**Food Research 7 (2) : 137 - 142 (April 2023)**
Journal homepage: https://www.myfoodresearch.com

FOOD RESEARCH

FULL PAPER

# Total flavonoid of dry extract and fraction of selected shallot (*Allium ascalonicum* L.) using ultraviolet-visible spectrophotometry and HPLC

[1,2]Nurcahyo, H., [1,*]Sumiwi, S.A., [1]Halimah, E. and [1]Wilar, G.

[1]*Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia*
[2]*Politeknik Harapan Bersama, Central Java, Indonesia*

***Article history:***
Received: 8 October 2021
Received in revised form: 26 November 2021
Accepted: 7 March 2022
Available Online: 30 March 2023

***Keywords:***
Shallot,
Flavonoid content,
Fractionation,
UV-Vis spectrophotometry,
HPLC

***DOI:***
https://doi.org/10.26656/fr.2017.7(2).765

**Abstract**

Shallots (*Allium ascalonicum* L.) of Brebes are plants with a high flavonoid content. Furthermore, the flavonoids in shallots were extracted using the proper methods and solvents. In this study, shallot simplicia was dried at 30, 40, 50, and 60°C before being macerated in a 70% ethanol solvent. Water, ethyl acetate, and n-hexane were used as solvents to fractionate the ethanol extract. Ultraviolet visible (UV-Vis) spectrophotometry was used to determine the total flavonoid levels in the extract. The best results were obtained from HPLC readings of the quercetin chromatogram profile. The ethanol extract contained the most flavonoids, with 13.484 mg EQ/g after drying at 40°C.

## 1. Introduction

Shallot (*Allium ascalonicum* L.) is one of many vegetables that can be used to enhance the flavour of various fish and meat dishes. It can also be usually consumed, as in "satay" in Indonesia, or as daily food. (Mang *et al*., 2019). Shallots (*Allium ascalonicum* L.) contain flavonoids with numerous benefits (Arshad *et al.,* 2017). High flavonoid contents in shallot are thus recommended for their major health benefit (Perez-Gregorio *et al*., 2010).

Diet refers to how a person consumes a variety of foods rather than just one, and now it has become an alternative and complementary approach to understanding the relationship between food and chronic disease. Several dietary patterns in the world show positive and negative impacts on increasing the risk of non-communicable diseases. The patterns known as metabolic syndrome (Bakker *et al*., 2014) increase the risks of obesity, high blood pressure, diabetes, and hyperlipidaemia (Cespedes and Hu, 2015). Hyperlipidaemia is a risk factor for cardiovascular diseases. Controlling hypercholesterolemia is important to prevent hyperlipidaemia. Reducing triglyceride levels in the bloodstream is one of the treatments for patients with cardiovascular-related disease through inducing LDL receptors and limiting VLDL secretion with certain drugs.

Reducing blood cholesterol concentrations is an interesting subject for functional food and drug development to help lower the risk of cardiovascular disease. Natural plant or organism components are potential candidates for reducing the chances of disease outbreaks. Shallot (*Allium ascalonicum* L.) has traditionally been used to limit blood cholesterol levels. It was traditionally used as medicine in Asia due to its fever-reducing, antiparasitic, detoxification, and anti-inflammation effects in the intestine. The primary components of shallot are flavonoids (quercetin) and sulfuric compounds (allyl propyl disulfide, diallyl disulfide), which both have health-promoting properties (Mang *et al*., 2019).

Flavonoid quercetin is flavonoid that belongs to the flavanol group and has polar-less or semi-polar habits (Pratoko *et al*., 2018). Ethyl acetate which is semi-polar, based on the like dissolve-like principle, will attract compounds with the same degree of polarity (Pratoko *et al*., 2018). Shallots contain flavonoids (Aryanta, 2019) with levels of 14.57% flavonoids, 21.42 m/100 g quercetin, 0.62 mg/100 g kaempferol, 0.02 mg/100 g myricetin. Flavonoid glycosides are more polar than their aglycone forms, such as a more polar routine than quercetin due to the presence of a 3-O-glycoside group (Heim *et al*., 2002). However, ethyl acetate solvent is capable to attract slightly both polar and non-polar compounds. Accordingly, although the 3-O-glycoside

*Corresponding author.
Email: *sri.adi@unpad.ac.id*

eISSN: 2550-2166 
Published by Rynnye Lyan Resources

group on the routine causes polarity to increase, the routine may also be attracted to semi-polar solvents, such as ethyl acetate (Pratoko *et al*., 2018). This finding is reinforced by research conducted by (Chavan and Amarowicz, 2013) which shows that the content of phenolic compounds is found more in semi-polar solvents than in polar solvents.

Flavonoid is a class of compounds that cannot withstand high temperatures and are easily oxidized (Rompas *et al.*, 2013). Consequently, it is necessary to determine the best extraction method. According to a study on the optimization of flavonoid extraction with ethanol solvent by Setiani *et al*. (2017) the highest flavonoid content was obtained at 70% ethanol concentration with raw material: solvent ratio of 1:10. To obtain a high total flavonoid substance, optimization is necessary (Sukmawati *et al.*, 2018). However, the flavanol group has less polar or semi-polar properties (Pratoko *et al*., 2018).

According to some similar studies, fractionation with different polarity levels adheres to the principle of like dissolves like, i.e. a solvent will tend to dissolve compounds with the same polarity level (Sukmawati *et al.*, 2018). The highest total phenolic contents are obtained by fractionation with ethyl acetate. The fractionation method is highly recommended to obtain the highest flavonoid level. However, it is necessary to develop solvents from polar, semi-polar and non-polar to get the highest quercetin from the shallots. Therefore, the focus of the current study was to determine the content of the quercetin equivalent flavonoid in a variety of shallot taken from Brebes the largest shallot-producing area in Indonesia which have the potential for lipid metabolism.

## 2. Materials and methods

### *2.1 Materials*

Bima is a variety of shallots that are cultivated by farmers in Brebes, Central Java, Indonesia with GPS latitude location: -6° 52 '59.99 "S and longitude location: 109° 02' 60.00" E, alcohol, aquadest, ethyl acetate, N hexane, quercetin, methanol, silica Gel GF254, UV-Vis spectrophotometer and HPLC.

### *2.2 Sample treatments*

The shallots were first cleaned to remove any foreign or unnecessary materials such as soil, grass, and dirt. Then, under running water, they were cleaned to remove soil residue and other impurities. The process was carried out immediately in order to keep all nutritious substances. The shallots were then chopped by using a special chopper machine to obtain similar slices. The study set four different drying temperatures: 30, 40, 50, and 60°C to obtain 10% water in the shallots.

### *2.3 Fractionation methods*

Water, ethyl acetate, and n-hexane as solvents were chosen for the fractionation process. A total of 10 g of viscous extract were dissolved in 100 mL of the solvent fraction. In a separating flask, the solution was mixed for 30-60 min resulting in a layer of shallot (polar-semi-polar layer, upper layer fraction layer). Furthermore, the segmented fractions were separated and accommodated using Erlenmeyer's method. The evaporation process was then carried out using a pot of water at 50°C to obtain a thick layer. The results of the fraction were then measured by the total flavonoid levels using a UV-Vis spectrophotometer.

### *2.4 Spectrophotometric*

The following steps were taken to test the samples using Uv-Vis spectrophotometry. To begin, 1000 ppm quercetin standard solution in the volumetric flask was prepared by weighing 10 mg quercetin standard powder dissolved in 10 mL methanol and determining the maximum wavelength by designing a standard solution containing 10 ppm quercetin. Here, a UV-Vis spectrophotometer was used to read all data in the 200-400 nm wavelength range. Next, make the quercetin standard series solution by diluting the 1000 ppm quercetin standard solution with methanol to 10, 20, 30, 40, and 50 ppm. The results were obtained by using Excel Software to solve the equation y = ax + b. Finally, the total flavonoid content of shallot extract was measured by weighing samples of ethanol extract and ethyl acetate fraction to obtain a concentration of 1000 ppm extract. A UV-vis method was used to measure the solution's absorption at the maximum wavelength obtained. The following equation was used to calculate the final flavonoid levels:

$$\text{Flavonoid content} = \frac{C \times V}{m}$$

Where $c$ = standard curve formula to calculate the concentration of quercetin, $v$ = Extract volume and $m$ = Extract weight

### *2.5 Quercetin chromatogram profile using HPLC*

Quercetin standard manufacturing procedure involved weighing the standard quercetin and dissolving it all in 10 mL of methanol, to obtain a standard solution of 1000 ppm. Diluting the 1000 ppm standard solution was determined by collecting 125 L of the 1000 ppm standard solution in a 5 mL measuring flask and adding methanol up to the limit mark to get a 25 ppm solution before filtering with Whatman paper number 1 and

https://doi.org/10.26656/fr.2017.7(2).765 

injecting it into HPLC Shimadzu Prominence 2030C3D.

The sample was prepared by diluting the extract and pipetting 0.1 mL of shallot extract at a 10% concentration. Methanol was used to mix the results to a volume of 10 ml. The procedure was then continued by diluting the fraction to a 10% concentration and piping 0.1 mL of the shallot fraction into a volume of 10 mL of methanol. The sample was then filtered with Whatman paper number 1 before being injected into an HPLC Shimadzu Prominence 2030C3D.

High-Performance Liquid Chromatography (HPLC) test conditions applied a mobile phase of methanol: distilled water: phosphoric acid (54:45:1) by employing a stationary phase on column C18 with a UV wavelength of 370 nm and a flow time of 1 mL/mins.

## 3. Results and discussion

Drying simplicia in particular temperatures ranging from 30, 40, 50, and 60°C was accompanied by distillation through a maceration method using 70% ethanol solvent (Table 1). The process was set to determine the effect of drying the shallots at different temperatures. This was due to the fact that the higher the temperature, the content of the flavonoid will decrease. Also, it was essential to find out the optimum or best temperature to get the best flavonoid content.

Table 1. Flavonoid levels from 70% shallot ethanol extract

| Simplicia Drying | Extract Flavonoid level |
|---|---|
| 30 | 13.359±0.018 |
| 40 | 13.493±0.018 |
| 50 | 10.365±0.017 |
| 60 | 7.200±0.001 |

At 400°C, the highest concentration of flavonoid level was discovered by creating polar solvents to non-polar solvents such as water, ethyl acetate, and n-hexane. This is consistent with the higher temperature of flavonoid drying levels proposed by (Syafrida *et al.,* 2018). After obtaining a linear equation, the extract was fractionated using a shift in solvent from polar to non-polar solvent at a wavelength of 375 nm (Table 2).

Table 2. Shallot flavonoid levels from various fraction

| Solvent | Flavonoid Fraction Level (mg EQ/g) |
|---|---|
| Water | 7.200±0.001 |
| Ethyl acetate | 96.776±0.015 |
| N-hexane | 5.436±0.001 |

To determine the quercetin absorption peak, two procedures were conducted by adding $AlCl_3$ shear reagent and using the original compound (Nurcahyo *et al*., 2020). A standard curve was then formed on the basis of a series of standard solutions within linear limits. This was performed to demonstrate that the levels of concentration of the sample solution from the measurement can be easily obtained using a standard curve.

A standard curve is a curve formed by a series of standard solutions that are straight enough to be linearly regressed. This serves to display the concentration of the sample solution based on the measurement results and enables the concentration of the sample solution to be easily obtained using such a curve.

Linearity is subject to two conditions. The first is that the value of the correlation coefficient is said to be good if it is greater than 0.9977, and the coefficient of the regression function (Vx0) is greater than 5%. The measurement revealed a Vx0 worth of 0.0005%. This is stated in accordance with the requirements. In order to determine and calculate the levels of quercetin in the sample. The area of the standard quercetin solution series was plotted with its concentration to obtain a standard quercetin curve with the line equation y = 0.0374x - 0.1053, correlation value (r) 0.9977, and Vx0 value of 0.0005%.

The standard curve represents the relationship between the solution's concentration (x) and area (y), where y = the dependent variable, x = the independent variable, a = the intercept, and b = the regression coefficient/slope. The obtained results were processed in a Spreadsheet to form the equation y = ax+b with the correlation coefficient of $R^2 = 0.998$ (Figure 1).

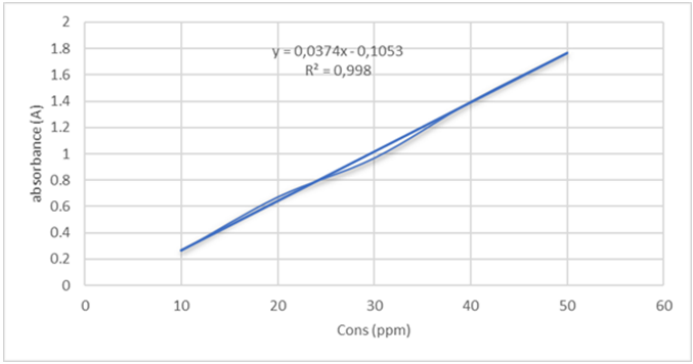


Figure 1. Linear equation of quercetin series

The identification of quercetin compounds was performed using HPLC with a UV-Vis detector (photodiode array) dedicated to detecting absorbance at wavelengths ranging from 200 to 600 nm, with 364 nm as the best wavelength. The stationary phase, mobile phase, and instrument conditions are all factors to consider while using an HPLC system. In this study, the stationary phase is reversed-phase C18 (Shim-pack VP ODS). This phase is commonly used because it is responsible for identifying the major portion of natural compounds. Methanol: distilled water: phosphoric acid in a ratio of (54:45:1) was chosen as a mobile phase

https://doi.org/10.26656/fr.2017.7(2).765

compatible with reverse phase C18, among other objects (Ozcan *et al*., 2012).

In Figure 2, the quercetin levels in the area of the result of a series of concentrations of standard quercetin solutions were plotted with their concentrations. The maximum wavelength (λmax) is the wavelength at which electronic excitation occurs which provides the maximum absorbance. The purpose of measuring the maximum wavelength in this study was to find out with respect to changes in absorbance for each concentration unit reached the greatest at the maximum wavelength for maximum analysis sensitivity (Sayuthi *et al*., 2015). Quercetin has two spectra peaks with two characteristics of chromophores; cinnamoyl and benzoyl. The prior contributes to the absorption of UV light at 375 nm wavelength, while the latter contributes at 258 nm region due to entering the wavelength range of 200-400 nm.

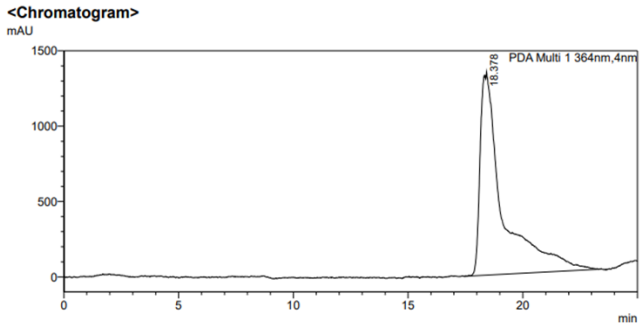


Figure 2. Chromatograms of quercetin

The wavelength quercetin determination results obtained two peaks of maximum absorption at a wavelength of 210 nm and 375 nm. The later wavelength was applied to determine the linear curve of the equation, and this assess the levels of extract flavonoids and fractions from the shallot.

Figure 3 shows that based on the absorbance measurement results on various concentrations of quercetin. These provide a linear equation of y = 0.0374x - 0.1053. The coefficient of determination ($R^2$) was obtained at 0.9977. The coefficient determination obtained was the relationship between the concentration of quercetin and its absorbance which met the linear criteria near 1. As a result, the line equation can be used to determine the validation of the method in determining flavonoid levels total using a UV-Visible spectrophotometer.

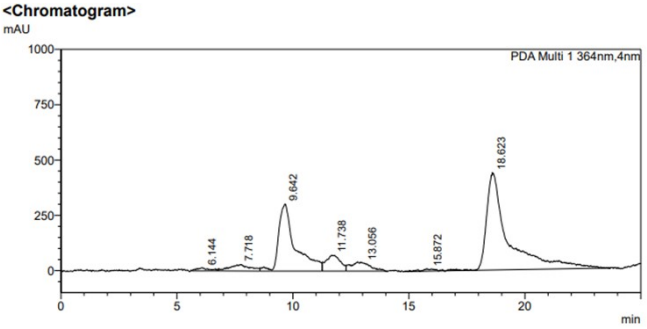


Figure 3. Chromatograms of ethyl acetate fraction

Figure 4 shows the obtained flavonoid levels of 70% shallot ethanol extract with a wavelength of 375 nm and a linear statistical equation.

Retention time is a qualitative parameter that demonstrates the identity of a compound in the form of the amount of time required for the analysis of the ingredients. This means that a High-Performance Liquid Chromatography (HPLC) tool can be used to determine the retention time of a substance. This method has the advantage over other separation methods of its analytical accuracy and high sensitivity, and it is suitable for separating non-volatile compounds with heat-resistant. The development of the HPLC process is crucial in drug discovery, drug development, and pharmaceutical product analysis (Cholifah *et al.*, 2020).

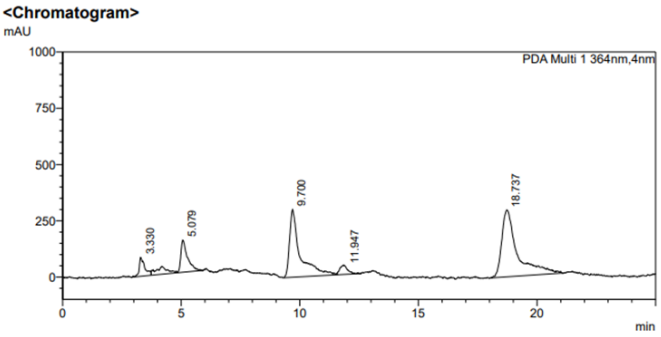


Figure 4. Chromatograms of ethanol extract

Figure 5 shows the results of HPLC chromatogram retention time for standard quercetin, with ethyl acetate fraction, and ethanol extract of Rt 18.378, 18.623 and 18.737. This means that quercetin standard, ethyl acetate fraction and ethanol extract indicate the same spectrum with almost the same retention time value.

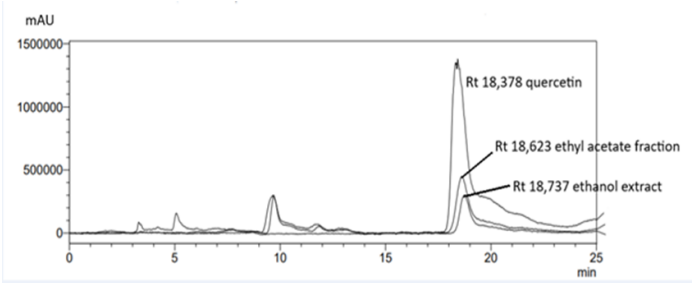


Figure 5. Profile chromatograms of HPLC

The chromatogram results are shown in Figures 2, 3, 4 and 5. Whereas, Figure 2 shows that the standard chromatogram profile of quercetin is used as a reference to compare the chromatogram characteristics of the ethanol extract and the ethyl acetate fraction by analysing the peaks and retention times that emerge. The quercetin compound was detected in the samples of the ethyl acetate fraction and the ethanol extract of shallots with the same peak based on the retention time (see Figure 5). Peak 1 with Rt 18.378 represents standard quercetin. With Rt 18.623, the ethyl acetate fraction was

https://doi.org/10.26656/fr.2017.7(2).765

shown at the 7th peak. The ethanol extract, on the other hand, was shown at peak 5 with Rt 18.737. This can be used to confirm that the ethyl acetate fraction and the onion fraction contain quercetin. Based on the chromatogram profile, the two samples of obtained ethyl acetate fraction and ethanolic extract of shallots showed that there were other compounds with large peak areas and heights. It is necessary to carry out further research to determine the compounds contained in these fractions and extracts.

To conclude, the development of this current research applied the fractionation of various solvents with different levels of polarity: polar as water, semi-polar as ethyl acetate, and nonpolar as n-hexane. The abilities of the solvent in dissolving compounds in shallots are varied. This affects the number of compounds attracted to the solvent. The ability of semi-polar compounds to attract polar and nonpolar compounds reached higher. Thus, the possibility of the compounds being absorbed was more than polar and non -polar solvents. This study applied HPLC testing with time retention to show that the ethanol extract and ethyl acetate fraction of the shallots contain quercetin, where the ethyl acetate fraction gained higher levels of content than the ethanol extract.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgements

The author thanks Universitas Padjadjaran, Bandung Indonesia for financial support through dissertation research (awarded to Prof. Dr. apt. Sri Adi Sumiwi, MS.)

## References

Arshad, M.S., Sohaib, M., Nadeem, M., Saeed, F., Imran, A., Javed, A., Amjad, Z. and Batool, S.M. (2017). Status and trends of nutraceuticals from onion and onion by-products: A critical review. *Cogent Food and Agriculture*, 3(1), 1280254. https://doi.org/10.1080/23311932.2017.1280254

Bakker, L.E.H., Schinkel, L.D Guigas, B., Streefland, T.C.M., Jonke J.T., Klinken, J.B., Zon, G.C.M., Lamb, H.J., Smit, J.W.A., Hanno, P., Meinders, A.E. and Jazet, I. (2014). A 5-day high-fat, high-calorie diet impairs insulin sensitivity in healthy, young South Asian men but not in Caucasian men. *Diabetes*, 63(1), 248–258. https://doi.org/10.2337/db13-0696

Cespedes, E.M. and Hu, F. (2015). Dietary patterns: From nutritional epidemiologic analysis to national guidelines. *American Journal of Clinical Nutrition*, 101(5), 899–900. https://doi.org/10.3945/ajcn.115.110213

Chavan, U.D. and Amarowicz, R. (2013). Effect of various solvent systems on extraction of phenolics, tannins and sugars from beach pea *(Lathyrus maritimus* L.). *International Food Research Journal*, 20(3), 1139–1144.

Cholifah, S.C., Lazuardi, M., Rahardjo, D., Maslachah, L., Sukmanadi, M. and Kurnijasanti, R. (2020). Uji Penetapan Stabilitas Retention Time Megestrole Acetate dalam Eluent Mobile Phase Menggunakan High Performance Liquid Chromatography. *Journal of Basic Medical Veterinary*, 9(1), 37-45. https://doi.org/10.20473/.v9i1.21093 [In Bahasa Indonesia].

Heim, K.E., Tagliaferro, A.R. and Bobliya, D. (2002). Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry*, 13(10), 572–584. https://doi.org/10.1016/S0955-2863(02)00208-5

Aryanta. I.W.A. (2019). Bawang Merah Dan Manfaatnya Bagi Kesehatan. *Widya Kesehatan*, 1(1), 29–35. https://doi.org/10.32795/widyakesehatan.v1i1.280 [In Bahasa Indonesia].

Mang, D.Y., Edith, D.M.J., Abdou, A.B. and Njintang, N. (2019). Determination of phytochemical properties of dried onion slices (*Allium cepa* L. var. Violet of galmi). *Journal of Food Measurement and Characterization*, 13(3), 1924–1934. https://doi.org/10.1007/s11694-019-00111-w

Nurcahyo, H., Sumiwi, S.A., Halimah, E. and Wilar, G. (2020). Total Flavonoid Levels of Ethanol Extract and Ethyl Acetate Fraction Dry Shallots (*Allium cepa* L. var. Garden Onion of Brebes) with Maceration Methods Using UV-Vis Spectrophotometry. *Systematic Reviews in Pharmacy*, 11(10), 286–289. https://doi.org/10.31838/srp.2020.10.48

Ozcan, C., Dilgin, Y. and Yaman, M. (2012). Determination of quercetin in medicinal plants such as rose hip (*Rosa canina*), bettle (*Urticadioica*), terebinth (*Terebinthina chica*) and purslane (*Portulace oleracea*) using HPLC-MS method. *Asian Journal of Chemistry*, 24(8), 3396–3400.

Perez-Gregorio, R.M., Garcı´a-Falco´ n, M.S., Simal-Ga´ndara, J., Rodrigues, A.S. and Almeida, D. (2010). Identification and quantification of flavonoids in traditional cultivars of red and white onions at harvest. *Journal of Food Composition and Analysis*, 23(6), 592–598. https://doi.org/10.1016/j.jfca.2009.08.013

Pratoko, D.K., Wardhani, F.A., Kristiningrum, N.,

 

Fajrin, F.A. and Pangribowo, D. (2018). Kadar Fenolat dan Flavonoid Total serta Kapasitas Antioksidan Ekstrak Etanol dan Fraksi Jahe Merah *(Zingiber officinale var. Rubrum)*. *Al-Kimia*, 6(2), 171-183. [In Bahasa Indonesia].

Rompas, R.A., Edy, H.J. and Yudistira, A. (2013). Isolasi dan Identifikasi Flavoniod Dalam Daun Lamun (*Syringodium Isoetifolium*), p. 1689–1699 Indonesia: Kementrian Kesehatan Republik Indonesia. [In Bahasa Indonesia].

Tulandi, G.P., Sudewi, S. and Lolo, W.A. (2015). Validasi Metode Analisis Untuk Penetapan Kadar Parasetamol Dalam Sediaan Tablet Secara Spektrofotometri Ultraviolet. *Pharmacon,* 4(4), 168-178. [In Bahasa Indonesia].

Setiani, L.A., Sari, B.L. and Indriani, L. (2017). Penentuan Kadar Falvonoid Ekstrak Etanol 70% Kulit bawang Merah (*Allium cepa* L.) Dengan Metode Maserasi dan MAE (Microwave Assisted Extraction). *Fitofarmaka*, 87(1,2), 149–200. [In Bahasa Indonesia].

Sukmawati, Sudewi, S. and Pontoh, J. (2018). Optimasi dan validasi Metode Analisis Dalam Penentuan Kandungan Total Flavoniod pada Ekstrak Daun Gedi Hijau (*Abelmoscus manihot* L.) yang diukur menggunakan Spektrofotometri UV-VIS. *Pharmacon*, 7(3), 32–41. https://doi.org/10.35799/pha.7.2018.20102 [In Bahasa Indonesia].

Syafrida, M., Darmanti, S. and Izzati, M. (2018). Pengaruh Suhu Pengeringan Terhadap Kadar Air, Kadar Flavonoid dan Aktivitas Antioksidan Daun dan Umbi Rumput Teki (*Cyperus rotundus* L.). *Bioma: Berkala Ilmiah Biologi*, 20(1), 44-50. https://doi.org/10.14710/bioma.20.1.44-50 [In Bahasa Indonesia].

https://doi.org/10.26656/fr.2017.7(2).765 