Identification and Determination of Total Flavonoids in Ethanol Extract of Old and Young Angsana Leaves (Pterocarpus indicus Willd.) Using Visible Spectrophotometry

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Abstract: *Angsana* (*Pterocarpus indicus Willd*.) is a forest plant widely used as an ornamental garden and as a shade. In many countries, *Angsana* is used as a traditional medicine, such as an antidiabetic drug. The compounds that act as antidiabetes are flavonoids. The purpose of this study was to determine the total flavonoid content of ethanol extract of old and young *Angsana* leaves using visible spectrophotometry. The wilstater test was performed as a qualitative research test to show that the ethanol extract of *Angsana* leaves contains flavonoid compounds. $AlCl_3$ was selected as a reagent in visible spectrophotometry to determine the flavonoid of *Angsana* leaf. The result of the accuracy test was 83.67% with acceptance criterion 80-120% and the precision test is 8.96% with acceptance criterion ≤10%. The total flavonoid levels of ethanol extract of old and young *Angsana* leaves were respectively 7.53 ± 0.32% w/w and 3.31 ± 0.07 % w/w.

1 INTRODUCTION

Angsana is one type of forest plant that is widely used as a protective tree and decoration for city parks. *Angsana* is now widely cultivated. In big cities, *Angsana* easily found on the highway. *Angsana* trees are dense and have beautiful flowers, so they are widely used as urban decorative plants, especially as shade plants, noise absorber, and pollution absorber (Bramasto *et al*., 2015). In many countries, *Angsana* is used as a traditional medicine. In Indonesia, the young leaves of *Angsana* are used as an ulcer medicine and the rash of prickly heat. In recent years, the Philippines has launched an *Angsana* extract product in the form of herbal tea preparations and pills used to treat leprosy, menstrual pain, flu, rheumatoid arthritis, and diabetes (Thomson, 2006). The Chemical content of *Angsana* leaves showed positive tests of phenol, flavonoid, saponin, triterpenoid and tannin compounds (Junanto *et al*., 2008). The general public knowledge around utilizing old *Angsana* leaves is limited to shade and as animal feed. The public has not come to know that the leaves of old *Angsana* can also be used as a traditional medicine. Until now, there has been no research that identifies and determines the total flavonoid levels in old and young *Angsana* leaves. For that, the researchers intend to do research on the identification and determination of flavonoid levels of old and young *Angsana* leaves. Flavonoid compounds are generally slightly soluble in water since 96% ethanol was used as the extraction solvent. The identification was done by wilstater test and determination of flavonoid level by a visible spectrophotometric method with the $AlCl_3$ reagent.

2 MATERIAL AND METHOD

2.1 Plant Material

Angsana leaves were collected from the Sidoarjo and Gresik Regencies. Plant determination of *Angsana* was done by LIPI, Purwodadi, Pasuruan.

2.2 Chemical

96% ethanol, standard quercetin (Sigma), aluminium chloride ($AlCl_3$), sodium acetate, aquadest, and magnesium.

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2.3 Instrument

UV-Vis Spectrophotometer X-ma 1200 Human Corp., macerator, vacuum rotary evaporator, analytical scales, and analytic glassware.

2.4 Preparation and Extraction

Angsana leaves, both old and young, were taken at random and then sorted and washed until clean, then chopped and dried respectively. The dry leaves were then milled. The preparation of the extract was carried out by maceration by dissolving 200 g of simplicia with 1000 mL of 96% ethanol solvent. The result of maceration was then concentrated using Rotary Vaccum Evaporator until a viscous extract was obtained.

2.5 Qualitative Test

2 ml of *Angsana* leaves extract were taken and put into a tube. 0.5 ml of concentrated HCl and 0.02 mg of Magnesium were then added and mixed. The presence of flavonoids is characterized by the occurrence of discoloration. The reduction with concentrated Mg and HCl produced red, yellow or orange colors (Robinson, 1995).

2.6 Quantitative Test

2.6.1 Quercetin Standard Curve

Quercetin was weighed for as much as 50 mg and inserted into a 50 mL measuring flask, then dissolved with 96% ethanol. Then, it was diluted through 20 consecutive concentrations; 40; 60; 80; and 100 ppm. 5 ml were added in 15 ml of 96% ethanol, 1 ml of 10% aluminum chloride, 1 ml of 1 M sodium acetate, and 28 ml of aquadest. Then, the mixture was incubated at room temperature for 30 minutes. The blank sample production was done without the addition of aluminum chloride. The next stage was the measurement standard curve level using visible spectrophotometry with a wavelength of 439 nm (Chang *et al*., 2002). Then, a calibration curve was made by connecting the absorption value and the concentration.

2.6.2 Determination of Flavonoid Levels

Samples of 100 mg were weighed and inserted in a 100 mL measuring flask and then dissolved with 96% ethanol. Samples of 5.0 mL were each added with 15 ml of 96% ethanol, 1 ml AlCl3, 1 ml of 1 M sodium acetate, and 28 ml of aquadest. Then, they were incubated at room temperature for 30 minutes. The next stage was sample rate measurement using visible spectrophotometry with a wavelength of 439 nm.

2.6.3 Accuracy and Precision

A sample of 50 mg was weighed and inserted in a 50 mL measuring flask and then dissolved with 96% ethanol. A standard of 1000 ppm was made by weighing 50 mg quercetin dissolved in 50 ml ethanol 96%. 1 ml sample 1000 ppm was extracted using pipette and inserted into 100 ml measuring flask. 6 ml of 1000 ppm quercetin solution was added until the water surface reached the limit indicator. The flask was then shaken until the solution was perfectly mixed. 5 ml of each sample was extracted using pipette and added with 15 ml of 96% ethanol, 1 ml of aluminum chloride, 1 ml of 1 M sodium acetate and 28 ml of aquadest. Then, it was incubated at room temperature for 30 minutes. The next step was to measure the sample content using visible spectrophotometry with a wavelength of 439 nm (Chang *et al*., 2002). The procedure was replicated 6 times (Riyanto, 2014).

3 RESULTS AND DISCUSSION

Flavonoid compounds can be separated from various other compounds by maceration using 96% ethanol solvent for analysis. The maceration results were then concentrated using a vacuum rotary evaporator, so as to produce a sample in the form of thick extracts. Then, the extract was evaporated again through aeration to produce dry extract. The separated flavonoids were determined using visible spectrophotometry. The standard comparison used was quercetin, where quercetin is a type of flavonoid compound that is most widely distributed in nature.

The results of determination indicate that the sample used in this study was *Angsana* with *Pterocarpus indicus Willd* species. Extraction resulted in 200 g of *Angsana* leaf sample, which was produced through 96% ethanol solvent with as much as 1000 mL by maceration method, resulting in a thick extract of each 2.4 g for old *Angsana* leaves and 12.4 g for young *Angsana* leaves. Qualitative test results showed positive results with change of color from dark green to yellowish green. Flavonoids are compounds containing two aromatic rings with more than one hydroxyl group. Reduction with concentrated Mg and HCl produces red, yellow, or orange colors (Robinson, 1995).

Before conducting a quantitative test, the determination of the maximum wavelength was done using quercetin work standard with a concentration of 80 ppm in ethanol solvent 96% pa. The absorbance reading was carried out at a wavelength of 400 - 800 nm. The wavelength produced a determination of total flavonoid of 439 nm. The wavelength was then used to measure the uptake of the calibration curve and the samples of *Angsana* leaf extract.

This study began with a verification test. Verification testing is an analytical method used to prove that the laboratory concerned is capable of testing using the method with valid results (Gandjar and Rohman, 2007).

The calibration curve obtained linear regression equation, that is y = 0.008x - 0.002, with correlation coefficient r = 0.999. The *angsana* leaf extract was then tested by the quantitative colorimetric method. The principle of the method of colorimetry is the formation of a complex between aluminum chloride and ketone groups at C-4 and hydroxy groups at adjacent C-3 and C-5 of flavon and flavonol groups. The compound used as a standard for the determination of this flavonoid level is quercetin since quercetin is a flavonoid group having keto groups in C-4 atoms and also hydroxyl groups on adjacent C-3 and C-5 (Azizah *et al*., 2014).

In the determination of total flavonoid levels, the addition of sodium acetate was intended to detect the presence of a 7-hydroxyl group (Mabry *et al*., 1970), while the 30-minute incubation treatment carried out was intended to allow the reaction to run perfectly, thus providing maximum color intensity. The determination of total flavonoid content from old and young *Angsana* leaf extract resulted in *Angsana* content of 7.53% ± 0.32% w/w (Table 1) and 3.31 ± 0.07% w/w (Table 2), respectively. Based on the results obtained between old and young *angsana* leaves, it was found that the old *angsana* leaves contain more flavonoids. Therefore, the use of old *angsana* leaves is more recommended than young *angsana*, especially when traditionally used by the community.

Table 1: Quantitative test result of old *angsana* leaves.

No	The weight of the sample (g)	Absor bance	Flavonoids in the sample (mg/100 mL)	%w/w
1	0.1005	0.307	7.725	7.687
2	0.1004	0.313	7.844	7.844
3	0.1004	0.279	7.025	6.997
4	0.1006	0.301	7.575	7.530
5	0.1007	0.303	7.625	7.572
x				7.53
SD				0.32

Table 2: Quantitative test result of young *angsana* leaves.

No	The weight of the sample (g)	Absor bance	Flavonoids in the sample (mg/100 mL)	%w/w
1	0.1001	0.114	2.9009	2.898
2	0.1004	0.125	3.1769	3.164
3	0.1003	0.136	3.4525	3.442
4	0.1004	0.138	3.5031	3.489
5	0.1004	0.141	3.5784	3.564
x				3.31
SD				0.07

Previously, precision test and accuracy test with a recovery of 83.67% and RSD of 8,96% have been done (Table 3). The acceptance criteria used in the study are 80-120% for accuracy, and from the achievement of ≤ 20% precision it can be concluded that the precision and accuracy test has met the requirements (Anonim, 2004).

Table 3: Test Result of Accuracy and Precision.

No.	The weight of the sample (g)	Sample (Abs)	Conc. Sample (ppm)	Sample+ Quercetin (Abs)	Conc. Sample + Quercetin (ppm)	Accuracy (%)
1.	0.0503	0.068	8.7327	0.471	59.2974	84.275
2.	0.0500	0.075	9.6110	0.415	52.2710	71.100
3.	0.0500	0.042	5.4705	0.486	61.1794	92.848
4.	0.0500	0.059	7.6035	0.442	55.6587	80.092
5.	0.0506	0.071	9.1092	0.491	61.8086	87.832
6.	0.0500	0.077	9.8620	0.492	61.3920	85.883
x						83.67
SD						7.45
RSD (Precision)						8.96

4 CONCLUSIONS

Based on the results, it can be concluded that the extract of old and young *Angsana* leaves contains flavonoid compound with total levels of flavonoids of ethanol extract being 7.53 ± 0.32% w/w and 3.31 ± 0.07% w/w, respectively.

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