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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 H_2O_2 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 develop[1]. 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 scavenger[1-5]. Numerous spices and herbs have been extracted in regards to obtain its antioxidant compounds, however research on antioxidant from perrenial 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 organs[6-8]. Antioxidant that has been identified in teak leaves were: phenolic compounds, such as quercetin, gallic acid, tannins[9].

Teak is planted extensively in Java and cover over one million ha and about 8,000-10,000 ha has produced teak wood annually[10]. 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[11]. 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[12]. 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[13,14]. Compost has strong impact on fruit quality and antioxidant compounds of pepper plants under field conditions[15].

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[16]. 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[17] 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[18], 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, sedimentary rocks, margel, volcanic tuff, hard limestone and volcanic bases tuff[19].

In the previous times teak leaf antioxidant compounds have been extracted using conventional methods, such as maceration[6] and sohxletation[7]. Microwave-Assisted Extraction (MAE) can help improve the efficiency and effectiveness of extraction with a thorough mechanism of cell destruction by microwaves[20,21]. MAE has been widely used to extract a variety of ingredients, such as ginseng[22], *Galium humifusum* and *G. tinctorum*[23], *Cypripedium arietinum*[24], tobacco[25], *Magnolia officinalis*[26], *Angelica sinensis*[27], *Sylibum marianum*[28], *Pueraria lobata*[29] and ashwaganda[30].

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[31]. The DPPH scavenging activity of Teak leaves extract was examined using the method of[32]. The electro-donating activity examination was done using modified FRAP assay[32].

Results

Comparing the antioxidant properti 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 Na_2CO_3 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 H_2O_2 radical scavenging compared to MAE ($P<0.0001$).

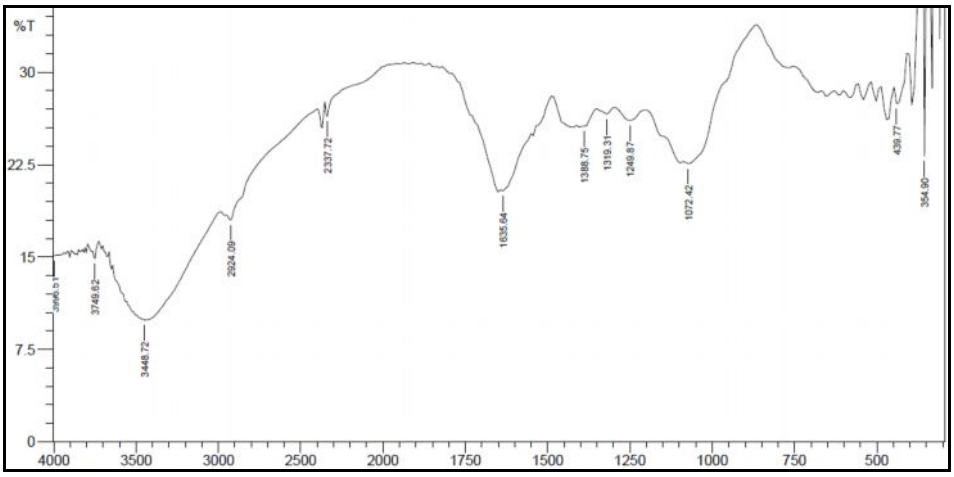
Table 1: Effect of Soxhlet and Microwave Assisted Extraction Methods to the Antioxidant Characteristic of Teak Leaves

Antioxidant Properties	Soxhlet Extraction	MAE
Total Phenolic Content (μg GAE/ml extract)	785.78±5.50a	872.22±5.93b
DPPH Radical Scavenging Activity (μg TE/ml extract)	52.35±0.27a	60.09±0.10b
Electro-Donating Activity (μg AAE/ml extract)	43.64±2.19a	50.52±1.82b
H_2O_2 Radical Scavenging Activity (μg AAE/ml extract)	45.54±5.00a	53.44±4.49b

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

MAE		
Frequency (cm^{-1})	**Functional Group**	**Transmittance (%)**
3448,72	O-H	9,88
1666,49	C=O	22,55
1635,64	C=C	20,36
1388,75	C-OH	25,62
1249,87	C-O-C	26,08

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[33]. 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).



Transmittance (%)

Wave Numbers (cm^{-1})

Figure 1: Spectra of quercetin using FTIR

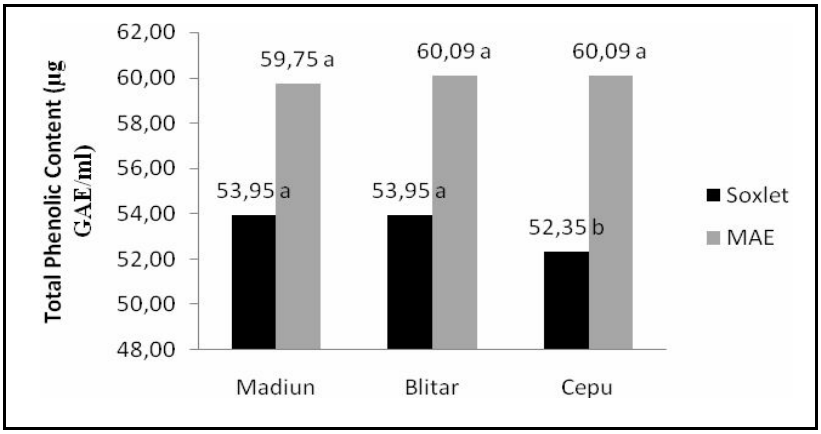


Figure 2: Total phenolic Content of antioxidant extracted from teak leaf grows in Madiun, Blitar and Cepu. Comparison was made among each extraction methods.

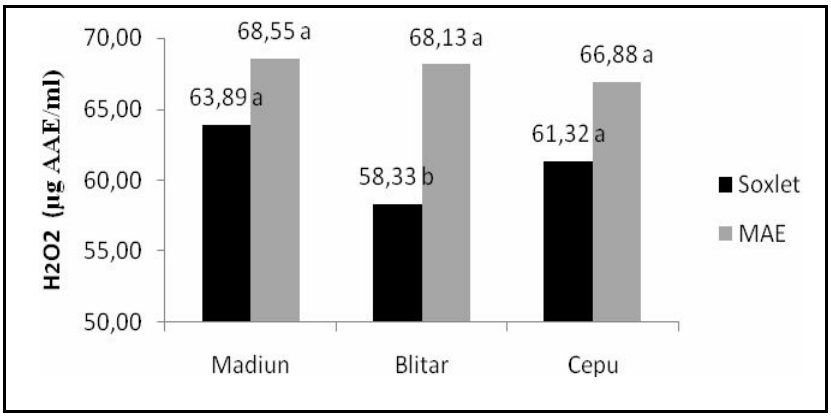


Figure 3: H_2O_2 scavenging activity of antioxidant extracted from teak leaf grows in Madiun, Blitar and Cepu. Comparison was made among each extraction methods.

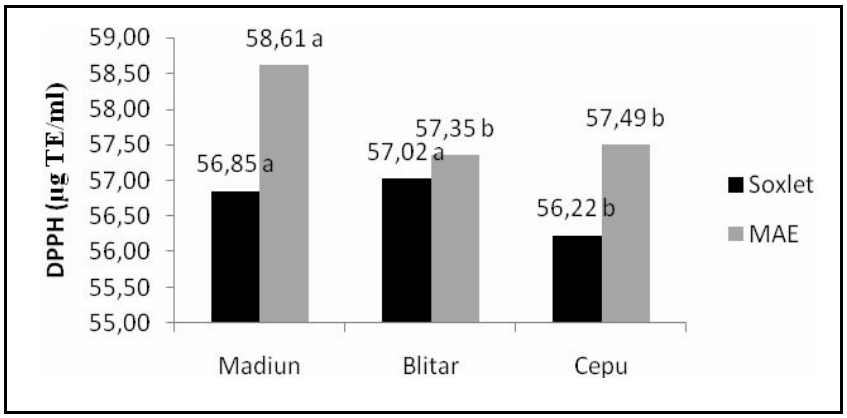


Figure 4: DPPH radical scavenging activity of antioxidant extracted from teak leaf grows in Madiun, Blitar and Cepu. Comparison was made among each extraction methods.

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_2O_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_2O_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

1. Greenlee H, Kwan M, Kushi L, Song J, Castillo A, Weltzien E et al. Antioxidant supplement use after breast cancer diagnosis and mortality in the Life After Cancer Epidemiology (LACE) cohort. Cancer. 2011,118(8):2048-2058.
2. Farag R, El-baroty G, Basuny A. Safety evaluation of olive phenolic compounds as natural antioxidants. Int J Food Sci Nutr. 2003, 54(3):159-174.
3. Reddy V, Urooj A, Kumar A. Evaluation of antioxidant activity of some plant extracts and their application in biscuits. Food Chem. 2005, 90(1-2):317-321.
4. Brewer M. Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. Comp Rev Food Sci Food Saf. 2011,10(4):221-247.
5. Kasote D. Flaxseed phenolics as natural antioxidants. Int Food Res J. 2013, 20(1):27-34.
6. Ghaisas M, Navghare V, Takawale A, Zope V, Deshpande A. In-vitro antioxidant activity of *Tectona grandis* Linn. Pharmacologyonline. 2008, (3):296-305.
7. Krishna M, Jayakumaran N. Antibacterial, Cytotoxic and Antioxidant Potential of Different Extracts from Leaf, Bark and Wood of *Tectona grandis*. Int J Pharm Sci Drug Res. 2010, 2(2):155-158.
8. Nayeem N, Karvekar M. Comparative phytochemical and pharmacological screening of the methanolic extracts of the frontal and mature leaves of *Tectona grandis*. Int J Pharm Bio Sci. 2010, 1(3):1-7.
9. Nayeem N, Karvekar M. Anti microbial and anti-oxidant properties of the isolated compounds from the methanolic extract from the leaves of Tectona grandis. J Basic Clin Pharma. 2011, 2(4):163-165.
10. Suseno O. Current status of tree improvement in Indonesia. In: Thielges BA, Sastrapradja S, Rimbawanto A, editors. Proceeding of the International Conference on In situ and Ex situ Conservation of Commercial Tropical Trees, 2001 Jun 11-13, Yogyakarta, Indonesia: CAB Direct, 2001.p.1-25.
11. Ichwandi I, Shinohara T, Cren B. Development of Teak Wood Production and Marketing in Cepu Forest District, Central Java, Indonesia. Bull. Fac. Sci. Univ. Ryukyus. 2009, (56):23-31.
12. Ahn T, Oke M, Schofield A, Paliyath G. Effects of Phosphorus Fertilizer Supplementation on Antioxidant Enzyme Activities in Tomato Fruits. J Agric Food Chem. 2005, 53(5):1539-1545.
13. Asami D, Hong Y, Barrett D, Mitchell A. Comparison of the Total Phenolic and Ascorbic Acid Content of Freeze-Dried and Air-Dried Marionberry, Strawberry, and Corn Grown Using Conventional, Organic, and Sustainable Agricultural Practices. J Agric Food Chem. 2003, 51(5):1237-1241.
14. Olsson M, Andersson C, Oredsson S, Berglund R, Gustavsson K. Antioxidant Levels and Inhibition of Cancer Cell Proliferation in Vitro by Extracts from Organically and Conventionally Cultivated Strawberries. J Agric Food Chem. 2006, 54(4):1248-1255.
15. Aminifard M, Aroiee H, Azizi M, Nemati H, Jaafar H. Effect of compost on antioxidant components and fruit quality of sweet pepper (capsicum annuum L.). JCEA. 2013, 14(2):47-56.
16. Samodra H, Gafoer S, Tjokrosapoetro S. Geologi Lembar Pacitan, Jawa. Bandung: Geological Center of Research and Development, 1992.
17. Sunaryo. Study on Mineralization Zone of Blitar District By Means of Simple Bouguer Anomaly. Int J Eng Innov Tech. 2012, 2(6):186-191.
18. Suhaendi H. Teak Improvement in Indonesia. Proceedings of the Second Regional Seminar on"Teak for the Future", 1995 May 29 – Jun 3, Yangon, Myanmar: TEAKNET Publication, 1998.1.
19. Perum Perhutani. Kelas Perusahaan Jati Dari Kesatuan Pemangkuan Hutan Cepu. 2nd ed. Cepu: RPKH, 2002. Indonesian.
20. Langat M, Nawrot D, Mulholland D. Chemical Constituents of East European Forest Species. Nairobi: Book of Extended Abstract The 14th NAPRECA Symposium and AAMPS Ethnoveterinary Medicine Symposium Nairobi, 2011.
21. Mandal V, Mohan Y, Hemalatha S. Microwave Assisted Extraction " An Innovative and Promising Extraction Tool for Medicinal Plant Research. Pharmacogn Rev. 2007, 1(1):7-18.
22. Shu Y, Ko M, Chang Y. Microwave-assisted extraction of ginsenosides from ginseng root. Microchem J. 2003, 74(2):131-139.

23. Dabiri M, Salimi S, Ghassempour A, Rassouli A, Talebi M. Optimization of microwave-assisted extraction for alizarin and purpurin in Rubiaceae plants and its comparison with conventional extraction methods. J Sep Science. 2005, 28(4):387-396.
24. Kerem Z, German-Shashoua H, Yarden O. Microwave-assisted extraction of bioactive saponins from chickpea (Cicer arietinum L). J Sci Food Agric. 2005, 85(3):406-412.
25. Zhou H, Liu C. Microwave-assisted extraction of solanesol from tobacco leaves. J Chromatogr A. 2006, 1129(1):135-139.
26. Martino E, Ramaiola I, Urbano M, Bracco F, Collina S. Microwave-assisted extraction of coumarin and related compounds from Melilotus officinalis (L.) Pallas as an alternative to Soxhlet and ultrasound-assisted extraction. J Chromatogr A. 2006, 1125(2):147-151.
27. Liu Z, Wang J, Shen P, Wang C, Shen Y. Microwave-assisted extraction and high-speed counter-current chromatography purification of ferulic acid from Radix Angelicae sinensis. Sep Purif Technol. 2006, 52(1):18-21.
28. Dhobi M, Mandal V, Hemalatha S. Optimization of Microwave Assisted Extraction of Bioactive Flavonolignan - Silybinin. J Chem Metrol. 2015, 1(3):13-23.
29. Wang Y, Xi G, Zheng Y, Miao F. Microwave-Assisted Extraction of Flavonoids from Chinese Herb Radix puerariae (Ge Gen). J Med Plant Res. 2010, 4(4):304-308.
30. Jyothi D, Khanam S, Sultana R. Optimization of Microwave Assisted Solvent Extraction of Withanolides from Leaves of Ashwagandha. Int J Pharm Pharm Sci. 2010, 2(4):46-50.
31. Rao K, Aradhana R, Banjii D, Chaitany R, Kumar A. In-Vitro Anti-Oxidant and Free Radical Scavenging Activity of Various Extracts of Tectona grandis Linn Leaves. J Pharm Res. 2011, 4(2):440-442.
32. Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal. 2006, 19(6-7):669-675.
33. Nayeem N, Karvekar M. Isolation of Phenolic Compounds from The Methanolic Extract of Tectona grandis. Res J Pharm Biol Chem Sci. 2010b, (1):221-225.
34. Borghetti G, Lula I, Sinisterra R, Bassani V. Quercetin/Î²-Cyclodextrin Solid Complexes Prepared in Aqueous Solution Followed by Spray-drying or by Physical Mixture. AAPS Pharm Sci Tech. 2009, 10(1):235-242.
35. Naidu P, Kinthada P, Kalyani P. Characterization and Biological Activities of Quercetin Thiosemicarbazone Derivatives: Potential Anti Cancer Drugs. Int J Pharm Biomed Sci. 2012, (3):24-27.
36. Gude V, Patil P, Martinez-Guerra E, Deng S, Nirmalakhandan N. Microwave energy potential for biodiesel production. Sustain Chem Process. 2013, 1(1):5.

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