

Qualitative and Quantitative Analysis of the Chemical Content of Hexane, Acetone, Ethanol and Water Extract from Avocado Seeds (*Persea americana* Mill.)

Harrizul Rivai^{1*}, Yolanda Triana Putri², Rusdi Rusdi²

¹Faculty of Pharmacy, Andalas University, Padang 25163, Indonesia

²School of Pharmaceutical Science (STIFARM), Padang 25143, Indonesia

*Corresponding author: Harrizul Rivai

| Received: 06.05.2019 | Accepted: 15.05.2019 | Published: 22.05.2019

DOI: [10.21276/sijtcn.2019.2.3.1](https://doi.org/10.21276/sijtcn.2019.2.3.1)

Abstract

Avocado seeds (*Persea americana* Mill.) have been recommended in traditional medicine which has therapeutic effects for hyperlipidemia, hypertension, and hypercholesterolemia. This study aims to analyze qualitatively and quantitatively the chemical compounds contained in hexane, acetone, ethanol and water extract from avocado seeds. The results obtained from the qualitative test showed that avocado seed hexane extract contained fatty acids. Avocado seeds acetone extract contains fatty acids, phenols, tannins, and flavonoids. Ethanol extracts of avocado seeds contain phenols, tannins, flavonoids, and alkaloids. Avocado seed water extract contains carbohydrates, phenols, and tannins. Quantitative tests of alkaloids are determined by the gravimetric method, as well as the ultraviolet spectrophotometer method for flavonoids, phenols, and tannins. The results obtained from the quantitative test showed the total alkaloid content of ethanol extract was 0.435 %, the total flavonoid levels of acetone and ethanol extract were 0.1068 % and 0.1084 % respectively, the total phenol content of acetone, ethanol and water extracts were 0.0476 %, 0.0309 %, and 0.0494 % respectively. The total tannin content of acetone, ethanol and water extracts were 0.1989 %, 0.2044 %, and 0.1804 % respectively.

Keywords: Avocado seeds, *Persea americana* Mill, qualitative analysis, quantitative analysis, extraction.

Copyright © 2019: This is an open-access article distributed under the terms of the Creative Commons Attribution license which permits unrestricted use, distribution, and reproduction in any medium for non-commercial use (NonCommercial, or CC-BY-NC) provided the original author and source are credited.

INTRODUCTION

Persea americana Mill is a family of Lauraceae commonly known as avocados. Avocados seed (Figure 1) has been recommended in traditional

medicine. Avocado seeds have a therapeutic effect on hyperlipidemia, hypertension, and hypercholesterolemia [1]. Therefore, avocado seeds are thought to have secondary metabolites.



Fig-1: Avocados fruit and seed

The results of phytochemical screening research by Marlinda *et al.*, [2] on ethanol extract of avocado seeds showed that avocado seeds contained alkaloids, flavonoids, triterpenoids, saponins, and tannins. The phytochemical test of the ethanol extract of

avocado seeds showed the presence of tannins, flavonoids, and anthocyanins [3]. Phytochemical analysis of water, ethanol, ether and chloroform extracts of avocado seeds showed that avocado seed water extract contained saponins, carbohydrates, and

terpenoids. Ethanol extracts contain alkaloids, saponins, carbohydrates, amino acids, glycosides, terpenoids, phenols, flavonoids, and coumarin glycosides. The ether extract contains steroids, while the chloroform extract of avocado seeds contains alkaloids, saponins, terpenoids, steroids and coumarin [4].

Quantitative analysis of avocado ethanol extract showed that the total phenol content was 289 ± 0.62 μ g Gallic Acid Equivalent (GAE)/mL, total flavonoid levels were 49.6 ± 0.02 μ g rutin/mL and proanthocyanin levels were 13.7 ± 0.01 μ g catechin/mL. Whereas avocado seed water extract contains a total phenol content of 243 ± 0.19 μ g GAE/mL, total flavonoid content is 37.2 ± 0.47 μ g rutin/mL and proanthocyanin level is 9.3 ± 0.61 μ g catechin/mL [4]. Based on research by Malangngi *et al.*, [5], total tannin content of dry avocado seed ethanol extract was 117 mg/kg while total tannin content of fresh avocado ethanol extract was 112 mg/kg. According to research by Githinji *et al.*, [6], the total phenolic content of avocado seed water extract was 18.55 ± 2.8 mg/g.

Based on the explanation above, it turns out that no research has been conducted on the qualitative and quantitative analysis of extracts of hexane, acetone, ethanol, and water from avocado seeds (*Persea americana* Mill.). Therefore, the researchers were interested in conducting a qualitative analysis and quantitative analysis of hexane, acetone, ethanol and water extracts from avocado seeds (*Persea americana* Mill.).

Experimental Section/Material and Methods

Tools And Materials

The tools used include UV-Vis Spectrophotometry (Shimadzu UV-1800), analytical scales (Precisa), rotary evaporator, Erlenmeyer (Iwaki), silica gel 60 F254, sonicator (Branson) and UV lamp (CAMAG).

The material used in this study is avocado seed simplicia (PT Temu Kencono). The chemicals used are purchased from Merck which include: hexane, acetone, ethanol, distilled water, chloral hydrate, concentrated sulfuric acid, chloroform, ferric (III) chloride, potassium bromide, lead acetate, sodium hydroxide, magnesium powder, hydrochloric acid, acetic acid anhydrous, potassium permanganate, ammonia, ether, gallic acid, sodium carbonate, sodium phosphate, ninhydrin, α -naphthol, copper sulfate anhydrous, bromine, mercury (II) chloride, potassium iodide, citric acid, salicylic acid, iodine, picric acid, sodium borohydride, copper acetate, chloroform, methanol, mercury (II) chloride, concentrated nitric acid, sodium phosphate, sodium dihydrogen phosphate, catechins,

ethyl acetate, aluminum chloride, ammonia, and quercetin.

Procedure

Provision of Simplicia

Avocado seed powder (*Persea americana* Mill) was purchased from PT Temu Kencono as much as 0.5 kg.

Simplicia characterization

Characterization of simplicia based on Indonesian Herbal Pharmacopoeia [7] included microscopic testing, drying losses, total ash content, acid insoluble ash content, water-soluble extract content, ethanol-soluble extract, and thin layer chromatography pattern.

Extraction

Fifty grams of simplicia powder of avocado seeds were macerated by soaking the simplicia into each solvent (hexane, acetone, ethanol) as much as 500 mL (ratio 1:10 w / v). Simplicia was soaked for the first 6 hours while stirring occasionally, then let stand for 18 hours. Maserate was separated by filtration; this process is repeated twice, using the same type and amount of solvent. All macerates were collected, then evaporated by rotary evaporator at temperatures below ± 50 °C to obtain the liquid extract.

The decoct method was extraction using water solvents, at a temperature of 96-98 °C for 30 minutes (calculated after the temperature of 96 °C is reached). The infusion vessel was immersed in water. Decoct was made by weighing as much as 50 grams of avocado seed simplicia and put into the infusion pan and added with 500 ml of water solvent, then put into a water bath for 30 minutes at 98° C, then filtered using the flannel cloth to obtain avocado seed water extract.

Qualitative Analysis

Qualitative analysis was carried out by Materia Medika Indonesia VI [8] and Hanani [9] towards Carbohydrate, Alkaloids, Flavonoids, Tannin, Terpenoid, Essential oil, Saponin, Phenol, Fatty acid, and Steroids.

Quantitative analysis

- Quantitative analysis of alkaloid was carried out following Indonesian Herbal Pharmacopoeia Edition I [7].
- Quantitative analysis of flavonoids was carried out following Indonesian Herbal Pharmacopoeia Supplement I [10].
- Quantitative analysis of phenol was carried out following Pharmacopoeia Hermal Indonesia Supplement II [11]
- Quantitative analysis of tannins was carried out by Indonesian Herbal Pharmacopoeia Supplement I [10].

RESULTS AND DISCUSSION

Simplicia Characterization

The results of microscopic standardization of avocado seed simplicia were shown in Figure-2. The microscopic images obtained were the file carrier, starch, epidermis and cork tissue.

In this study, a qualitative and quantitative analysis of extracts from hexane, acetone, ethanol, and water from avocado seeds was carried out. Avocado

seeds were purchased from PT Temu Kencono. Before the extraction process was carried out, simplicia characterization was carried out aimed at obtaining good quality simplicia and those that met the Indonesian Herbal Pharmacopoeia standard [7], which included microscopic testing, drying losses, total ash content, acid insoluble ash content, water-soluble extract content, soluble extracts of ethanol and thin layer chromatography patterns.

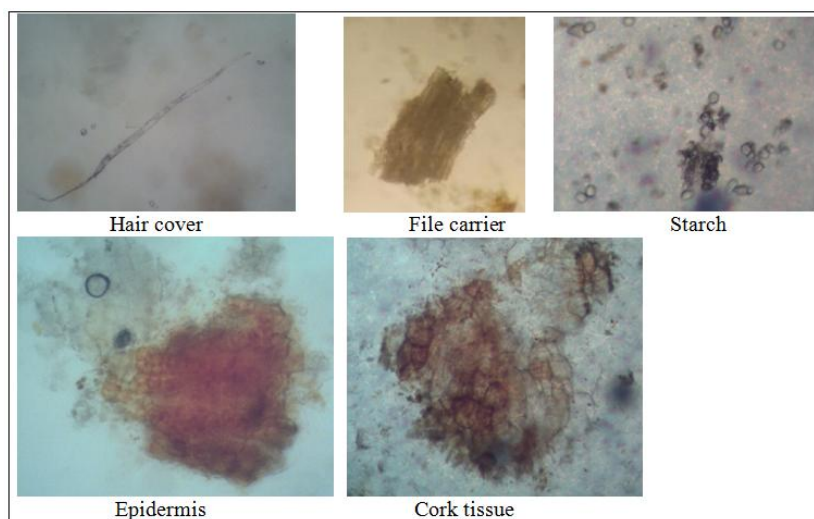


Fig-2: Microscopic of avocado seed simplicia

The average drying loss from avocado seed simplicia is $9.4018 \pm 0.0204\%$. The average total ash content of avocado seed simplicia is $4.996 \pm 0.012\%$. The average acid insoluble ash content of avocado seed simplicia is $0.6352 \pm 0.0023\%$. The average level of a water-soluble extract from avocado seeds simplicia is $21.0625 \pm 0.0153\%$. The average content of soluble ethanol extract from avocado seeds simplicia was $16.6933 \pm 0.0265\%$.

The thin layer chromatographic pattern of avocado seed simplicia was shown in Figure-3. The thin layer chromatography pattern of avocado seed simplicia showed $Rf_1 = 0.43$, $Rf_2 = 0.69$ and $Rf_3 = 0.86$. These Rf values were by Indonesian Herbal Pharmacopoeia [7].

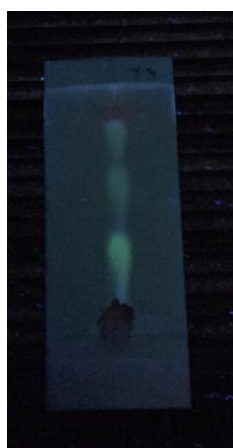


Fig-3: The thin layer chromatographic pattern of avocado seed simplicia

Description:

Stationary phase: Silica gel 60 F₂₅₄

Mobile phase: Chloroform-methanol-water (80: 12: 2)

Detection: UV λ 366 nm

Qualitative analysis of avocado seed extract

Avocado seeds that were extracted using four different solvents, namely hexane, acetone, ethanol and

water solvents were analyzed which showed the chemical compounds contained in each of these extracts (Table-1).

Table-1: Qualitative tests of hexane, acetone, ethanol and water extract from avocado seeds

No.	Testing	Extract of			
		Hexane	Acetone	Ethanol	Water
1.	Carbohydrate				
	- Molish	-	-	-	-
	- Benedict	-	-	-	+
	- Fehling	-	-	-	-
2.	Fatty acid				
	- Sulfuric acid	+	+	-	-
3.	Phenolic				
	- FeCl ₃	-	-	+	+
	- Lead acetate	-	+	-	-
4.	Tannin				
	- FeCl ₃	-	+	+	+
5.	Flavonoids				
	- Lead acetate	-	-	-	-
	- Shinoda	-	+	+	-
6.	Alkaloid				
	- Mayer	-	-	+	-
	- Wagner	-	-	+	-
7.	Terpenoid				
	- Acetate anhydride + sulfuric acid	-	-	-	-
8.	Volatile oil				
	- KMnO ₄	-	-	-	-
9.	Saponin				
	- Foam test	-	-	-	-
10.	Steroid				
	- Acetate anhydride + sulfuric acid	-	-	-	-

Information:

+ = Contain metabolites

- = Does not contain metabolites

The results of the phytochemical screening test from hexane extract of avocado seeds contain fatty acid compounds. Hexane extract can only attract fatty acid compounds because hexane is nonpolar, so it can only attract nonpolar compounds such as fatty acids. The results of the phytochemical screening test of acetone seed extract contain fatty acid compounds, phenols, tannins, and flavonoids. The results of the phytochemical screening test for the ethanol extract of avocado seeds contain phenol, tannin, flavonoids and alkaloid compounds. Phytochemical screening test results of avocado seed water extract containing carbohydrate compounds, phenols, and tannins.

Quantitative analysis of avocado seed extract

The total alkaloid content of the avocado ethanol extract was 0.435% (Table-2.). The total flavonoid content of acetone and ethanol extract from avocado seeds were 0.1068% and 0.1084%, respectively, calculated as quercetin (Table-2, Figures 4 and 5). Total phenol levels from acetone, ethanol and avocado seed water extracts were 0.0476%, 0.0309%, and 0.0494%, respectively, calculated as gallic acid (Table 2, Figures 6 and 7). Total tannin levels from acetone, ethanol and water extract from avocado seeds were 0.1989%, 0.2044%, and 0.1804% respectively calculated as catechins (Table-2).

Table-2: Quantitative analysis of hexane, acetone, ethanol and water extract from avocado seeds

Extract type	Total alkaloids (%)	Total flavonoids (%)	Phenol total (%)	Total tannin (%)
Hexane Extract	-	-	-	-
Acetone Extract	-	0.1068	0.0476	0.1989
Ethanol Extract	0.435	0.1084	0.0309	0.2044
Water Extract	-	-	0.0494	0.1804

Determination of total alkaloid levels from ethanol extract from avocado seeds using the gravimetric method is an analysis method based on weight measurements, which involves the formation of deposits, measurement of weight or isolation of deposits. Generally, alkaloids in plants are mostly salts of organic acids. Determination of the alkaloid content of ethanol extract was carried out by the addition of ammonia, hydrochloric acid, and chloroform. Ammonia is added aiming to release the alkaloid bond with its acid so that the alkaloid is again in a free condition because ammonia will bind to hydrochloric acid which forms water-soluble salts while the alkaloid in the free condition is alkaline and insoluble in water. With the addition of chloroform, two layers, namely the acid layer and chloroform layer, an alkaloid in the basic free form will be extracted with chloroform solvent, resulting in chloroform extract containing total alkaloids. The collection of chloroform phases is evaporated at 50 °C, then dry at a temperature of 100 °C to a fixed weight. Based on the experimental results, the total alkaloid content of ethanol extract was 0.435% (Table-2).

Determination of total flavonoid content from acetone extract and ethanol extract from avocado seeds was carried out using the colorimetric method with aluminum chloride reagent. In this study, the determination of the maximum absorption wavelength from the standard quercetin solution was 430.5 nm at a concentration of 50 µg/mL (Figure-4). To determine the quercetin calibration curve, dilution of solution was made with various concentrations namely 30 µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL and 70 µg/mL (Figure-5). Measurement of the test solution of acetone extract and ethanol extract of avocado seeds were performed by adding ethanol to increase the solubility of flavonoids, then by adding aluminum (III) chloride to form a complex so that a wavelength shift towards the visible light marked with a solution produced a yellow color. Sodium acetate was added as a stabilizer. After that, the mixture was incubated for 30 minutes, with the aim that the reaction between quercetin and the reagents added could take place so that the resulting color intensity was more maximal.

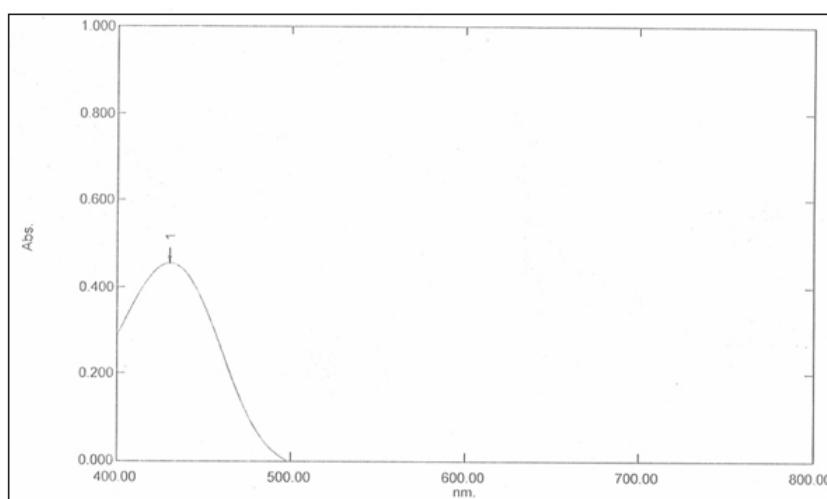


Fig-4: Absorption spectrum of quercetin at a concentration of 50 µg / mL with the addition of aluminum chloride (maximum absorption wavelength of 430.5 nm)

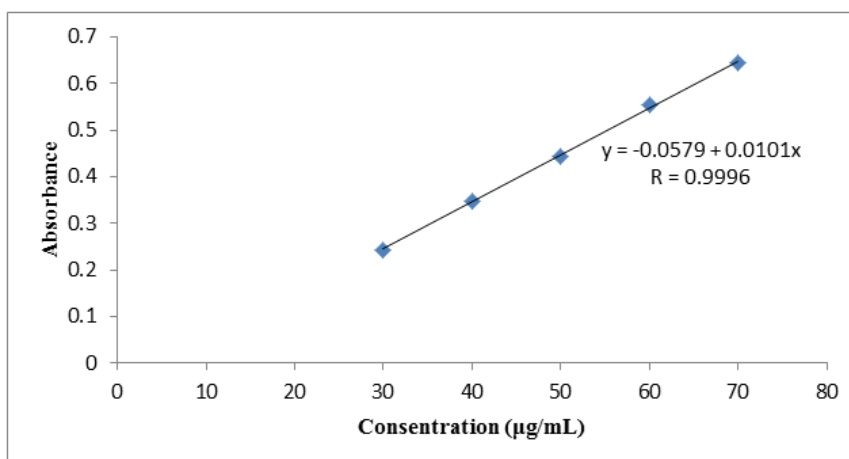


Fig-5: Quercetin calibration curve at maximum wavelength of 430.5 nm

Based on the calibration curve in Figure-5 the regression equation obtained $y = -0.0579 + 0.0101x$, the correlation coefficient $r = 0.9996$. The correlation coefficient is close to 1, which means that there is a very high correlation between absorptive and content levels. With this equation, the total flavonoid content of acetone extract was 0.1068%, and ethanol extract was 0.1084%.

The determination of the total phenol content was used by the Folin Ciocalteu method. This method was the most commonly used method for determining total phenol levels. The process was straightforward. Phenol compounds can react with Folin Ciocalteu reagents to form a colored solution that can be measured absorbance.

In determining total phenol levels, gallic acid was used as a comparison. The maximum absorption wavelength of the standard gallic acid solution is 764 nm at a concentration of 15 $\mu\text{g/mL}$ (Figure-6). In preparation of the calibration curve, the gallic acid mother solution was made in various concentrations namely concentrations of 15, 30, 45, 60, 75 and 90 $\mu\text{g/mL}$ (Figure-7). Furthermore, the test solution of each ethanol extract, water extract, and acetone extract was added with a dilute Folin-Ciocalteu reagent (7.5% in water) and 1% NaOH, incubated for 1 hour, then measured the absorption of each solution at the wavelength maximum of 764 nm. Furthermore, the regression equation obtained was used to calculate the total phenolic level in each avocado seed extract.

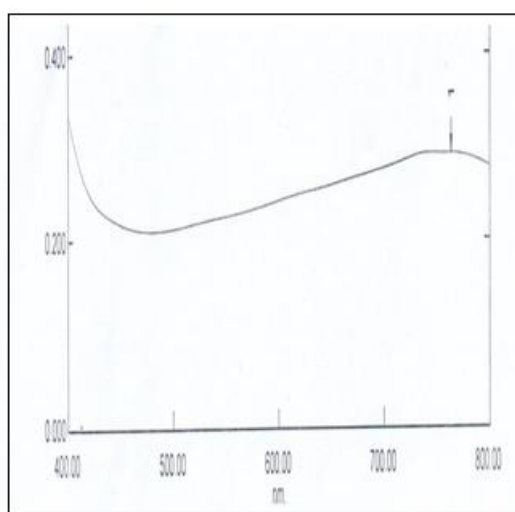


Fig-6: The gallic acid absorption spectrum at a concentration of 15 $\mu\text{g/mL}$ with the addition of Folin-Ciocalteu (maximum absorption wavelength of 764 nm).

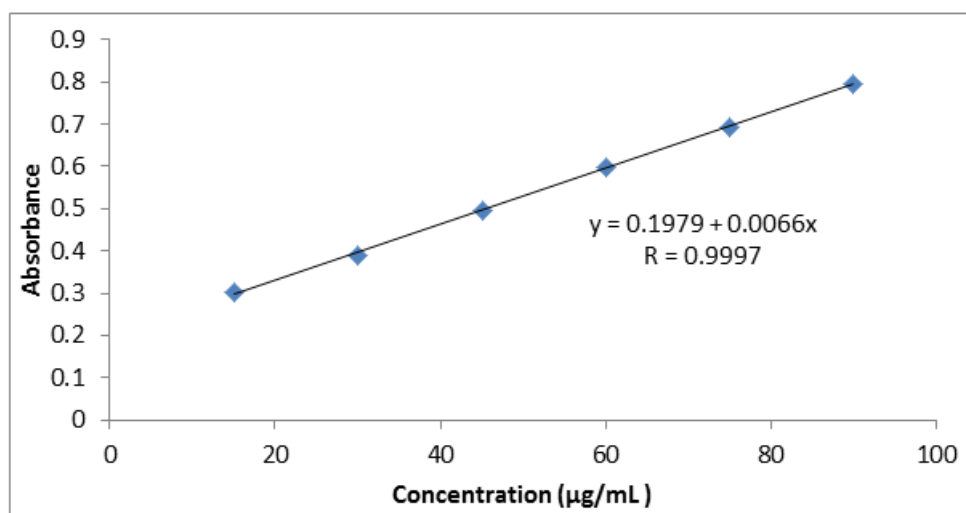


Fig-7: Gallic acid calibration curve at wavelength 764 nm

Based on the calibration curve in Figure-7, the regression equation $y = + 0.1979 + 0.0066x$, the correlation coefficient $r = 0.9997$. This correlation

coefficient is close to 1, which means that there is a very high correlation between absorptive and compound levels. By using this equation, the total

phenol content of acetone extract was 0.0476%, ethanol extract 0.0309% and water extract 0.0494%.

Determination of tannin is carried out by ultraviolet-visible spectrophotometry using standard catechin compounds. Catechins are relatives of condensed tannins which are often called polyphenols because of the large number of hydroxy functional groups they have. To determine the total tannin level using the ultraviolet-visible spectrophotometry method, first make a new solution, a comparison solution, and a test solution. The new solution used is ethyl acetate, the comparative solution is catechins, and the test solutions are acetone, ethanol and water extract from avocado seeds. The comparison solution and the test solution were added with ethyl acetate and then sonicated so that the solution with ethyl acetate was mixed homogeneously. The absorbance of the comparison solution, test solution, and the new solution was measured spectrophotometrically at wavelengths of 279 nm and 300 nm. Absorbance of the test solution at 300 nm no more than 0.03. Catechin levels in acetone, ethanol and water extracts were calculated at a wavelength of 279 nm. The absorbance of the comparative solution at wavelength 279 was 0.270, the test solution of acetone extract 0.537 with the obtained content was 0.1989%, ethanol extract 0.552 with a concentration of 0.2044% and water extract 0.487 with levels of 0.1804% (Table-2).

CONCLUSION

The chemical compounds of the hexane extract of avocado seeds are fatty acids. The chemical compounds of acetone extract from avocado seeds are fatty acids, phenols, tannins, and flavonoids. The chemical compounds from the ethanol extract of avocado seeds are phenols, tannins, flavonoids, and alkaloids. The chemical compounds of water extract from avocado seeds are carbohydrates, phenols, and tannins. The total alkaloid content in the ethanol extract was 0.435%. Total flavonoid levels in acetone and ethanol extracts were 0.1068% and 0.1084%. Total phenol content of acetone, ethanol and water extracts was 0.0476%, 0.0309%, and 0.0494%. The total tannin content of acetone, ethanol and water extracts was 0.1989%, 0.2044%, and 0.1804%.

REFERENCES

1. Minister of Health Regulation of the Republic of Indonesia Number 6 of 2016 concerning Formulary of Original Indonesian Herbal Medicine.
2. Marlinda, M., Sangi, M. S., & Wuntu, A. D. (2012). Analisis senyawa metabolit sekunder dan uji toksisitas ekstrak etanol biji buah alpukat (*Persea americana* Mill.). *Jurnal MIPA*, 1(1), 24-28.
3. Arifah, C. N., & Saleh, C. (2016). Uji fitokimia dan uji stabilitas zat warna dari ekstrak biji buah alpukat (*Persea americana* Mill) dengan metode spektroskopi UV-VIS. *Jurnal Atomik*, 1(1), 18-22.
4. Noorul, H., Mujahid, M., Badruddeen, Khalid, M., Vartika, S., Nesar, A., Zafar, K., & Zohrameena, S. (2017). Physico-phytochemical analysis & Estimation of total phenolics, flavonoids and proanthocyanidin content of *Persea americana* (avocado) seed extracts. *World Journal of Pharmaceutical Sciences*, 5(4), 70-77.
5. Malangngi, L., Sangi, M., & Paendong, J. (2012). Penentuan kandungan tanin dan uji aktivitas antioksidan ekstrak biji buah alpukat (*Persea americana* Mill.). *Jurnal MIPA*, 1(1), 5-10.
6. Githinji, P., Gitu, L., Marete, E., Githua, M., Mugo, M., & Mbaka, E. N. (2013). Quantitative analysis of total phenolic content in Avocado (*Persea americana*) seeds in Eastern Province of Kenya. *Chemistry and Materials Research*, 3(10), 48-51.
7. Indonesian Herbal Pharmacopoeia Edition I. (2008). Jakarta: Ministry of Health of the Republic of Indonesia.
8. Ministry of Health of the Republic of Indonesia. (1995). *Materia Medika Indonesia* (Edition IV). Jakarta: Ministry of Health of the Republic of Indonesia.
9. Hanani, E. (2017). *Analisis Fitokimia*, Jakarta: Penerbit Buku Kedokteran: EGC.
10. Ministry of Health of the Republic of Indonesia. (2010). *Indonesian Herbal Pharmacopoeia Supplement I*. Jakarta: Ministry of Health of the Republic of Indonesia.
11. Ministry of Health of the Republic of Indonesia. (2011). *Indonesian Herbal Pharmacopoeia Supplement II*. Jakarta: Ministry of Health of the Republic of Indonesia.