Antioxidant Activity of MAE Extracted Teak 
(Tectona Grandis L.F.) Leaves Collected from Different 
Plantation Site at Java Island, Indonesia

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Abstract : Antioxidant properties of teak leaves collected from Cepu, Central Java, Indonesia 
which was extracted using Microwave Assisted Extraction (MAE) and soxhlet extraction was 
identified. The antioxidant activity was measured based on total phenol content, DPPH radical 
scavenging activity and H2O2 scavenging activity. MAE significantly produced higher 
antioxidant properties in shorter time compared to soxhlet extraction, however, the antioxidant 
properties of teak leaves collected from Cepu, was not significantly different from that 
collected from Blitar and Madiun, East Java. Different types of soil seem not affect the 
antioxidant properties of teak. 

Keywords: Antioxidant, Cepu, MAE, Plantation site, Teak (Tectona grandis L.f.).

Introduction

Antioxidant has been reported to neutralize the effects of free radicals or reactive oxygen species (ROS) 
that naturally produce as the body's stress response. Many modern diseases including cancer have been 
identified to be induced by free radicals. The free radicals can damage cells and its DNA, so if it can be 
scavenged by antioxidant agent the cancer cell could not develop1. Excessive numbers of antioxidant agents 
have been uncovered to fight against free radicals. Various mechanisms of antioxidant activity have also been 
investigated. Researchers suggested natural antioxidant as safe and effective free radicals scavenger1-5. 
Numerous spices and herbs have been extracted in regards to obtain its antioxidant compounds, however 
research on antioxidant from perennial plant origin was limited.

Teak (Tectona grandis L.f.) has been known as Indonesian most important commercial tree species that 
has been reported to contain antioxidants in the plant organs6-8. Antioxidant that has been identified in teak 
leaves were: phenolic compounds, such as quercetin, gallic acid, tannins9.

Teak is planted extensively in Java and cover over one million ha and about 8,000-10,000 ha has 
produced teak wood annually10. The leaf of teak usually was not utilized and become waste product of teak 
timber production. There are three units of teak plantation in Indonesia : Unit I (Central Java), Unit II (East Java 
and Madura), and Unit III (West Java and Banten). These units are divided into forest districts. Now, Perum 
Perhutani has a total of 57 forests. Cepu, which is part of the district Blora (Unit I) produced the largest amount
and the best quality teak wood among other teak plantation in Indonesia. The second large amount of teak plantation is Madiun, whereas Blitar is considered as quite small teak plantation at Java island.

The antioxidant content and activity of plants has been reported to be influenced by the soil where the plant grows. Plantation media and fertilizer applied to plant influence the antioxidant concentration. Total phenolics in arionberries grown with organic agricultural methods significantly higher compared with conventional method. Strawberries grown in organic media showed higher total phenolic, ellagic acid, and flavonols, than the conventionally grown strawberries. Compost has strong impact on fruit quality and antioxidant compounds of pepper plants under field conditions.

The forest district of Indonesia teak plantation has various type and fertility of soil. Blitar and Madiun forest district as part of Unit II, geologically located in the Old Volcanic Metallogenic, and sediment rock from Arjosari formation. This areas were quite fertile because of the high mineral content such as kaolin, limestone, bentonite, manganese, feldspar, iron sand, copper, ball clay, onyx, calcites, zeolites, volcanic rocks, sand, trass, gold, piropilit, chalcedony, and other ore minerals and the addition of volcanic ash from Kelud Volcano made the areas even more fertile. The annual rainfall of these area is 1892 mm and 3 months dry season, on the other hand, Cepu forest district as part of Unit I has the annual rainfall of 2062 mm and 4 months dry season. Cepu Teak forests located at the altitude of 30-250 m above sea level, sloping contour conditions, partly rocky (limestone), slightly hilly. The soil type is Latosol, Grumusol, Mediterranean, and Alluvial derived from igneous rocks, cement rocks, margeal, volcanic tuff, hard limestone and volcanic bases tuff.

In the previous times teak leaf antioxidant compounds have been extracted using conventional methods, such as maceration and soxhletation. Microwave-Assisted Extraction (MAE) can help improve the efficiency and effectiveness of extraction with a thorough mechanism of cell destruction by microwaves. MAE has been widely used to extract a variety of ingredients, such as ginseng, Galium humifusum and G. tinctorum, Cypripedium arietinum, tobacco, Magnolia officinalis, Angelica sinensis, Sylibum marianum, Pueraria lobata and ashwaganda.

The usage of MAE in this study was expected to improve the effectiveness and efficiency of antioxidant compound extraction of teak leaves. Comparison of antioxidant activity of teak leaves collected from Blitar, Madiun and Cepu which has different soil type, annual rainfall and dry season period was done.

Material and Methods

Fresh Teak leaves were harvested from Perhutani KPH Cepu, East Java, Indonesia. Fifty grams of shredded leaves (1 cm width) were extracted using various method of extraction by ethanol 50%. All chemical reagent used in this study were analytical standard (E-Merck). MAE was conducted by modified home microwave oven Samsung E45 for 2 minutes (power 80 watt). Extract was evaporated in vacuum condition (40°C) and preserved in 2°C. Extraction was conducted 9 times for each method, the extracts were observed for its antioxidant properties in triplicates.

The total phenolic content of the extracts was determined using the Folin-Ciocalteau method. The DPPH scavenging activity of Teak leaves extract was examined using the method of. The electro-donating activity examination was done using modified FRAP assay.

Results

Comparing the antioxidant properties of teak leaves extracted with MAE and soxlet extraction, there were evident that MAE could increase the concentration of extractable phenolic compound from Teak leaves (P<0.0001) (Table 1).

The phenolic compound content of Teak leaves extract showed linear relationship with other examined antioxidant properties. Alkaline conditioning of Teak leaves phenolic compounds by Na₂CO₃ brought the changes of dissociated phenolic proton into anion. Hence, those anionic antioxidant compounds reduced Mo (V) ion of polyphosphotunstates-molybdates inside Folin-Ciocalteau reagent to produce blue colour.
Teak leaves antioxidant extracted using MAE showed better activity of DPPH radical scavenging than that extracted using soxhlet (Table 1.). Phenolic compounds of Teak leaves changed the colour of these stable radicals into colourless or pale yellow. This color change was brought by DPPH radical conversion becoming diamagnetic molecules by receiving electron or hydrogen radicals. Antioxidant produced by soxhlet extraction indicated lower activity of H₂O₂ radical scavenging compared to MAE (P<0.0001).

Table 1: Effect of Soxhlet and Microwave Assisted Extraction Methods to the Antioxidant Characteristic of Teak Leaves

<table>
<thead>
<tr>
<th>Antioxidant Properties</th>
<th>Soxhlet Extraction</th>
<th>MAE</th>
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<tbody>
<tr>
<td>Total Phenolic Content (μg GAE/ml extract)</td>
<td>785.78±5.50a</td>
<td>872.22±5.93b</td>
</tr>
<tr>
<td>DPPH Radical Scavenging Activity (μg TE/ml extract)</td>
<td>52.35±0.27a</td>
<td>60.09±0.10b</td>
</tr>
<tr>
<td>Electro-Donating Activity (μg AAE/ml extract)</td>
<td>43.64±2.19a</td>
<td>50.52±1.82b</td>
</tr>
<tr>
<td>H₂O₂ Radical Scavenging Activity (μg AAE/ml extract)</td>
<td>45.54±5.00a</td>
<td>53.44±4.49b</td>
</tr>
</tbody>
</table>

Table 2: The IR Spectra Maximum Absorption of Quercetin from Teak Leaves

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Frequency (cm⁻¹)</th>
<th>Transmittance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-H</td>
<td>3448,72</td>
<td>9,88</td>
</tr>
<tr>
<td>C=O</td>
<td>1666,49</td>
<td>22,55</td>
</tr>
<tr>
<td>C=C</td>
<td>1635,64</td>
<td>20,36</td>
</tr>
<tr>
<td>C-OH</td>
<td>1388,75</td>
<td>25,62</td>
</tr>
<tr>
<td>C-O-C</td>
<td>1249,87</td>
<td>26,08</td>
</tr>
</tbody>
</table>

Quercetin has been known as the antioxidant component which is effective in preventing cancer. In this study, quercetin was identified by FTIR according to Nayeem & Karvekar method. The spectra showed that Microwave-Assisted Extraction method also could extract quercetin from Teak leaves (Figure 1). However, the transmittance value from Teak leaves extract of MAE lower than of soxhlet extraction (Table 2).
Comparison between the antioxidant activities of teak leaves collected from Central Java (Cepu) and East Java (Blitar and Madiun) based on the total phenolic content (Figure 2) and H$_2$O$_2$ scavenging activity (Figure 3), showed no significant differences especially for the antioxidant that was extracted using MAE. However the antioxidant property of teak leaves collected from Cepu measured using DPPH scavenging activity (Figure 4) was significantly lower than the other site of teak plantation.
Discussion

Teak leaves extracted by MAE gave higher activity than some previous study\(^6,7,31\). In this study, the electron-donating activity assay of Teak leaves extract was implemented by Ferric-Reducing Antioxidant Power method\(^32\). By the same manner of electron transfer, Teak leaves antioxidant also showed reducing activity towards Fe\(^{3+}\) from FeCl\(_3\). Blue complex gained from TPTZ and Fe (II) binding was measured using spectrophotometry method. It was revealed that MAE could give increment against activity of Teak leaves electron donation (Table 1) (P<0.0001).

Hydrogen peroxide was known as one of cancer main cause that leads to lipid peroxidation and DNA damage. Therefore, Teak leaves extract also expected to suppress radical formation (OH\(^*\)) of hydrogen peroxide in the presence of oxygen and water. Previous studies showed that quercetin is the one of main antioxidant compound from Teak leaves obtained from conventional method of extraction. Researchers revealed that quercetin has good ability of anti-cancer activity. Special characteristic of quercetin absorption frequencies happen on 3409–3144 cm\(^{-1}\) (O–H); 1667 cm\(^{-1}\) (C=O); 1610 cm\(^{-1}\) (C=C); 1381 cm\(^{-1}\) (C–OH); and 1264 cm\(^{-1}\) (C–O–C) \(^{33-35}\).

Irradiative heating mechanism of microwave, especially towards moisture of plant material, expected to give overall rupture throughout the cell materials. Frictional heat induced by ionic conduction and dipoles rotation leads to internal moisture vaporization\(^21\). Excessive internal pressure of cells caused by water vapours pushed the cells membrane out to swell and rupture. The temperature increment also damaged the structure of polyphenol oxidase, thence this undesired enzyme lost its functionality. Conversely, polyphenol oxidase could not be inactivated by soxhlet extraction due to its lower temperature condition than MAE. By relying on appropriate polarity between the solvent and the solute, the mass transfer process of target compound also limited by its equilibrium concentration\(^36\). Therefore, prolonged extraction duration was needed in order to obtain whole extraction. In this case, soxhlet extraction was considered as less effective and less efficient than MAE.

Microwave-Assisted Extraction could extract the antioxidant of Teak leaves, which possessed higher total phenolic content and other antioxidant activities compared to soxhlet extraction. Quercetin also found in Teak leaves extract produced by MAE. From this study, it was indicated that novel extraction method gave better performance of Teak leaves extraction while shortened extraction time and facilitated polyphenol oxidase inactivation during the process. This study may attribute to natural medicine development of cancer.

The effect of soil type and fertility on antioxidant content and activity of plants did not confirmed in this study. Cepu has been known as the biggest producer and the center of the best quality teak wood in Indonesia\(^11\). However, the best quality of teakwood was not revealed the quality of antioxidant content in the plant. Two of antioxidant property used in this study, total phenolic content and H\(_2\)O\(_2\) scavenging activity did not show the differences between teak leaves collected from Cepu with two other sites; however DPPH radical scavenging activity of antioxidant extracted from teak leaf grows in Cepu significantly lower than Madiun, Blitar and Cepu.

Most secondary metabolites, including phenolic compound as the main antioxidant compounds in teak leaves, was increasingly produced when plant subjected to stress condition. Generally Cepu has lower fertility of soil, slightly higher annual rainfall but longer dry season compared to Madiun and Blitar\(^17-19\). This condition may caused more stress to teak plantation in Cepu compared to those grown in Blitar and Madiun, however the differences of soil and climate showed by those areas was not significant enough to cause the differences in the antioxidant produced. Higher rainfall experienced by teak plantation at Cepu may suppress the potential of low fertility and longer dry season to trigger higher production of antioxidant.

Conclusion

MAE significantly produced higher antioxidant properties in shorter time compared to soxhlet extraction. However the antioxidant properties of teak leaves collected from Cepu, was not significantly different from that from Blitar and Madiun.
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References


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