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Research Article

Rambusa (*Passiflora foetida* L) vs. Free Radicals: In Vitro Study with DPPH Method

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ABSTRAK

Rambusa (*Passiflora foetida* L) adalah tumbuhan obat yang banyak terdapat di berbagai daerah di Indonesia, termasuk di Kalimantan Tengah. Beberapa bagian tumbuhan dari rambusa diketahui memiliki aktivitas antioksidan termasuk di bagian daunnya. Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan dari daun rambusa yang berasal dari Kalimantan Tengah. Metode yang digunakan adalah metode 2,2-diphenyl-1-picrylhydrazyl (DPPH) yang diukur serapannya dengan Spektrofotometer UV Vis. Hasil yang diperoleh menunjukkan nilai IC_{50} dari ekstrak etanol daun rambusa senilai 93,269 µg/mL. Meskipun nilai IC_{50} yang diperoleh lebih rendah dibandingkan senyawa antioksidan standar seperti kuersetin, nilai IC_{50} dari ekstrak etanol daun rambusa asal Kalimantan Tengah masih lebih tinggi dibandingkan daun rambusa yang diperoleh dari daerah lain

Kata Kunci—antioksidan, DPPH, Kalimantan Tengah, Passiflora foetida, rambusa

ABSTRACT

Rambusa (Passiflora foetida L) is a medicinal plant that is widely found in various regions in Indonesia, including in Central Kalimantan. Some parts of the plant of rambusa are known to have antioxidant activities including in the leaves. This study aims to determine the antioxidant activity of rambusa leaves from Central Kalimantan. The method used was 2,2-diphenyl-1-picrylhydrazyl (DPPH) method which was measured by UV Vis spectrophotometer. The results obtained showed IC_{50} values of ethanol extract of rambusa leaves worth 93.269 µg/mL. Although the IC_{50} values obtained were lower than the standard antioxidant compounds such as quercetin, the IC_{50} value of the ethanol extract of the leaves of rambusa from Central Kalimantan was still higher than that of the rambusa leaves obtained from other regions or some other medicinal plant extracts.

Keywords— antioxidants, DPPH, Central Kalimantan, Passiflora foetida, rambusa

I. INTRODUCTION

Free radicals are molecules that contain unpaired electrons in their outer orbitals. Free radicals are unstable and very reactive, so to achieve stability must react with other molecules. This high-reactive molecule can initiate a chain reaction in its formation to give rise to abnormal compounds and can damage important cells in the body (Phaniendra *et al*, 2015). Free radical compounds that attack the body's cells can relax various cell damage in the body. Cell damage can cause homeostasis and can also cause cancer, atherosclerosis, inflammation, asthma, diabetes, aging and cell death (Lobo *et al*, 2010). Free radicals not only come from radiation exposure, but from several sources that are very close to our daily environment such as cigarette smoke, drugs, vegetables contaminated with pesticides, stress and emotions (Sharma *et al*, 2013). When the amounts of free radicals exceed the body's capacity to neutralize it, oxidative stress is formed which causes damage to cell structures, tissues and organs, including those that play a role in the aging process (Suratno *et al*., 2019; Nita & Grzybowski, 2016).

Various efforts have been made to produce compounds that can inhibit or counteract free radicals commonly called antioxidants. Antioxidants are molecules that can donate electrons to free radical molecules, thus stopping the chain reaction (Pratama & Suhartono, 2018; Nimse & Pal, 2015). This type of antioxidant consists of two, namely natural antioxidants and synthetic antioxidants. Many natural antioxidants are found in plants, vegetables and fruits, while those included in synthetic antioxidants are butyl hydroxy anisole (BHA), butyl hydroxy toluene (BHT), propyl gallate, and ethoxyquin (Taghvaei & Jafari, 2015).

The ability of natural plants to ward off free radicals is inseparable from the content of secondary metabolites. For example, the Kelakai root (Stenochlaena palustris Bedd) reported by Adawiyah and Rizki (2018) has very high antioxidant activity. Another study by Wahdaningsih *et al* (2011) reported that the Fern Plants stem isolates (*Alsophila glauca* J. Sm) had lower radical capture antioxidant activity than quercetin as a positive control (IC_{50} 178.4 μg/mL compared with 2.17 μg/mL from quercetin). Yuhernita and Juniarti (2011) in their study also reported that there were potential antioxidant effects in surian methanol extract although lower than vitamin C. However, quite a lot of traditional medicinal plants that have antioxidant activity are not too high as found in the akar kuning (*Arcangelisia*

flava) stem ethanol extract as reported by Suratno *et al* (2019).

One natural plant that contains secondary metabolites and has the potential to counteract free radicals is rambusa (*Passiflora foetida* L). Previous studies have shown that various parts of plants from rambusa have high antioxidant activity, such as leaves, flowers, fruit peels, and seeds (Song *et al,* 2018; Sasikala *et al*, 2011; Sathish *et al*, 2011). However, research on antioxidant activity from rambusa has never been done before for samples originating from the island of Borneo, especially from Central Kalimantan. Though these plants are spread evenly in almost all areas of Central Kalimantan (Mulyani, 2019).

The purpose of this study was to determine the antioxidant activity of rambusa from Central Kalimantan. The part of the plant that will be used is the leaves, considering that the leaves of rambusa are known to have high antioxidant activity. The method used was with 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent as free radicals. The principle is the reaction of hydrogen capture by DPPH from antioxidant compounds contained in medicinal plant extracts. DPPH method itself is one method that is quite often used in testing antioxidant activity of medicinal plant extracts, as was done in the study of Rosawanti *et al* (2018) with extract of damang mahar leaves.

II. METHODS

The equipment and instruments used in this study include the maceration chamber, rotary evaporator, analytic balance, water bath, oven, micropipette, cuvette, vortex, and UV-Vis spectrophotometer. While the materials used were simplisia of rambusa leaves, ethanol 96%, methanol pro analytic, and 0.4 mM DPPH reagent.

The research was carried out in several stages consisting of the determination process of rambusa leaves, making simplicia, extraction, testing antioxidant activity with DPPH, and data analysis. Rambusa leaves are obtained from the region of Palangka Raya, Central Kalimantan and are determined using Materia Medika Indonesia. The leaves are then sorted and then dried and mashed until dry simplicia is formed. Extraction was carried out using 96% ethanol with maceration method for 24 hours followed by evaporation using rotary evaporator until thick extract was obtained.

The 0.4 mM DPPH reagent was made by dissolving 4 mg DPPH powder in pro analytic methanol on a 25 mL volumetric flask. The DPPH reagent absorbance is measured at the theoretical maximum wavelength of DPPH which is 512 nm.

The absorbance results from these solutions are used as controls. The test sample solution was made by dissolving the thick ethanol extract of rambusa leaves (EERL) using pro analytic methanol with various concentrations of 25, 50, 75, and 100 µg/mL. One mL of 0.4 mM DPPH solution was put into a 5 mL volumetric flask, then added with 4 mL sample solution. The solution was left to stand for 30 minutes then absorbance was measured with a UV Vis spectrophotometer at a wavelength of 512 nm.

Data analysis was done by making a standard curve equation with x axis in the form of sample concentration and y axis in the form of% inhibition from the sample. The sample inhibition value was calculated as the percentage difference between the absorbance of the sample and the absorbance control (DPPH) compared to the absorbance of the control. The IC_{50} value was calculated against the concentration of the sample which caused % inhibition of 50 percent of the control.

III. RESULTS AND DISCUSSION

The results of the antioxidant activity of EERL using the DPPH method showed relatively low results. Of the four test concentrations used, the decrease in absorbance which reached more than half the control absorbance was only shown at a concentration of 100 µg/mL. Roughly speaking, this implies that the decrease in the amounts of free radicals from the DPPH reagent which reaches 50% of the original amount is only shown at a concentration of 100 µg/mL, or in other words, the IC_{50} value of EERL is in the range of 100 µg/mL. This value is certainly very low when compared to standard comparative antioxidants such as quercetin with IC_{50} values of 2.17 µg/mL (Wahdaningsih *et al*, 2011), although the value is also still higher than other plant extracts such as akar kuning which has an IC_{50} value of 136.81 µg/mL (Suratno *et al*, 2019). The absorbance results from EERL along with% inhibition is presented in Table I.

Table I. The absorbance results from the ethanol extract of the leaves of rambusa

Sample	**Absorbance (nm)**		**% inhibition (µg/mL)** (Δ absorbance/ control)*100 %
	Measurement	**Δ absorbance** (control - sample)	
DPPH (control)	0.776	-	-
25 µg/mL EERL	0.652	0.124	15.979
50 µg/mL EERL	0.523	0.253	32.603
75 µg/mL EERL	0.448	0.328	42.268
100 µg/mL EERL	0.375	0.401	51.675

Note: DPPH = 2,2-diphenyl-1-picrylhydrazyl; EERL = ethanol extract of rambusa leaves

Through the absorbance value obtained, a standard curve for EERL is then made to obtain the standard curve equation. The equation is then used for IC_{50} calculations from EERL. From the calculation results obtained by the standard curve equation y = 0.467x + 6.4433 with the value R = 0.989, as shown in Figure 1. With this equation, IC_{50} of EERL is calculated from with the value obtained is 93.269 µg/mL.

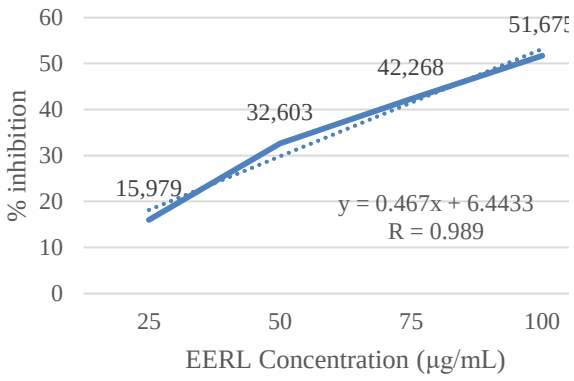

Figure 1. Graph of % Inhibition of EERL against DPPH

The IC_{50} value of the antioxidant from EERL although still lower than the standard antioxidant from natural ingredients such as quercetin, but the results turned out to be still higher than the IC_{50} value of other parts of rambusa or extract with different solvents. One of these studies is Fadillah *et al* (2017) who reported IC_{50} values from rambusa leaves extracted with methanol, ethyl acetate, n-hexane, and n-butanol solvents, respectively valued at 237.68, 648.912, 105.84 and 140.3 µg/mL. These results are in line with the research conducted by Sasikala *et al* (2011) which states that among other plant parts, the highest inhibition of DPPH reagents from rambusa is shown by the ethanol extract of the leaf part with IC_{50} 595.23 µg/mL. These results indicate that compared to other studies, the results obtained in this study showed higher antioxidant activity. One of the differences in results was caused by differences in the content of secondary metabolites from rambusa which grew in Central Kalimantan with other regions, so that there were differences in the antioxidant activity shown.

In addition, the results obtained also showed higher antioxidant activity compared to other plants that were both derived from the genus Passiflora. One of them is the research from Bandara *et al* (2018) which used methanol extract of *P. suberosa* leaves only showed IC_{50} values with DPPH method of 418.67 µg/mL. However, compared to the *P. ligularis* fruit peels acetone extract, the IC_{50} antioxidant value with the DPPH method obtained was still lower at 19.13 µg/mL (Saravanan & Parimelazhagan, 2014).

IV. CONCLUSION

Overall, this study has found the antioxidant activity of ethanol extract of rambusa leaves from Central Kalimantan

with the IC_{50} value of the DPPH method valued at 93.269 µg/mL. Although the IC_{50} value is still lower than standard compounds such as quercetin, but still higher than some other medicinal plants, especially those that are both derived from the genus Passiflora. Furthermore, isolation and identification of secondary metabolites from rambusa which have the highest antioxidant activity can be done to optimize the antioxidant activity of leaves of rambusa, especially those from Central Kalimantan

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