Antioxidant Profile of Heartwood and Sapwood of *Caesalpinia sappan* L. Tree’s Part Grown in Imogiri Nature Preserve, Yogyakarta

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Abstract. The usage of natural antioxidant from medicinal plants has been grown recently. One of them is wood of *Caesalpinia sappan* L. In community usage, the tree would be cut down in order to get the wood. The extensive usage might lead to the extinction of *C. sappan* wood because it grows wildly. This research aims to investigate antioxidant profile of each wood parts of *C. sappan* grown in Imogiri Nature Preserve Yogyakarta. Wood were collected based on the main, middle, and branch of tree. Each wood was separated into heartwood and sapwood part. The collected wood material was macerated using ethanol 50%. The extracts were investigated for their phytochemistry content qualitatively. The antioxidant profile was obtained based on their DPPH radical scavenging activity and Folin-Ciocalteau phenolic content. The result showed all wood part contained alkaloid, tannin and saponin. Triterpenoid and flavonoid were found in wood part except for branch sapwood. Total phenolic content of *C. sappan* wood was in the range from 443,20 ± 8,87 to 885,12 ± 11,56 mg GAE/100 g dry extracts. All the wood part resulted very strong antioxidant activity based on the IC$_{50}$ value (< 50 ppm) range from 7.1 to 24.4 ppm. These results of this study showed that in order to use the *C. sappan* wood as natural antioxidant agent sustainably, there is no need to cut down all the *C. sappan* tree. This study has demonstrated, for the first time, that wood part of *C. sappan* has very strong antioxidant activity, even in branch sapwood part.

1. Introduction

The usage of natural antioxidant obtained from medicinal plants has gained popularity recently. They can be used for food preservative [1] and known to have health benefits for humans [2]. Since they are valuable resources, medicinal plants are more vulnerable than non-medicinal plants [3]. Thus, their excessive usage might lead to their extinction [4] and might be threat to environmental sustainability as well.

In recent years the usage of *Caesalpinia sappan* L. as antioxidant has been increased recently [5-7]. Antioxidant properties of *C. sappan* wood were exerted from secondary metabolites and successfully isolated such as brazilin and sappanchalcone [8,9]. The part used of *C. sappan* are dominated for its wood [10,11]. *C. sappan* woods are grown wildly in forest and some are planted in the house yard. In the community usage, in order to get *C. sappan* wood they will cut down the tree. It could lead to the extinction of *C. sappan* wood.

There is a need to take the wood part such from branch or middle part of tree in order to conserve the *C. sappan* wood. This action could allow *C. sappan* to grow back and the extinction could be prevented. Besides that, there are concern in the wood part that is divided into heartwood and sapwood [12] Heartwood and sapwood have different secondary metabolites properties [13] and might affect the antioxidant activity respectively. The *C. sappan* grown in Imogiri Nature Preserve is a good model for antioxidant activity determination. It will express the nature of *C. sappan* wood.

However, to the best of author knowledge, no report has been found so far investigating the antioxidant activity of *C. sappan* wood based on the tree and wood structure part. The aim of this
research is to examine the antioxidant activity of *C. sappan* wood based on the tree part and wood part. The long-term implication of this study will impact the procurement of *C. sappan* wood as the natural antioxidant agent in community and health industry.

2. Materials and methods

2.1. Plant materials

*C. sappan* woods were collected from Imogiri Nature Preserve, Bantul Yogyakarta Province, Indonesia. The tree wood part was classified into the main (50 cm above ground), center, and branch. Each of wood was taken for 50 cm length. Each tree’s part was divided again into its heartwood and sapwood. Wood were grinded and dried using oven.

2.2. Extraction

Each of dried wood part 50 gram was macerated for 24 hours using 500 mL ethanol 50% as solvent. The extract was filtered and evaporated using vacuum rotary evaporator. The yield of extract was measured. Concentrated extract then was preserved in refrigerator and will be used as sample.

2.3. Phytochemicals analysis

The phytochemical analysis method of alkaloid, tannin, triterpenoid, steroid, flavonoid, and saponin was carried out by Harborne (1984) with some modification [14].

2.4. Total phenolic content

The total phenolic content of the *C. sappan* wood extract was analysed by using Folin-Ciocalteau method [15] with some modification. Gallic acid was used as a reference standard for plotting calibration curve. a volume of 0,4 mL of the plant extract was mixed with 0,4 mL of the Folin-Ciocalteau reagen and were added with 4 mL of sodium carbonate solution (7 %). The reaction mixture was incubated at room temperature for 1 hour. The absorbance was measured at 750 nm using UV-VIS spectrophotometer. The total phenolic contents were calculated from linear regression of a standard curve prepared with gallic acid. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of dry extract. Each of sample were done triplicate.

2.5. Antioxidant assay - 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical activity was carried out by DPPH assay [16] with some modification. DPPH 1mM was dissolved in methanol was prepared in 1 mM concentration. The extract was dissolved in ethanol and was prepared in series concentration with final concentration from 2.5-500 ppm. Control solution was prepared by DPPH solution and ethanol. Ascorbic acid, as the control positive, was prepared into final series concentration from 2.5 -100 ppm. The assay was carried out by adding 2.5 mL of extract to 2.5 mL DPPH solution. The solution was then placed in dark condition for 30 minutes. After 30 minutes, the absorbance was measured at 517 nm. The percentage inhibition was calculated using the following formula:

\[
\text{Inhibition (\%)} = \frac{(\text{Abs}_{\text{sample}} - \text{Abs}_{\text{control}})}{\text{Abs}_{\text{control}}} \times 100
\]  

IC$_{50}$ values were calculated using linear regression. The antioxidant activity was expressed as IC$_{50}$ value whereas the ability of sample to inhibit 50% of free radical. Each of assay was done triplicate.

3. Results and discussions

The yield of extraction and antioxidant activity would be depend on the solvent and method of extraction. In order to regain secondary metabolites optimally, the *C. sappan* woods were extracted using ethanol 50% by maceration for 24 hours. Ethanol has been known to have suitable property to extract polyphenol from medicinal plant. It is also generally known as safe (GRAS) solvent [17]. Maceration method was selected because this method is simple and suitable for secondary metabolites that is sensitive to heat [18]. Table 1. shows the yield of *C. sappan* wood extraction based on tree and
wood part. This table reveals that the sapwood showed low yield compared to heartwood for all part of tree. Heartwood showed high yield because of high secondary metabolites in it. Heartwood often stored phenolic compounds and could increase the natural durability of tree [19]. What stands out in this table is the yield of main heartwood showed the highest yield (8.7%) followed by middle and branch part. The finding is consistent with de Paula Protásio et. Al that the extractive components tend to increase by the age of wood [20].

### Table 1. The yield of C. sappan wood extraction

| Part of Tree | Part of wood | Yield (%) |
|--------------|--------------|-----------|
| Branch       | Heartwood    | 3.2       |
|              | Sapwood      | 2.8       |
| Middle       | Heartwood    | 5.4       |
|              | Sapwood      | 2.0       |
| Main         | Heartwood    | 8.7       |
|              | Sapwood      | 2.2       |

The extracts then were carried to the analysis of phytochemical screening. Phytochemicals contained in medicinal plants are responsible to exert antioxidant activity may lead to the finding active compound [21]. The qualitative phytochemical screening was tested for alkaloid, triterpenoid, steroid, flavonoid, tannin, and saponin content. The result in Table 2. showed that all wood part contained alkaloid, tannin and saponin. Triterpenoid and flavonoid were found in wood part except for branch sapwood. Srinivasan et. al. reported that ethanolic extract of C. sappan wood do not contain alkaloid but contain steroid [22]. This result is different with this study. Setiawan et. al. reported C. sappan wood did not contain tannin and saponin but contained flavonoid and alkaloid [23]. The differences might be because of the different growth place since biosynthesis of secondary metabolite affected by growth place [24].

### Table 2. The result of qualitative phytochemical analyses

| Part of tree and wood | Part of wood | Alkaloid | Triterpenoid | Steroid |
|-----------------------|--------------|----------|--------------|---------|
|                       |              | Dragendorf| Wagner        | Mayer   |
| Branch                | Sapwood      | ++       | -            | ++      |
|                       | Heartwood    | ++       | -            | +       |
| Middle                | Sapwood      | +        | +            | ++      |
|                       | Heartwood    | +        | ++           | -       |
| Main                  | Sapwood      | +        | +            | +       |
|                       | Heartwood    | +        | +            | +       |

Antioxidant activity exerted by the secondary metabolites in the medicinal plants. It is reported that phenolic compound is one of the secondary metabolites for determining the antioxidant activity [25]. Table 3. shows the result of total phenolic content in wood part of C. sappan. Interestingly, the total phenolic content of sapwood at branch (776,86 ± 5,45 mg GAE/100 g) and middle part (885,12 ± 11,56 mg GAE/100 g) higher than the main sapwood (443,20 ± 8,87 mg GAE/100 g). From the sight of the heartwood, main part showed the highest total phenolic content. It this possible that the phenolic was biosynthesized in the sapwood part then moved into the cell plant in heartwood [19].

### Table 3. Total phenolic content of C. sappan wood based on its part

| Part of Tree | Part of wood | Total Phenolic Content (mg GAE/100 g) |
|--------------|--------------|---------------------------------------|
| Branch       | Sapwood      | 776,86 ± 5,45                         |
|              | Heartwood    | 487,43 ± 12,37                        |
|              | Sapwood      | 885,12 ± 11,56                        |
| Middle       | Heartwood    | 533,96 ± 6,49                         |
|              | Sapwood      | 443,20 ± 8,87                         |
| Main         | Heartwood    | 825,05 ± 17,85                        |
Antioxidant activity is classified based on IC$_{50}$ value which shows the capability of extracts to scavenge 50% of DPPH. Based on Table 3. All the extract showed IC$_{50}$ value below 50 ppm in range from 7.1 to 24.4 ppm. According to [26], IC$_{50}$ < 50 ppm was categorized as very active antioxidant. All the wood part exerted very strong antioxidant activity based on the IC$_{50}$ value (< 50 ppm) [26]. This value is higher than reported by Setiawan et. al [23] that the IC$_{50}$ value was 101.8 ppm. Interestingly, even though branch sapwood has high total phenolic content (776.86 ± 5.45 mg GAE/100 g), it does not result in strong IC$_{50}$ value (24.4 ppm) compared to middle sapwood (885.12 ± 11.56 mg GAE/100 g; IC$_{50}$=9.6 ppm) and main heartwood (825.05 ± 17.85 mg GAE/100 g; 7.1 ppm). It might be caused by the profile of phenolic contents in branch sapwood differ from middle sapwood and main heartwood. These results of the study indicate that *C. sappan* wood grown in Imogiri Nature Preserve has the good quality as the raw material for natural antioxidant agents. This study has demonstrated, for the first time, that wood part of *C. sappan* has very strong antioxidant activity, even in branch sapwood part.

| Table 4. Antioxidant activity (IC$_{50}$ value) of *C. sappan* wood based on its part
| Part of Tree | Part of wood | IC$_{50}$ (ppm) |
|-------------|-------------|----------------|
| Branch      | Sapwood     | 24.4           |
|             | Heartwood   | 15.6           |
| Middle      | Sapwood     | 9.6            |
|             | Heartwood   | 7.3            |
| Main        | Sapwood     | 8.5            |
|             | Heartwood   | 7.1            |
| Control positive | Ascorbic acid | 4.02          |

4. Conclusion

This paper presented results of the antioxidant activity of *C. sappan* wood grown in Imogiri Nature Preserve based on the tree and wood part. This study has found that generally all part of wood contains alkaloid, tannin and saponin which might responsible for the antioxidant activity. It was also shown that the total phenolic content relates to antioxidant activity. The most obvious finding to emerge from this study is that all wood part of *C. sappan* has very strong antioxidant activity. This study provides recommendation that in order to get antioxidant activity, cutting down all part of the tree can be prevented. It offers the procurement of *C. sappan* wood sustainably.

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