Antioxidant activity from ethanol extract of kupa leaves (Syzygium polycephalum (Miq.) Merr & L. M. Perry) using DPPH (2,2-diphenyl-1-picrylhydrazil) method

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Abstract. Kupa is one of the endemic plants from Indonesia belong to guava family (Myrtaceae). This research conducted to determine antioxidant activity of kupa leaves (Syzygium polycephalum (Miq.) Merr & L. M. Perry) ethanol extract. Extraction of kupa leaves was carried out by maceration method using ethanol 96%. The antioxidant activity test was done qualitatively and quantitatively. The qualitative antioxidant test was carried out by Thin Layer Chromatography (TLC) using mobile phase chloroform: methanol: formic acid (8: 2: 1) and DPPH 0.2% spotting appearance. Quantitative testing of antioxidant was carried out using DPPH (2,2-diphenyl-1-picrylhydrazil) assays. The TLC test results showed yellow spot on the DPPH with Rf value 0.381. Kupa leaves ethanol extract had the lowest IC50 of DPPH scavenging activity 10.327 ppm which was a very strong antioxidant.

1. Introduction
Traditional medicinal plants were natural ingredients that had been traditionally used for treatment of disease and health related problem. Moreover, traditional medicines were also used in maintaining health, stamina, and treating diseases [1]. One of the medicinal plants empirically used in traditional community was kupa plant. Kupa was one of the endemic plants of Indonesia belong to guava family (Myrtaceae). Previous research on kupa stated that fruits of kupa had an antioxidant activity [2]. Degenerative diseases today had been related with oxidative stress. Antioxidant had potential to protect oxidative stress [3]. There were some methods to determined antioxidant activity, for example DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC (Cupric ion Reducing Antioxidant Capacity), etc. [4]. This study aimed to determined the antioxidant activity of ethanol extract from kupa leaves (Syzygium polycephalum (Miq.) Merr & L. M. Perry) using DPPH assays.

2. Method
Kupa leaves harvested from Tasikmalaya, West Java. Kupa leaves identification was done by macroscopic and microscopic examination. Extraction was conducted by maceration method. About 500 g of kupa leaves macerated using ethanol 96% in 3x24 h. The extracts were evaporated then using rotary evaporator.
The antioxidant activity test was carried out qualitatively and quantitatively. Qualitative antioxidant assay was carried out using Thin Layer Chromatography (TLC) and quantitatively tested using DPPH (2,2'-diphenyl-1-picrylhydrazil) assays [5].

Determination of antioxidant activity using DPPH assay was done by using UV-Visible Spectrophotometry. DPPH solution was used as control while ascorbic acid was used as standard. Analysis was done in triplicate for each group [6]. Antioxidant activity of each extract was determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity [7]. IC$_{50}$ of DPPH scavenging activity of each extract can be calculated using calibration curve.

3. Result and discussion

3.1 Result

![Figure 1. Macroscopic of Kupa Leaves.](image1)

![Figure 2. Microscopic of Kupa Leaves.](image2)

Noted:
- a) Lower Epidermis;
- b) Upper epidermis;
- c) Lower Epidermis with Stomata
- d) Hair Tissue Cover;
- e) Tissue Fiber; and
- f) Calcium oxalate crystal with prim shape.
Figure 3. TLC of ethanol extract kupa leaves.

Note:
A) Visible;
B) UV 254nm;
C) UV 366nm;
D) UV366 nm, after being sprayed with Sitroborat;
E) Visible after being sprayed with DPPH0.2%

Table 1. Antioxidant activity of Ascorbic Acid.

| C (ppm) | Average Absorbance | Absorbance Control | Average % Inhibition | Average IC50 (ppm) | SD    |
|---------|---------------------|--------------------|----------------------|--------------------|-------|
| 2       | 0.562               |                    | 22.222               |                    |       |
| 4       | 0.477               |                    | 34.025               |                    |       |
| 6       | 0.421               | 0.723              | 41.817               | 7.386              | 0.028 |
| 8       | 0.338               |                    | 53.204               |                    |       |
| 10      | 0.264               |                    | 63.485               |                    |       |

Table 2. Antioxidant activity of extract.

| C (ppm) | Average Absorbance | Absorbance Control | Average % Inhibition | Average IC50 (ppm) | SD    |
|---------|---------------------|--------------------|----------------------|--------------------|-------|
| 6       | 0.538               |                    | 25.542               |                    |       |
| 8       | 0.455               |                    | 37.114               |                    |       |
| 10      | 0.370               | 0.723              | 48.778               | 10.327             | 0.0419|
| 12      | 0.283               |                    | 60.811               |                    |       |
| 14      | 0.226               |                    | 68.741               |                    |       |
3.2 Discussion

Based on macroscopic study, the kupa leaves had a crossed, oval shaped and tapered tip [8]. The color was green and had no smell. The TLC results obtained a greenish color on the sitroborat and yellow on the DPPH with an rf value of 0.381. In the antioxidant test the kupa leaf ethanol extract obtained an IC\textsubscript{50} value of 10,327 ppm which is a very strong antioxidant [9]. DPPH dissolved in methanol or ethanol had specific characteristic as absorption at wavelength 517 nm. DPPH color would be changed when the free radicals were scavenged by antioxidant [10]. The IC\textsubscript{50} of DPPH scavenging activities in kupa leaves ethanol extract using DPPH were shown in Fig 3.

The IC\textsubscript{50} of DPPH scavenging activities in extract then compared to IC\textsubscript{50} of ascorbic acid standard [11]. IC\textsubscript{50} of DPPH scavenging activity is the concentration of sample or standard that can inhibit 50\% of DPPH scavenging activity. The lowest IC\textsubscript{50} means the highest antioxidant capacity. The IC\textsubscript{50} were used to categorize antioxidant activity of a sample compared to standard. Sample that had IC\textsubscript{50} less than 50 ppm was very strong antioxidant, 50-100 ppm was a strong antioxidant, 101-150 ppm was a medium antioxidant, while IC\textsubscript{50} greater than 150 ppm described as weak antioxidant [12].

4. Conclusion

Kupa leaves ethanol extract had the lowest IC\textsubscript{50} of DPPH scavenging activity 10.327 ppm which was a very strong antioxidant. And the extract have the component antioxidant based on TLC.

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