daun Alpukat (Persea americana mill) dengan Variasi Teknik dan Lama Pengeringan*. Surakarta : Universitas Muhammadiyah Surakarta.
- [16] Sembiring, B. 2007. *Teknologi Penyediaan Siplisa Terstandar Tanaman Obat*. *Warta Puslitbang* Vol 13 No 12 .Balitro.litbang.depta.go.id

- [17] S hakhashiri. 2009. Chemical of the Week : Ethanol.Scifun Online. Home page online. Available from : http://scifun.chem.wisc.edu/chemweek/pdf_/ethanol.pdf. Internet; accessed 1 Juni 2021.
- [18] Sriningsih. 2008. Analisa Senyawa Golongan Flavonoid Herba Tempuyung (*Sonchusarvensis* L) : www.indonesia.com/intisari/1999/juni/tempuyung.htm. Diakses tanggal 1 Juni 2021
- [19] Sunarjo, H. 1998. Prospek Berkebun Buah. Penebar Swadaya : Jakarta.
- [20] Supriatna, A. (2008). *Uji Performansi Dan Analisa Teknik Alat Evaporator Vakum*. Bogor: Institut Pertanian Bogor.
- [21] Vanessa, M. Munhoza, R. L., José R.P., João, A.C., Zequic, E., Leite, M., Gisely, C., Lopesa, J.P., Melloa. (2014). Extraction Of Flavonoids From Tagetes Patula: Process Optimization And Screening For Biological Activity. *Rev Bras Farmacogn*, 24, 576-583.