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Antioxidant Extraction of Teak (Tectona grandis) Leaves Using Microwave-Assisted Extraction

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Abstract: The awareness of natural healthy lifestyle is growing these days. The spices and herbs as natural medicine have been widely used. Teak (*Tectona grandis*) is one of native herbs from Indonesia. The Teak timbers have been valued and majored for decades, whereas, its leaves have been used as traditional food packaging and medicine. Many researches purposed on its development have been conducted. In this research, novel method of extraction names Microwave-Assisted Extraction (MAE) was applicated to gain antioxidant compound of Teak leaves effectively. This work highlighted a comparative qualitative phytochemical screening on Teak leaves. Antioxidant activities of each extract were brought by Total Phenolic Content Folin-Ciocalteau method; DPPH (2-2-diphenyl-1-picrylhydrazyl) radical scavenging assay; Ferric-Reducing Antioxidant Power assay; and H_2O_2 (hydrogen peroxide) radical scavenging assay. MAE possessed higher antioxidant activity than Soxhletation. The aim of this study is comparing novel and conventional extraction method of Teak leaves antioxidant related to its effectivity and efficiency towards antioxidant activity.
Keywords: Antioxidant, *Tectona grandis*, microwave, soxhlet, extraction, leaves.

Introductions

Teak (*Tectona grandis*) is common tropical tree in Indonesia. Teak plantation has promising future to be developed, but its waste could have been a significant problem. Teak leaves, one of its organic wastes, have been used traditionally as food packaging and medicine. Nowadays, many researchers have revealed its phenolic compound in order to develop its utility, such as cotton coloring agent[1], mosquito larvicidal[2], metal chelator[3-4], active carbon[5-6], organic fertilizer composite[7].

It has been known that the phenolic compounds of Teak leaves, such as quercitine[8], gallic acid[9], ellagic acid[10], and tectoquinone[11], have great potency to be antioxidant agent[12-13]. Many solvents and methods have been used to obtain the antioxidant compound of Teak leaves. Due to economical challenge of extraction process, those conventional methods have been evaluated to be less effective and efficient related to its time and solvent consumptions.

MAE (Microwave-Assisted Extraction) is a novel method of extraction process that use microwave to increase its efficiency and effectivity[14]. Many materials have been extracted using MAE method, such as Ginseng[15], *G. humifusum* and *R. tinctorum*[16], *C. arietinum*[17], tobacco[18], *M. officinalis*[19], *R. angelicae*[20], *S.*

marianum[21], *R. puerariae*[22], Ashwagandha[23], *Z. budrunga*[25], and coffee bean[25]. MAE condition is expected to enhance the effectivity and efficiency of plant material extraction. The comparison of soxhletation and MAE effect towards the antioxidant of Teak leaves extraction was observed.

Experimental

Frontal Teak leaves were collected from Perhutani KPH Hutan Blitar, East Java, Indonesia (October 2012). The leaves were washed, sorted, and shredded into 1 cm width. The leaves (50 g) were water-juiced by modified method of Rao[26] and extracted by soxhletation (79.9°C; 12 hours; ethanol 50%) and MAE (80 watt; 2 minutes, ethanol 50%). All extract evaporated at vacuum condition (40°C) and stored at 2°C until the completion of all assay. All antioxidant activity assays for each sample were examined in triplicate.

The total phenolic content of the extracts was determined using the Folin-Ciocalteau method by Rao[26]. An aliquot (1 ml) of extract or a standard solution of gallic acid (1-100 µg/ml) was prepared. Distilled water was used as reagent blank. The Folin-Ciocalteau reagent (1.5 ml) was added to the mixture and shaken. The mixture was allowed to stand for 5 min. The mixture was introduced with 4 ml of 20% Na_2CO_3 solution. After incubation for 30 min at room temperature, the absorbance against the prepared reagent blank was determined at 738 nm with an UV-Vis Spectrophotometer Shimadzu. The absorbance was calculated with the following formula: Absorbance = 0.010 [Total phenolic (µg Gallic Acid Equivalent/ml)] + 0.041 (R^2 = 0.996).

The DPPH scavenging activity of Teak leaves extract was examined using the method of Thaipong[27]. DPPH solution was freshly prepared by dissolving 24 mg DPPH in 100 ml methanol, stored at -20±1°C before use. Stock solution (10 ml) was mixed in 45 ml methanol to obtain an absorbance 1.1±0.02 units at 517 nm using UV-Vis Spectrophotometer Shimadzu. Teak leaves extract (150 µl) were allowed to react with 2850 µl of the DPPH working solution for 24 hours in the dark room. The absorbance of sampel was taken at 517 nm.The curve was linear between 25 and 800 µM Trolox. Results are expressed in µM TE/g fresh mass. Additional dilution was needed if the DPPH value measured was over the linear range of the standard curve. The percentage of DPPH radical by the samples was calculated according to formula, % inhibition = [{Abs control – Abs sample}/Abs control] x 100, Where Abs control is the absorbance of DPPH radical, Abs sample is the absorbance of DPPH radical with extract/standard.

The electro-donating activity examination was done modified FRAP assay by Thaipong[27]. Acetate buffer pH 3.6 300 mM was made from 3.1 g $C_2H_3NaO_2.3H_2O$ and 16 ml $C_2H_4O_2$ in a liter distilled water. TPTZ 10 µM was mixed with 40 mM HCl. The FRAP working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ solution, and 2.5 ml $FeCl_3.6H_2O$ 20 mM and then warming at 37±1°C for 10 mins. An aliquot (150 µl) of extract or standard ascorbic acid (1-8 µg/ml) was allowed to react with 2850 µl FRAP working solution for 30 mins in dark condition. Distilled water was used as reagent blank. The change of mixture's color was read by UV-Vis Spectrophotometer Shimadzu at 593 nm. Additional dilution was needed if the FRAP value measured was over the linear range of the standard curve. The following formula was used to calculate the absorbance: Absorbance = 0.084 [Ferric reduced (µg Ascorbic Acid Equivalent/ml)] + 0.209 (R^2 = 0.993).

Hydrogen peroxide scavenging assay was carried out according to procedure of Ghaisas[28]. H_2O_2 solution (40 mM) was introduced in phosphate buffer 50 mM pH 7.4 and its absorbance was read using UV-Vis Spectrophotometer Shimadzu at 230 nm. Extract is added to hydrogen peroxide and the absorbance is determined after 10 minutes. The blank solution contained with phosphate buffer without H_2O_2. The percentage of hydrogen peroxide scavenging by the extract was calculated as following: % scavenging = [{Abs control – Abs sample}/Abs control] x 100, Where Abs control is the absorbance of DPPH radical, Abs sample is the absorbance of DPPH radical with extract/standard.

Results and Discussions

Medicinal plants have been used widely in therapeutic and traditional medicine throughout the world. Studies have shown that plants have phytochemical component and biological activities that produce definite physiological action in body. The most important bioactive phytoconstituents of plant are phenolic compounds. Several studies have shown that they also have known properties, such as free radical scavenging activity, wound-healing activity, antibacterial activity, and ferric-reducing activity.

Phenolic compound is the major active compound of Teak[4]. Some phenolic compounds, such as quercitin, gallic acid, rutin, and ellagic acid of Teak leaves have already reported as polar antioxidant agent[10]. Its activity and concentration are affected by proper extraction settings. The amount of extractable phenolic compund and its activity have been examined by following assays.

Microwave-Assisted Extraction (MAE) possessed statistically significant higher yield of Teak leaves antioxidant than soxhletation method ($P<0.001$). The total phenolic concentration of Teak leaves extract affected other antioxidant properties, such as radical scavenging and electro-donating activity. Many researchers found excellent linear correlations between the "total phenolic profiles" and "the antioxidant activity"[30]. The following explanation would describe the relationship of total phenolic content and antioxidant activity.

Folin-Ciocalteau method has been used to determine the total phenolic content of Teak leaves extract. The phenolic compound of Teak leaves reduced the heteropolyphosphotunstates-molybdates in Folin-**Ciocalteu reagent (Table 1).** Dissociated phenolic proton in alkaline medium (pH 10) becomes phenolate anion, that can reduce Mo (V). Thence, its reaction with phenols produced blue color complex of Mo (IV) or $(PMoW_{11}O_{40})^{4-}$. Thus, this method can reflect the reducing capacity of Teak leaves antioxidant through electron-transfer mechanism[30].

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay is mostly applied to rapidly examine the antioxidant capacity of samples. This method is widely used, as its reliability on giving information about the main property of antioxidant potential for many medicinal plants. DPPH is purplish stable free radical. It has been converted into diamagnetic molecule by accepting hydrogen radical or electron. Because DPPH radical quenched by Teak leaves antioxidant molecule, the color changes from purple to yellow (Table 1). This color changes was observed quantitatively using spectrophotometer UV-Vis at 517 nm[29].

The electron-donating activity of Teak leaves extract, which is an important action of phenolic antioxidant, was examined by FRAP assay (Table 1). Fe (III) from ferric chloride in FRAP reagent has been reduced into Fe (II) by donating electron. Fe^{2+} was introduced in TPTZ (2,4,6-tri-(2-pyridyl)-s-triazine)) to produce blue complex. This color change has been measured spectrophotometrically at 593 nm. This reaction is pH-dependent and optimum at pH 3.6. The decreasing of absorbance is proportional to the antioxidant reducing activity[30].

Table 1. The Activity of Teak Leaves Antioxidant ($P<0.001$) tested by ANOVA and HSD test ($P<0.05$)

Antioxidant Properties of Teak Leaves Extract	Soxhletation Method	Microwave-Assisted Extraction Method
Total Phenol Content (μg GAE/ml)	785.67 ± 24.33a	858.11±16.41b
DPPH Radical Scavenging Activity (%)	53.85 ± 2.52a	60.09 ± 1.87b
Ferric-Reducing Activity (μg AAE/ml)	41.79±4.21a	50.52±2.34b
H2O2 Radical Scavenging Activity (%)	53.95±2.52a	60.09±1.87b

H_2O_2 is rapidly decomposed into oxygen and water and this may produce hydroxyl radicals (OH*) that can initiate lipid peroxidation and cause DNA damage. The more H_2O_2 concentration decreased by Teak leaves antioxidant compound, the lower the absorbance value (Table 1). Nevertheless, it is quite usual that samples also absorb the wave at 230 nm length, requiring the measurement of blank solution. However, these peroxidase-based approaches do not allow determining whether the Teak leaves antioxidant is reacting directly with H_2O_2, or reacting with intermediates formed from the enzyme and H_2O_2. The superoxide radical is possibly produced during enzyme activity[29].

The high temperature of soxhletation will help to open the natural openings and damage the material cell slightly by heating internal water cell on material surface[31]. These phenomena allow the solvent to contact with solute (desired compound inside the material cell). Meanwhile, liquid solvent has ability to separate desired solute if it has proper polarity betwixt the solvent and the solute[32]. When solute dissolved in solvent, the solvent solution contained within solute diffuses unto the material surface then all solvent on the material surface transferred into bulk solution.

Continuous soxhletation process gives higher yields than the batch system process[33]. However, the soxhletation process requires more time to dissolve the entire target component. The combination of high temperature, high pressure and long extraction time could cause damage and volatilization to target compound in form of volatile hydrocarbon compound and thermolabile[34]. Proper choosing of flammable solvent is also important to be done in soxhletation extraction[35].

Microwave is able to maximize the cell rupturing process during extraction. Molecular vibration of radiated water molecule and also dissipation factor of material and solvent produced frictional heat that affected its internal temperature[36]. Furthermore, water inside the cell vaporized and increased its internal pressure. Excessive internal pressure would enhance the rupturing process thoroughly. The internal temperature would proportionally increase as the increment of extraction time[37]. On the contrary, overheating inside the material cell could thermally damage the heat-labile active compound[38], such as phenolic compound.

The frictional heat significantly enhanced cell-rupturing process[39] during microwave-assisted extraction. Also, the activity of polyphenoloxidase enzyme might be inhibited by thermal degradation[40]. This action would prevent the undesired enzymatical hydrolysis of phenolic compound mechanism. In other hand, those excessive local heating could damage its phenolic compound and vaporized any volatiles. Thus, proper microwave-assisted extraction method and settings has effect that is more advantageous than soxhletation.

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