Advances in Social Science, Education and Humanities Research, volume 521
Proceedings of the First International Conference on Health,
Social Sciences and Technology (ICoHSST 2020)

Comparison of the Antioxidant Activity of Cashew (*Anacardium occidentale* L.) Leaf Extract with the Soxhletation and Reflux Extraction Methods

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***Abstract*-Cashew is a plant that has antioxidant benefits. Extraction by hot method is more perfect and the extraction process is faster than cold extraction. This study aims to compare the antioxidant activity of cashew leaf extract obtained by means of soxhletation and reflux. This research is an experimental method by measuring the percentage of attenuation on DPPH and IC_{50} of cashew leaf extracts extracted by soxhletation and reflux methods. The result of this research is that the antioxidant activity of soxhletation cashew leaf extract is 9.08 ppm and reflux is 9.56 ppm. It was concluded that the antioxidant activity of soxhletation cashew leaf extract was greater than that of reflux cashew leaf extract.**

Keywords: antioxidant activity, cashew leaves, soxhletation, reflux.

I. INTRODUCTION

Indonesia has biodiversity potential as medicinal plants, one of which is the cashew plant (*Anacardium occidentale* Linn). Cashew are a plant that has many health benefits, young cashew leaves are consumed as a medicine for high blood pressure (hypertension), diabetes (diabetes mellitus), malaria, rheumatism, canker sores, and skin rashes [1].

Cashew leaves contain flavonoid compounds that act as antioxidants that can ward off free radicals in the body [2]. According to [3], it is known that the ethanol extract and all ethanol fractions of cashew leaves contain flavonoids, tannins / polyphenols, and steroids (except for the ethanol fraction which does not contain steroids).

Antioxidants are electron-giving compounds or reductants. Antioxidants can inhibit oxidation reactions, by binding to free radicals and highly reactive molecules so that cell damage is inhibited [4]. Free radicals are molecules or molecular fragments that contain one or more unpaired electrons in their outer orbitals. To achieve stability, these free radicals will look for their electron pairs so that they are very reactive [4]. Free radicals can come from within the body as part of the metabolic process. Meanwhile, free radicals from outside the body can be caused by infections, exposure to pollutants, toxins, alcohol, drugs, radiation and poor diet [5].

One of the most common methods for testing antioxidant activity is using the free radical 1,1-diphenyl-2- picrylhydrazil (DPPH). Measuring antioxidants with DPPH is a simple, fast method, and does not require a lot of reagents like other methods [6]. DPPH which reacts with antioxidants will change color from purple to yellow, the intensity of the color depends on the antioxidant ability.

One of the factors that affect the quality of the extract is the extraction method [7]. In this study, the comparison of the extraction method of soxhletation

and reflux was tested. Both are hot extraction, where the process is faster and more complete than the cold requires less solvent and is relatively constant so that the extraction process is efficient [8].

In [9] showed that the antioxidant activity of refluxed parang romang ethanol extract was greater than the macerated extract, with IC_{50} of 30.58 ppm and 100.89 ppm, respectively. In the research of [7], it was found that the antioxidant activity of the ethanol extract of guava leaves using the soxhletation method was greater than the antioxidant activity of the maceration extract with IC_{50} of soxhlet extract was 37.67 ppm and the maceration extract was 47.80 ppm.

Based on the above, the hot extraction method has higher antioxidant activity than the cold extraction method. This study was to test the antioxidant activity of the ethanol extract of cashew leaves extracted by soxhletation compared to the antioxidant activity of the reflux extract.

II. RESEARCH METHODS

This research is an experimental study, by comparing the antioxidant activity of cashew leaf extract obtained by soxhletation and reflux extraction. Measured the attenuation power of cashew leaf extract against DPPH free radicals (1,1- diphenyl-2-picrilhydrazil) using UV-Vis spectrophotometry and calculating the IC50 of each cashew leaf extract resulted from shock and reflux.

The research sample is young cashew leaves obtained from Kayuagung Ogan Komering Ilir.

2.1. Work Procedures

2.1.1. Extraction of Cashew Leaves

Young cashew leaves are washed, finely chopped, and dried, then extracted in two different ways, namely soxhletation and reflux, using 96% ethanol solvent.

2.1.2. Identification of secondary metabolites

The extracts resulting from soxhletation and reflux were identified respectively for the presence of flavonoids and tannins using Mg metal + concentrated HCl to identify flavonoids and $FeCl_3$1% for tannin identification.

2.1.3. Preparation of Test Solutions

Consists of DPPH solution, blank solution, sample solution, and comparison solution

2.1.4. Antioxidant Activity Test

2.1.4.1. Measurement of the Percentage of Free

Radical Reduction for DPPH Namely by measuring the amount of DPPH radical absorption resistance by calculating the percentage of DPPH absorption inhibition using the formula: % DPPH Inhibition = (Abs blank- Abs sample) / (Blank abs) x 100%

2.1.4.2 Measurement IC_{50} sample

Based on the relationship between the percentage of inhibition and the concentration of each extract, a linear regression equation is obtained:

$$y = Ax + B$$

IC_{50} is obtained from the value of x, after y is replaced by 50. The smaller the IC_{50}, the greater the antioxidant activity of a sample (12).

2.2. Data Analysis

To analyze the differences in antioxidant activity between the two extraction methods, the Independent Sample T Test analysis was used.

III. RESULT

In this study, it was found that the average yields of cashew leaf extract with the soxhletation and reflux methods were 31.8237% and 23.8781%, respectively.

Table 1. Yield of Cashew (Anacardium occidentale L.) Leaf Extract

Sample	Time	Linear regression equation	IC_{50}
Cashew Leaf Extract with Soxhletation Method	30 minute	y = 7,985x - 22,537	9,08
Cashew Leaf Extract with Reflux Method	30 minute	y = 9,898x – 44,906	9,56

In table 1, it was found that the cashew leaf extract with the soxhletation and reflux methods contained flavonoids and tannins which were marked by changing the color of the sample solution to red for flavonoids and black for tannins.

Table 2. Identification Results of Flavonoids and Tannins in Soxhletated and Reflux Cashew Leaf Extract

Sample	t (minute)	Concentration	% Inhibition
Cashew Leaf Extract with Sokletation Method	30 minute	6 ppm	25,2014%
		8 ppm	41,6935%
		10 ppm	57,1431%
Cashew Leaf Extract with Reflux Method	30 minute	6 ppm	15,4038%
		8 ppm	32,4248%
		10 ppm	54,9941%

Soxhletasi cashew leaf extract has an IC50 of 9.08 ppm. Meanwhile, reflux cashew leaf extract has an IC50 of 9.56 ppm.

Table 3. Average percent of inhibition of cashew leaf extract with the soxhletation and reflux method

	Flavonoids		Tanins	
	Color with reagent	The result	Color with reagent	The result
Cashew Leaf Extract with Sokletation Method	Red	Contains Flavonoids	Black	Contains tannins
Cashew Leaf Extract with Reflux Method	Red	Contains Flavonoids	Black	Contains tannins

In table 3 it can be seen that the percent inhibition of cashew leaf extract with the soxhletation method is higher than the inhibition percent of the cashew leaf extract with the reflux method.

Table 4. The IC50 average of the cashew leaf extract with the soxhletation and reflux methods

Sample	Replication	Weight of Simplicia (g)	Weight of Extract (g)	Yield (%)	Mean (%) ± SD
Sokletasi	1	100	23,8307	23,8307	31,8237 ± 11,30
Extraction	2	100	39,8167	39,8167	
Refluks Extraction	1	100	22,5878	22,5878	23,8781 ± 1,82
	2	100	25,1684	25,1684	

IV. DISCUSSION

In this study, cashew (Anacardium occidentale L.) leaves were extracted by two methods, namely the soxhletation method and the reflux method. This study aims to measure the antioxidant activity of the cashew leaf extract from both extraction methods.

The selection of cashew leaves in this study is based on empirical use as a treatment for hypertension and diabetes mellitus [1]. Hypertension and diabetes mellitus are degenerative diseases that can be treated with antioxidants [10]. According to [3], the compounds in cashew leaves are flavonoids, tannins / polyphenols, and steroids. Flavonoids and tannins are antioxidant compounds that can ward off free radicals in the body. In this study, it was found that the cashew leaf extract with the soxhletation and reflux methods contained flavonoids and tannins which were marked by changing the color of the sample solution to red for flavonoids and black for tannins.

In this study, it was found that the average yields of cashew leaf extract with the soxhletation and reflux methods were 31.8237% and 23.8781%, respectively. This is in accordance with the research of [11], which states that the yield of pineapple peel extract with the soxhletation method is higher at 3.29% compared to the reflux and maceration methods, respectively 3.20% and 1, 23%. Then measured the antioxidant activity of soxhletation and reflux cashew leaves extract.

In table 3 it can be seen that the percent inhibition of cashew leaf extract with the soxhletation method is higher than the inhibition percent of the cashew leaf extract with the reflux method. In accordance with the research of [12], that the pineapple peel extract soxhletation method has the highest free radical inhibiting ability of 91.3% compared to the reflux method of 81.2% and the maceration method of 63.8%. Based on the relationship between concentration and percent inhibition of each extract, a linear regression equation is obtained, y = Ax + B, where the IC50 value is obtained from the x value after y is replaced by 50. Soxhletasi cashew leaf extract has an IC50 of 9.08 ppm. Meanwhile, reflux cashew leaf extract has an IC50 of 9.56 ppm. The lower the IC50, the higher the antioxidant activity of a sample [13],[14] . So extract cashew leaves soxhletasi has greater antioxidant activity than reflux cashew leaf extract. This is in accordance with the results of previous studies that the antioxidant activity of soxhletated pineapple peel extract was highest with an IC50 of 2.78 ppm compared to reflux and maceration method with an IC50 of 2.95 ppm and 3.18 ppm respectively [11]. This is because the soxhletation extraction is carried out repeatedly continuously which causes the chemical compounds in the sample to be completely extracted. However, the two extracts using the soxhletation and reflux methods both produce very strong antioxidant activity. Antioxidant activity is very strong if IC50 ppm, strong (IC50=50-100 ppm), moderate (IC50= 100-150 ppm), weak (IC50 =150-200 ppm) [13].

Furthermore, the antioxidant activity data were analyzed by using the Independent Sample T Test to determine the difference in antioxidant activity between the soxhletation and reflux extracts. The test results of the Independent Sample T Test obtained a sig value of 0.141 (p>0.05), which means that the antioxidant activity of the cashew leaf extract with the soxhletation and reflux methods did not have a significant difference.

V.CONCLUSIONS

Antioxidant activity of soxhletasi cashew leaf extract is greater than reflux cashew leaf extract, but not statistically significant. Shown by the percentage of inhibition of the soxhletation extract is greater than that of the reflux extract, likewise the IC50 of the soxhletation extract is smaller than that of the reflux extract.

ACKNOWLEDGMENT

This work was supported by Politeknik Kesehatan Kemenkes Palembang and Department of Chemical Engineering State Polytechnic of Sriwijaya Palembang.

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