ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Application of Microwave-Assisted Extraction on Teak (*Tectona grandis*) Leaves Antioxidant Extraction

Cynthia Setiawan [1]*, Hari Purnomo[2], and Joni Kusnadi[1]

[1]Department of Food Science and Technology, University of Brawijaya, Malang, East Java, Indonesia.
[2]Department of Animal Husbandry, University of Brawijaya, Malang, East Java, Indonesia.

ABSTRACT

Microwave-Assisted Extraction is a novel kind of extraction method that recently used for herbs and spices. Many studies have shown that the irradiative heating mechanism of microwave works effectively throughout the medicinal plant cell. Teak (*Tectona grandis*) is a native and common tropical tree from Indonesia. Its timbers have become the main commodity of forestry industry in Madiun (East Java). Recently, its leaves are known to possess antioxidant activity. Thus, this method was expected to increase the activity of Teak leaves antioxidant. This research highlighted a qualitative phytoconstituents screening on Teak leaves. The aim of this in-vitro study was to compare the effectivity and efficiency of extraction method (soxhletation and Microwave-Assisted Extraction) towards Teak leaves antioxidant. The examination of total phenol content by Folin-Ciocalteau method; DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity; electro-donating activity by Ferric-Reducing Antioxidant Power assay; and hydrogen peroxide scavenging activity of Teak leaves antioxidant were carried out in respect to show the significance of extraction method towards the antioxidant activity. The utilization of microwave during extraction could increase the yield and concentration of phenolic compound in Teak leaves extract, further affects its antioxidant activity. Microwave-Assisted Extraction exhibited higher antioxidant activity than soxhletation.

Keywords: Microwave, Soxhletation *Tectona grandis*; Leaves; Antioxidant; Madiun.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Microwave-Assisted Extraction (MAE) becomes the desirable option of extraction as it has shorter extraction time and less the use of solvent. Microwave is a non-ionizing electromagnetic energy wave that can produce heat [1]. Microwave uses two effective mechanisms of heating that leads to the increment of cells burst. Dissipation and dipoles rotation, which are types of irradiative heating mechanisms, have been used to extract many medicinal herbs, such as, *O. basilicum* and *C. ambrosioides* [1]; *C. zeylanicum*, *C. sativum*, *C. sativus* [2]; *C. cyminum* [3]; *C. phaeocaulis* and *M. haplocalyx* [4].

Teak (*Tectona grandis*) is a major tropical tree that grows in Indonesia, especially Madiun area (East Java). The timbers of Teak become the commodity of forestry industry that take place there, whereas, its leaves produced highly as the main organic waste. Many researchers have investigated its phenolic compounds for medicinal use. The phenolic compounds of Teak leaves, such as quercitin, gallic acid, ellagic acid, tectoquinone, and tannins, have been known to possess antioxidant activity. Some conventional methods have been conducted in order to obtain its active compounds.

Previous study showed that soxhletation could give the highest Teak leaves antioxidant activity. Continuous extractions in long-period and high amount of solvent usage, which are the limitation of soxhletation, have been assessed as undesirable cost. Reviewed as less effective and efficient, in this study, MAE is expected to increase the Teak leaves antioxidant activity instead of soxhletation. Therefore, the effect of MAE and soxhletation towards the Teak leaves antioxidant activity was examined.

MATERIALS AND METHODS

Frontal Teak leaves were obtained from Perhutani KPH Madiun, East Java, Indonesia (October 2012). Its size has been reduced into 1 cm width. The pre-treatment of sample (50 g) has been done by modified juicing method of Rao [5] using ethanol 50% as solvent. MAE (modified Samsung E45, 2450 MHz) has been conducted on 80 watt for 2 minutes, while soxhletation has been set on 79.9°C for 12 hours. Extract of samples have evaporated in vacuum condition (40°C) and stored at 2°C until all examinations completed. All assays have been brought in triplicate. Total phenolic content assay was examined by Rao method [5]. The activity of electron donating assay and DPPH radical quenching assay were conducted by Thaipong method [6]. The hydrogen peroxide scavenging assay was done by Ghaisas method [7]. The standard curve of ascorbic acid and gallic acid were shown in Figure 1 and Figure 2 respectively

ISSN: 0975-8585

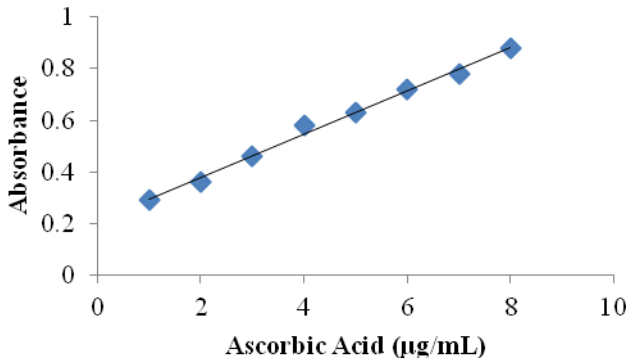

Figure 1: Absorbance of Standard Ascorbic Acid on FRAP assay (593 nm)

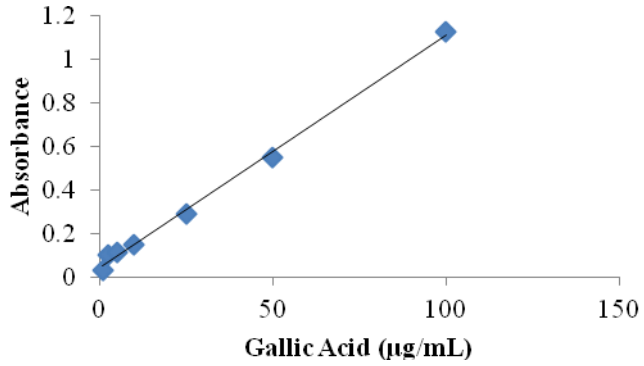

Figure 2: Absorbance of Standard Gallic Acid on FC Reagent

RESULTS AND DISCUSSIONS

The Effect of Extraction Method towards the Concentration of Teak Leaves Phenolic Compound

Microwave is electromagnetic energy waves at frequencies 0.3–300 GHz. These waves can penetrate materials that have dissipation factor and then convert it into heat energy [8]. It also interacts selectively with polar molecule, such as water and alcohol. The heating mechanism of microwave occurs in closed system (no heat loss to the environment) that makes microwave can produce heat effectively in very short time [9]. It has been revealed that this mechanism can significantly reduce the time of extraction as compared to soxhletation.

The moisture inside the Teak leaves cell and polar solvent serve as the target of microwave heating [1]. The frictional heat, that produced by ionic conduction and dipoles rotation, increase the internal temperature of cells and evaporate its moisture content [10]. Immense amount of vapors were generated according to the time function. Thus, the increasing internal pressure of cell pushes, stretches, and ruptures the cell wall [11]. This

phenomenon facilitates the active compound to be leached into the surrounding solvent. Therefore, microwave-heating can improves the yield of active compounds.

The heating effectivity of microwave was shown in the phenolic compound content and other antioxidant activity of Teak leaves extract (Table 1). Microwave indicated better activity of antioxidant as the effect of higher antioxidant yield. With its longer extraction time, soxhletation has less extraction effectivity. This experiment showed that the concentration of phenolic compound obtained from soxhletation was only 780.111±4.857 µg/mL. Ergo, according to the antioxidant activity of Teak leaves, the extraction method using soxhlet were less desired.

Method of Extraction	Total Phenolic Content (µg/ml GAE)
Soxhletation	780.111 ± 4.857a
Microwave-Assited Extraction	858.889 ± 19.814b

Table 1: Total Phenolic Content of Teak leaves tested by ANOVA (P<0.001) and HSD test (P<0.05)

Soxhletation is a conventional method of antioxidant extraction. It occupies the solubility of solute into the solvent to obtain the antioxidant compound inside the Teak leaves cell. Whereas, the heat, that is used for vaporizing the solvent, also used for enlarging the natural opening of cells [12]. These openings allowed the phenolic compounds to be dissolved certainly on the polarity of solvent used [13]. Thence, the effectivity of Teak leaves antioxidant yielding decrease when the equilibrium state has already obtained.

Former study showed that continuous process of soxhletation gave higher yield of extract[14]. In contrary, the soxhlet condition (pressure, and heat) encourage the phenolic compounds to be volatilized and damaged [15]. Yet, soxhletation does not provide any enzyme deactivation of material cells like on MAE [16]. Thus, the undesired enzymatic hydrolysis of Teak leaves antioxidant, such as polyphenoloxidase activity, was allowed during long extraction time of soxhletation.

The Antioxidant Capacity of Teak Leaves

DPPH (2,2-diphenyl-1-[2,4,6-trinitrophenyl]hydrazyl) is dark purplish stable free-radical molecules, that majorly used as indicator in order to measure the antioxidant capacity of medicinal plant [17]. DPPH radical has strong absorption of ultraviolet on wavelength 517 nm (dark violet). Other radicals (R*) are rapidly trapped by DPPH. Meanwhile, DPPH is also easily scavenged by Teak leaves antioxidant molecules [18]. When it is neutralized by accepting hydrogen radical or electron, the color changed into pale yellow or colorless diamagnetic molecule. This change is measured as the capacity of Teak leaves antioxidant [19]. This experiment showed that MAE could significantly increase the activity of DPPH radical quenching ($p<0.001$) (Figure 3), compared to soxhletation. The Teak leaves antioxidant extracted by soxhletation could inhibit 53.952±1.913% of DPPH radicals. The application of microwave on extraction increased the activity of DPPH radical scavenging until 59.751±0.520%.

ISSN: 0975-8585

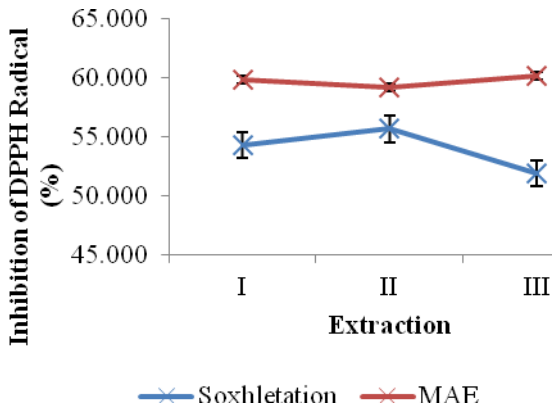

Figure 3: DPPH Radical Scavenging Activity by Teak Leaves Antioxidant

FRAP (Ferric-Reducing Antioxidant Power) is commonly used to examine the electron-donating activity of antioxidant. This assay allows the Teak leaves antioxidant molecule to reduce Fe^{3+} from ferric chloride in FRAP reagent. By electron donating mechanism, the production of Fe (II) is linearly correlated to the activity of Teak leaves antioxidant [19]. Fe^{2+} binds into blue complex in the presence of TPTZ (2,4,6-tri-(2-pyridyl)-s-triazine)) [17]. This pH-dependent reaction provides visible color change that can be measured by UV-Vis spectrophotometer at 593 nm pH 3.6 [16]. The soxhletation process resulted in lower electro-donating activity of Teak leaves than MAE did ($p<0.001$) (Figure 4). From the soxhletation, the antioxidant activity of Teak leaves worked equally as 42.315 ± 2.392 µg/mL of ascorbic acid. The effect of microwave heating could increase the activity as much as 49.722±1.375 µg/mL AAE.

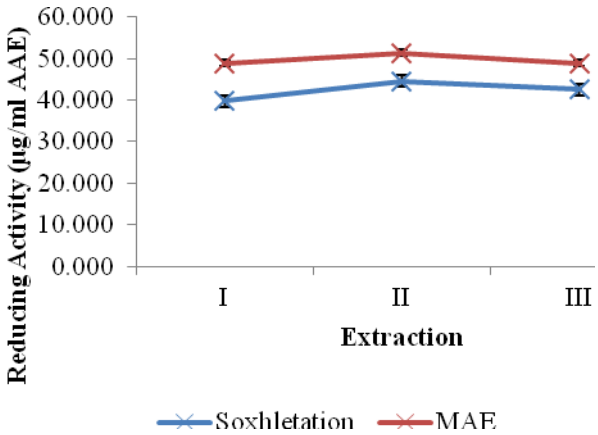

Figure 4: Teak Leaves Antioxidant Activity by FRAP Assay

The production of radical species by H_2O_2 causes lipid peroxidation and DNA damage. H_2O_2 is effortlessly decomposed into H_2O, O_2, and may produce OH* (hydroxyl radical) [20]. Hydrogen peroxide optimally absorbs the ultraviolet at wavelength 230 nm [21]. In other hand, some samples also absorb the wave at same length. Thus, this peroxide-based approach requires the measurement of blank solution. Howbeit, the mechanism of superoxide radical

inhibition cannot be detected, whether the Teak leaves antioxidant reacts directly towards H_2O_2, or reacts with intermediate compounds. Both enzyme (during its activity) and H_2O_2 are possible to form the intermediates [18]. The H_2O_2 radical suppressed by antioxidant can decrease the formation of another radical species. Using soxhlet, Teak leaves antioxidant could inhibit H_2O_2 radical by 63.889±1.334%. Meanwhile, the H_2O_2 radical scavenging of Teak leaves extracted by MAE was 68.553±1.258%. Microwave could significantly increase the activity of H_2O_2 radical scavenging ($p<0.001$) (Figure 5).

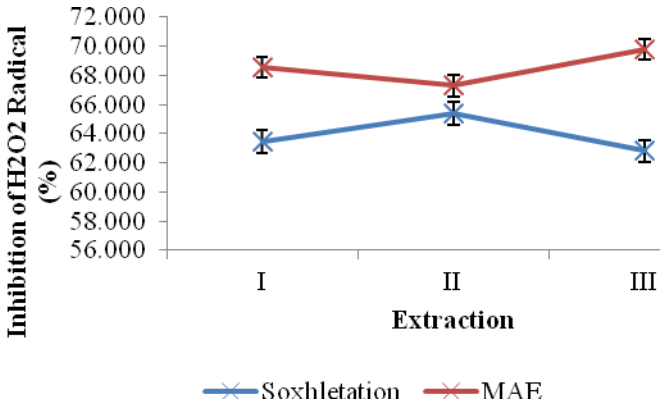

Figure 5: The H_2O_2 Radical Scavenging Activity by Teak Leaves Antioxidant

CONCLUSIONS

The application of microwave during Teak leaves extraction significantly increase the activity of Teak leaves antioxidant. Based on the total phenolic content, DPPH radical scavenging activity, electron-donating activity, and H_2O_2 radical scavenging activity of Teak leaves extract, Microwave-Assisted Extraction (2 minutes of extraction) gave higher activity of antioxidant than soxhletation (12 hours of extraction). Thus, Microwave-Assisted Extraction is more desirable as the extraction method of Teak leaves antioxidant.

ACKNOWLEDGEMENT

PHBI Batch II LPPM University of Brawijaya supported this work. The authors thank Mrs. Estri Laras Arumningtyas and Mr. Christianto Gunawan for the technical assistance.

REFERENCES

[1] Zou X, Li J, Ma B, Yu Y. Zhongguo Zhong Yao Za Zhi 2011; 36 (17): 2329-2333.
[2] Gallo M, Ferracane R, Graziani G, Ritieni A, Fogliano V. Molecules 2010; *15*: 6365-6374.
[3] Benkaci AF, Mekaoui R, Eppe G, Pau ED, Faucont JF. World Academy of Science, Engineering and Technology 2012; 68: 1569-1572.
[4] Ugarte GA, Becerra GP, Morales ME, López MA. Journal of Microwave Power and Electromagnetic Energy 2013; 47 (1): 63-72.

[5] Rao K, Aradhana R, Banjii D, Chaitanya R, Kumar AA. Journal of Pharmacy Researc*h* 2011; *2* (4): 440-442.
[6] Thaipong K, Boonprakob U, Crosby K, Cisneros ZL, Byrn DH. Journal of Food Composition and Analysis 2006; 19: 669–675.
[7] Ghaisas MM, Navghare VV, Takawale AR, Zope VS, Deshpande AD. Pharmacologyonline 2008; *3*: 296-305.
[8] Afoakwah A, Owusu J, Adomako C, Teye E. Global Journal of Bio-Science and Biotechnology 2012; 1 (2): 132-140.
[9] Kaufmann B, Christen P. *Phytochemical Analysis,* 2002; *13*: 105-113.
[10] Mandal V, Mohan Y, Hemalatha S. Pharmacognosy Reviews 2007; *1* (1): 7-18.
[11] Steed L, Truong V, Simunovic J, Sandeep K, Kumar P, Cartwright G, Swartzel KR. Food Engineering and Physical Properties 2008; 73 (9): 455-462.
[12] Berk Z, Elsevier Inc, New York 2009, pp. 259-278
[13] Brennan JG, Grandison AS, Lewis MJ. WILEY-VCH Verlag GmbH & Co. KGaA., Weinheim, 2006, pp. 429-513.
[14] Shivani P, Khushbu P, Faldu N, Thakkar V, Shubramanian RB. African Journal of Biotechnology 2011; *10* (79),:18210-18213.
[15] Oluseyi T, Olayinka K, Alo B, Smith RM. African Journal of Environmental Science and Technology 2011; *5* (7): 482-493.
[16] Hernandez DS. Journal of Microwave Power and Electromagnetic Energy 1999; *34* (4), 240-252.
[17] Badarinath AV, Rao KM, Chetty CM, Ramkanth S, Rajan TV, Gnanaprakash K. International Journal of PharmTech Research 2010; *2* (2): 1276-1285.
[18] Huang D, Ou B, Prior RL. Journal of Agricultural and Food Chemistry 2005; *53*: 1841-1856.
[19] Apak R, Güçlü K, Demirata B, Özyürek M, Çelik SE, Bektaşoğlu B, Berker KI, Özyurt D. Molecules 2007; 12: 1496-1547.
[20] Malik A, Kushnoor A, Saini V, Singhal S, Kumar S, Yadav YC. Journal of Chemical and Pharmaceutical Research 2011; *3* (3): 659-665.
[21] Keser S, Celik S, Turkoglu S, Yilmaz Ö, Turkoglu I. Chemistry Journal 2012; *2* (1): 9-12.