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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

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ABSTRACT

Introduction: Shallots (*Allium cepa* L. var. Garden Onion of Brebes) are one of abundant plants from Indonesia with high flavonoid content. Different drying processes can characterize different of flavonoid contents with two different solvents as a comparison.
Objective: This study aims to determine the total flavonoid levels of ethanol extract of dry shallot leaves obtained from maceration extraction methods and fractionation.
Methods: In this study, the simplicia of shallots were dried at a temperature 30, 40, and 60 °C and each was macerated using 96% ethanol as a solvent. The highest flavonoid content was fractionated using ethyl acetate as a solvent. Comparison between the simplicia and the solvent used is 1:10, then the extract obtained was carried out with of the total flavonoid levels were determined using UV-Vis spectrophotometry.
Results: The result showed that the highest flavonoid content of shallots ethanol extract are 2.715±0.005 mg QE/g samples and ethyl acetate fraction 115.7±0.025 ng QE/g samples with dried at 40°C.

Keywords: Shallot, flavonoid content, maceration, UV-Vis spectrophotometry

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INTRODUCTION

Shallots (*Allium cepa* L.) are one of the important vegetables to add flavor, such as processed fish and meat dishes. It can also be consumed raw as in processed "satay" in Indonesia and as daily food (Mang *et al*., 2019). Shallots (*Allium cepa* L) contain flavonoids wich have pharmacological effects that are good for health (Fossen *et al*., 1998). High flavonoids have benefits that can reduce of the hypolipidemic effects (Pérez-Gregorio *et al*., 2011; Prakash *et al*., 2007). The main compounds in shallots are flavonoids such as quercetin, quercitrin, and routine, saponins, sulfur compounds such as pectin, allyl propyl disulfide and allisin (Cassol *et al*., 2019) with health enhancing effects.

Flavonoids are one of the compounds that cannot withstand heat and are easily damaged at high temperatures in the extraction process (Katsampa *et al*., 2015). The extraction methods that has been reported such as subcritical water, ultrasound assisted extraction, extractor machine (Fossen *et al*., 1998; Ko *et al*., 2011; Piechowiak *et al*., 2020; Sinha *et al*., 1992). These extraction methode was a method that has been used as an advancement, but these methods considered expensive in terms of production and maintenance. However, conventional methods become an alternative to use.

Macerated extraction method is a simple method of extraction with the principle of immersion and stirring of the sample in a suitable solvent in extracting flavonoid compounds in the sample, compared to other methods in maceration extraction having the advantage of using more solvents than other extraction methods. This method is better because the extraction process is at room temperature to prevent flavonoid damage (Hidayati *et al*., 2018). Optimization that can be done on the maceration method is to vary the ratio of the number of samples and solvents used. Factors that can affect the amount of yield and total levels of extracted compounds include the type of solvent and the amount of solvent used (Sapiun *et al*., 2020). Maceration is a method that has been widely used for the extraction that are easily damaged. This methods effectively used for volatile compound such as flavonoid (Kiassos *et al*., 2009; Setiani *et al*., 2017). Hence it could be hypothesised that flavonoids can have extracted by maceration because it is cheaper methods and easily to use.

MATERIALS AND METHODS

Materials

Bima variety of shallots purchased from Brebes farmers, Central Java, Indonesia with GPS latitude location: -6° 52' 59.99" S and longitude location: 109° 02' 60.00" E. Dry onions using an oven (Sharp, Japan) at a temperature of 30, 40 and 60^{0}C, alcohol, ethyl acetate, $AlCl_3$, distilled water, acetic acid, quercetin, methanol (pa merck) and silica Gel GF_{254} (Merck), UV-Vis spectrophotometer (Genesys, China), HPLC (Shimadzu Prominance 2030C3D, Japan)

Sample treatments

Shallots are separated by dirt from foreign materials such as soil and unnecessary parts such as epidermis and grass residue. The shallots are then washed to remove soil residue and other impurities under running water. Washing is carried out as short as possible so as not to remove nutritious substances from the onions. The shallots are then chopped to speed up the drying process, chopping is done using a special chopper machine to obtain thin slices and the same size. Shallots are dried with

three temperature variations of 30, 40 and 60°C as long as constant weight is obtained (water content ≤ 10%).

Extraction methods

Maceration is a technique used to withdraw or remove the desired compound from a solution or solid by immersion in the material to be extracted. The refined sample was immersed in an organic solvent for some time (Ibrahim and Marham, 2013).

According to (Zhang *et al.*, 2018), this process is very beneficial in isolating compounds from natural materials because in addition to being cheap and easy to do, immersion of plant samples will result in the breakdown of cell walls and membranes due to differences in pressure between inside and outside the cell, so that the secondary metabolites inside The cytoplasm will dissolve in organic solvents and compound extraction will be perfect because it can be adjusted for the length of immersion that is carried out. The solvent that flows into the cell can cause the protoplasm to swell and the cell content will dissolve according to its solubility. The choice of solvent for the maceration process will provide high effectiveness by taking into account the solubility of natural compounds in the solvent.

Ethanol is a common solvent and is widely used by industry, has a low boiling point and tends to be safe to use. Ethanol has a boiling point of 70°C so that the extraction temperature used can attract all the components in the raw material (Kealey *et al*, 2004). The physical properties of ethanol are mainly influenced by the presence of the hydroxyl group and the short chain of ethanol carbon (Suarasa, 2011). Based on the results of research by Umar (2008) on optimization of flavonoid extraction with ethanol solvent, the highest flavonol content was obtained at 70% ethanol concentration with the ratio of raw material: solvent is 1:10. The solvents used in this study were ethanol and ethyl acetate.

Spectrophotometric analyzes

Sample testing was carried out by UV-Vis spectrophotometry following the steps as follows: Preparation of a 1000 ppm quercetin standard solution by weighing 10 mg of quercetin standard powder dissolved with 10 ml methanol in a volumetric flask. Determining the maximum wavelength is done by making a standard solution of quercetin 100 ppm which is then reacted with 1 ml of 2% AlCl3 in a test tube. Then 8 ml of 5% acetic acid was added to the solution and readings were carried out over a wavelength range of 400-500 nm using a UV-Vis Spectrophotometer. Making quercetin standard series solutions is made by diluting the standard 1000 ppm quercetin solution to 10 ppm, 20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm with the solvent is methanol. The results obtained were then processed by Ms. Excel so that the equation y = ax + b is obtained. The ethanol extract sample and ethyl acetate fraction were weighed each to obtain a concentration of 1000 ppm extract. From the extract, 1 ml was taken and added by volume of aluminum chloride ($AlCl_3$) 2% into the test tube. Add 8 ml of 5% acetic acid so that the volume is exactly 10 ml. The solution mixture was shaken until homogeneous and the solution incubated for 20 minutes. Furthermore, the absorption of the solution was measured using a UV-vis spectrophotometer at the maximum wavelength that had been obtained. The final flavonoid value was calculated using a formula Flvonoid content: $\frac{C \times V}{m}$. The quercetin concentration is calculated using the equation of the standard standard curve, V = volume of extract, M = weight of extract.

RESULTS AND DISCUSSION

After the shallots becomes simplicia, research is conducted on non-specific and specific characterization, so that the following results are obtained: The sample used was *Allium cepa* L. var. Garden Onion of Brebes taken in the area of Brebes, Central Java which has been carried out an identification test at the Biology Laboratory of the State University of Semarang, Central Java, Indonesia with the results of the *Allium cepa* L. var specimen. Garden Onion of Brebes After the plants are harvested, wet sorting is carried out, washing with running water, drying in three ways, namely drying, drying, direct sunlight and using an oven. Then do dry sorting, packing and storage. After that, it is continued with simplicia testing which aims to obtain good quality simplicia and meet the standardization of the Indonesian Herbal Pharmacopoeia Edition I (2008):

Table 1. Yield of dry shallots Allium cepa

Temp drying (°C)	Weight of wet shallot (gram)	Time of drying (hour)	Dry shallots (gram)
30	1005,59±0,123	120	193,72±0,023
40	1005,83±0,132	96	182,58±0,104
60	1005,89±0,143	90	140,29±0,124

Based on the results of drying shrinkage research that has been carried out with three drying temperatures of drying with temperatures of 30 (193,72±0,023), 40 (182,58±0,104), 60 (140,29±0,124) and based on the results of ANOVA calculation of shrinkage drying for the three drying temperatures shows that the sig value <0.05, which means that Ho is rejected or the average drying loss for the three drying temperatures is significantly different. It can be seen that the method of drying onion simplicia has an effect on drying shrinkage of shallot simplicia.

Table 2. TLC profile of shallots

No	Sample	Temp (°C)	Rf	HRf
1	Quersetin standart		0.9375±0,0135	93.75±1,35
2	Ethanol exctract 70%	30	0,9240±0,0043	92,40±0,43
		40	0,8734±0,0072	87,34±0,72
		60	0,8734±0,0095	87,34±0,95

Using TLC with drying done by drying has an Rf value, namely Rf ethanol extract with drying 30, 40, 60℃ were 0,9240, 0,8734, 0,8734 approaching the Rf value of the comparison contained in the Indonesian Herbal Pharmacopoeia Edition and the standart of quersetin Rf value 0,9375.

Total flavonoids from shallots ethanol extract were determined by measuring the color absorption with $AlCl_3$ reagent. Quercetin is used as a standard flavonoid because it is a flavonol group that has a keto group at C-4 and has a hydroxy group at C-3 or C-5 atoms so that it can form a color complex with $AlCl_3$ (Desmianty, 2009). Acetic acid is added as a stabilizer to C-4 carbonyl and 3 or 5-OH. The maximum wavelength and operating time are determined before determining the concentration of flavonoids. The maximum wavelength was obtained at 415 nm according to research conducted by Ipandi *et al* (2016). Total flavonoids were determined with the addition of $AlCl_3$, namely 415 nm and operating time obtained at 23 minutes. Quercetin standard curves were made by connecting the concentration of six quercetin series solutions with the absorbance at a maximum wavelength of 415 nm.

The results of determining the standard quercetin curve can be seen in the following figure.

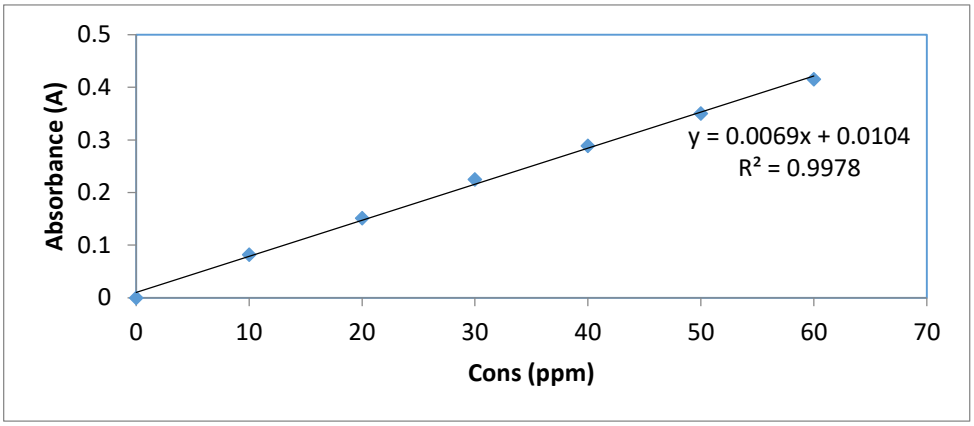

Figure 1. Absorbance vs consnetrasi series of quersetin

Measurement of quercetin standard absorbance as shown in Table 3. produces a linear regression equation y = 0.0069x + 0.0104 with a value of r = 0.9978. the resulting r value indicates that the standard curve produced has an accuracy of 99.78%, a method is said to be good if the r value produced is close to 1 or in the range 0.95-1. Furthermore, the linear regression equation is used in determining total flavonoid level in ethanol extracts of shallots.

Table 3. Flavonoid content of shallots extracts and fraction

Extraction	Solvent	Temp (⁰C)	Weight of Exctract	Yield (%)	Flavonoid content (mg QE/g extract)	Flavonoid content (mg QE/g fraction)
Maceration	Ethanol 70%	30	46,75±2,99	23.37±1,49	1,815±0,315	96,2±0,128
		40	30,01±5,76	15,00±2,88	2,715±0,005	115,7±0,535
		60	38,93±1,28	19,47±0,64	2,159±0,151	102,3±0,432

Spectrophotometry UV-Vis analysis revealed that a shalloots extract and fraction are good source of flavonoid content (Piechowiak *et al*., 2020). Ethyl acetate is a semipolar solvent, so it is thought to attract polar and nonpolar chemical compounds. Ethyl acetate can dissolve semipolar compounds in cell walls such as flavonoid aglycones. Flavonoid aglycones in plants are polyphenols that have chemical properties such as phenolic compounds (Mangkasa *et al*., 2018). Research by (Muhridja *et al*., 2016), extraction using semi-polar solvents such as chloroform, diethyl ether or ethyl acetate can bind low polarity flavonoids such as isoflavones, flavanones, methyl flavones and flavonols.

CONCLUSION

Shallots ethanol extract contains the highest levels of total flavonoids at drying temperature 40⁰C, extracted by maceration method with a ratio of 1:10, quantitative test of onion ethanol extract contains flavonoids with quercetin standard 2,715±0,005 mg QE/g and ethyl acetate fraction with a level of 115.7 mg QE /g.

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