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To cite this article: Hartati *et al* 2019 *IOP Conf. Ser.: Mater. Sci. Eng.* **551** 012111

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IOP Conf. Series: Materials Science and Engineering **551** (2019) 012111 doi:10.1088/1757-899X/551/1/012111

Effect of Extraction and Fractionation on Antioxidant Activity of Extract and Fraction *Crescentia Cujete* L Leaves

Hartati[1], Abd Muis[1], Narhaeda[1], Hasmida Mohd Nasir[2] and Nur Salsabila Md Norodin[3]

[1]Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Negeri Makassar, Indonesia
[2]Centre of Lipids Engineering & Applied Research (CLEAR), Ibnu Sina Institute for Scientific & Industrial Research, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia
[3]School of Chemical and Energy Engineering, Faculty Engineering, Universiti Teknologi Malaysia, 81310 Johor

E-mail: hartati@unm.ac.id

Abstract. Antioxidant compunds like phenols and flavonoids scavenge free radicals and thus inhibit the oxidative mechanisms that lead to control degenerative and other diseases. The aim of this study was to investigate the antioxidant activity in extracts and fractions of *Crescentia cujete* leaves. 1,1-difenil-2-pikrilhidrazil (DPPH) assay was performed to measure the free-radical scavenging activity of extracts. The results of the study show that leaves of *C. cujete* possesses significant free radical scavenging properties. All the ethanol extract, etyl acetat extract and fractions exhibited antioxidant activities, however, ethanol fractions of leaves showing the highest antioxidant activity based on the results of DPPH tests.

1. Introduction

Natural antioxidants occur in all parts of plants. These antioxidants include vitamins, carotenoids, flavonoids, phenols, dietary glutathionine, and endogenous metabolites. Plant-derived antioxidants have been shown to function as singlet and triplet oxygen quenchers, free radical scavengers, peroxide decomposers, enzyme inhibitors, and synergists [1]. The phytochemicals in plant tissues responsible for the antioxidant capacity can largely be attributed to the phenolics, anthocyanins, and other flavonoid compounds [2]

Crescentia cujete L Family of Bignoniaceae is commonly known as calabash tree. It is widely distributed in the Caribbean region, Mexico, Northern and Southern American and later introduced to tropical Africa from Senegal to Cameroon then to other parts of Africa [3]. In Indonesia, it is known as Maja or Bila. Maja leaves in traditional medicine are used to treat new wounds and reduce hypertension. The powdered leaves are used for headaches, and internally as a diuretic and in the treatment of tumors and hematomas [4]. This plant contains active compounds namely tartrate acid, stenohidric, citric acid, stearic acid, palmitic acid, flavonoid and quercetin [5]. The aim of this study was to investigate the antioxidant activity in extracts and fractions of *Crescentia cujete* leaves.

2. Experimental

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2.1. Preparation Extracts

The leaves of the *C. cujete* was made into coarse powder. Five hundred gram of *C. cujete* was macerated in 70% ethanol for 3 days. The liquid component was filtered through whatman no.1 filter paper and evaporated to dryness under vacuum at 40°C using a rotary evaporator. All the step will be repeated using ethyl acetate solvent. The sample was stored under refrigeration (-20°C) condition for further analysis.

2.2. Fractionation of Active Extracts

The extract which showed the highest antioxidant activity was carried out fractionation. Fractionation is done by using a vacuum chromatography column to separate the compounds contained in it. The column was inserted PF254 silica gel as a stationary phase, then eluted using a mobile phase system using solvents which began with the lowest to high polarity level. Each fraction obtained was visualized to determine its profile using TLC aluminum plate GF254 (E-Merck) to determine the fraction to be combined. The combined fractions obtained were tested for antioxidant activity by using DPPH test.

2.3. Antioxidant activity of C. cujete

Antioxidant activity was measured by using DPPH assay. This assay was carried out according to the method [6] with a slight modification. Extract solution was prepared by dissolving 0.025 g of dry extract in 10 ml of methanol to give final concentration at 2.5 mg/ml. Then, 77 µL of the extract solution was mixed with 3 ml of 6 x 10^{-5} M methanolic solution of DPPH. After that, the mixture was placed in the dark for 30 minutes at room temperature and the decrease in the absorption was measured at 517 nm by using spectrophotometer. The DPPH radical concentration was calculated by using the following equation:

$$\text{DPPH radical concentration } (\%) = \frac{\text{A Control} - \text{A Sample}}{\text{A Control}} \text{ x } 100 \quad (1)$$

Where $A_{Control}$ is the absorbance value of the control reaction and A_{Sample} is the absorbance value with the presence of the tested extracts in the sample.

2.4. Analysis of Data

The results of these experiments are expressed as mean ± S.E, of three animals in each group. The data were evaluated by one-way ANOVA followed by Tukey's pair-wise comparison test. The values of $p < 0.05$ were considered as statistically significant.

3. Results and Discussions

3.1. Yield extract of C. cujete

Figure 1 shows that the result of yield on *C. Cujete* extract. There are two different solvents were used to extract the *C. cujete* which are 70% ethanol and acetate ethyl.

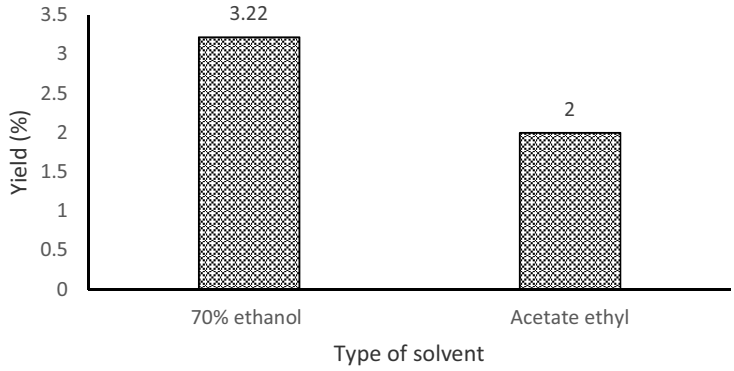

Figure 1. Percentage of extraction yield on different solvent

The figure 1 shows that the highest extraction yield was found with 70% ethanol solvent. On the other hand, acetate ethyl resulted in lowest extraction yield. Extracts using 70% ethanol solvent were higher than ethyl acetate solvents. The yield extract value was related to the number of secondary metabolites, the greater the yield value the more the content of secondary metabolites [7].

3.2. Activity antioxidant of C. cujete

The presented figure 2 shows that the DPPH free radical scavenging assay result of five extract from *C.cujete* leaves. The figure 2 show that the 70% ethanol fraction (75.43 %) gives highest DPPH radical scavenging activity compared to the other. This indicates the antioxidant ability of 70% ethanol fraction is stronger than the other extract. The radical scavenging activity of the extracts could be related to the nature of phenolics, thus contributing to their electron transfer/hydrogen donating ability. This plant contains active compounds namely tartrate acid, stenohidric, citric acid, stearic acid, palmitic acid, flavonoid and quercetin [5]. *C.cujete* leaves contain flavonoid and phenol which give antioxidant activity in vitro [8]. Flavonoid found in *C.cujete* can act as antioxidants and protect the cells of the body from radical damage [9]. Alkaloid in *C. cujete* may explain why it is being used as anti-inflammatory agents [5,10]. Other studies have shown that C. cujete contains Flavonoid-quercetin [11], tannins, phenols, saponins, anthraquinones and cardenolides [5]. tartaric acid, cyanohydric, citric acid, cresentia acid, beta-sitosterol, stigmastrol, alpa and beta amyrine, esteric acid, palmitic acid, apigenin, naphthaquinone, iridoid glycosides, 3-hydroxyoktanol glycosides [11]. Flavonoid-quercetin also found in *C.cujete* has activity as an antioxidant that protects the body cells from free radical damage that contribute to cell damage and various health-related problems [5]. *C. cujete* has significant wound healing activity [12].

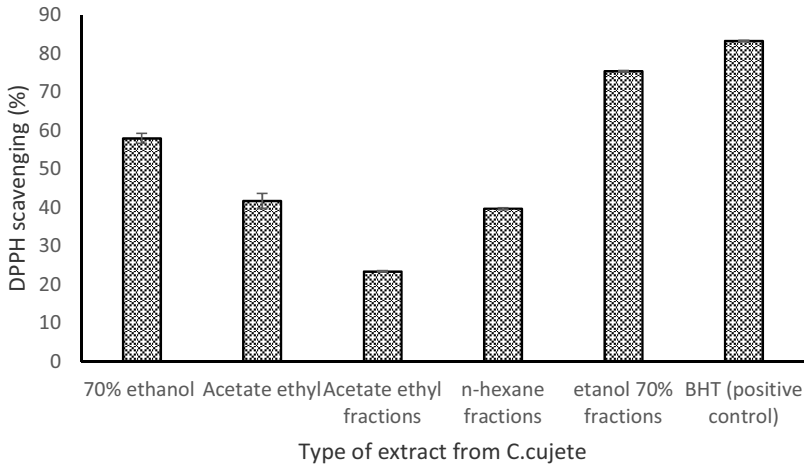

Figure 2. Percentage of DPPH Free radical scavenging from extract and fractions of *C.cujete.*

4. Conclusions

Crescentia cujete Leaves of the ethanol extract, etyl acetat extract and fractions exhibited antioxidant activities, however, ethanol fractions of leaves showing the highest antioxidant activity based on the results of DPPH tests.

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