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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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 which have pharmacological effects that are good for health (Fossen et al., 1998). High flavonoids have benefits that can reduce the hypolipidemic effects (Pérez-Gregorio et al., 2011; Prakash et al., 2007). The main compounds in shallots are flavonoids such as quercetin, quercitrin, and rutin, saponins, sulfur compounds such as pectin, alky 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 methods 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 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°C, alcohol, ethyl acetate, AlCl3, distilled water, acetic acid, quercetin, methanol (pa merck) and silica Gel GF254 (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 78°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 (Suarsa, 2011). Based on the results of research by Umair (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 100 ppm extract. From the extract, 1 ml was taken and added by volume of aluminum chloride (AlCl₃) 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 Flvnonoid content: \( \frac{C \times V}{m} \). The quercetin concentration is calculated using the equation of the standard curve, \( V = \) volume of extract, \( M = \) weight of extract.

**RESULTS AND DISCUSSION**

After the shallots become 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 1 (2008):

### Table 1. Yield of dry shallots *Allium cepa*

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

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

<table>
<thead>
<tr>
<th>No</th>
<th>Sample</th>
<th>Temp (°C)</th>
<th>Rf</th>
<th>HRf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Quersetin standart</td>
<td>0.9375±0,0135</td>
<td>93.75±1.35</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Ethanol extract 70%</td>
<td>0.9240±0,0043</td>
<td>92.40±0.43</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.8734±0,0072</td>
<td>87.34±0.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.8734±0,0095</td>
<td>87.34±0.95</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>0.8734±0,0095</td>
<td>87.34±0.95</td>
<td></td>
</tr>
</tbody>
</table>
Using TLC with drying done by drying has an Rf value, namely Rf ethanol extract with drying 30, 40, 60°C were 0.9240, 0.8734, 0.8734 approaching the Rf value of the comparison contained in the Indonesian Herbal Pharmacopoeia Edition and the standard of quersetin Rf value 0.9375. Total flavonoids from shallots ethanol extract were determined by measuring the color absorption with AlCl₃ 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 AlCl₃ (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 AlCl₃, 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](image)

Measurement of quercetin standard absorbance as shown in Table 3. produces a linear regression absorbance 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

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Solvent</th>
<th>Temp (°C)</th>
<th>Weight of Extract</th>
<th>Yield (%)</th>
<th>Flavonoid content (mg QE/g extract)</th>
<th>Flavonoid content (mg QE/g fraction)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maceration</td>
<td>Ethanol 70%</td>
<td>30</td>
<td>46.75±2.99</td>
<td>23.37±1.49</td>
<td>1,815±0.315</td>
<td>96.2±0.128</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40</td>
<td>30.01±5.76</td>
<td>15.00±2.88</td>
<td>2,715±0.005</td>
<td>115.7±0.535</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60</td>
<td>38.93±1.28</td>
<td>19.47±0.64</td>
<td>2,159±0.151</td>
<td>102.3±0.432</td>
</tr>
</tbody>
</table>

Spectrophotometry UV-Vis analysis revealed that a shallots 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., 2016). 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 40°C, 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.

**REFERENCES**

Ultrasonication Methods on Indigofera Tinctoria Linn Leaf Extraction. Jurnal Bahan Alam Terbarukan, 7(1), 54–58. https://doi.org/10.15294/jbat.v7i1.11405


