Proximate analysis and bioactivity study on acoustically isolated *Elaeis guineensis* leaves extract

Cite as: AIP Conference Proceedings **2155**, 020001 (2019); https://doi.org/10.1063/1.5125505
Published Online: 06 September 2019

Nissha Bharrathi Romes, Roswanira Abdul Wahab, and Mariani Abdul Hamid

Proximate Analysis and Bioactivity Study on Acoustically Isolated *Elaeis guineensis* Leaves Extract

Nissha Bharrathi Romes[1,2, a)],Roswanira Abdul Wahab [1, 2, b)] and Mariani Abdul Hamid[3,c)]

[1] *Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia*
[2] *Enzyme Technology and Green Synthesis Group, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia*
[3] *Institute of Bioproduct Development, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Malaysia*

[b)] Corresponding author: roswanira@kimia.fs.utm.my
[a)] nisshach_27@yahoo.com
[c)] mariani@utm.my

Abstract. Extracts of bioactive compounds from *Elaeis guineensis* leaves previously acoustically isolated using a probe sonicator that yielded the highest total phenolic content (TPC) was subjected to proximate analysis and antioxidant capacity tests such as ferric reducing antioxidant power assay (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) and antibacterial activity. These tests were aimed to gauge the feasibility of incorporating the extract as an active ingredient in a topically applied nanoemulsion. Results revealed that the leaves extract with a TPC of 209.70 mg gallic acid equivalent (GAE)/g) yielded a FRAP value of 1,417.40 µM Fe^{2+}/mg and exhibited strong DPPH radical scavenging capacity with an IC_{50} value of 9.899 ± 0.050 µg/mL. The value seen here was equivalent to that of positive controls, Trolox and butylated hydroxytoluene (BHT). Consequently, the proximate composition of the leaves was found to comprise of 18.8% moisture content, 5.2% ash, 11.2% protein, 7.1% fat, 57.7% carbohydrate and energy value of 339.5 kcal/100g. *E. guineensis* leaves extract showed resistance towards the Gram-positive bacteria, *Bacillus subtilis* at 50 and 100 mg/mL, respectively, but did not inhibit the growth of the Gram-negative bacteria, *Escherichia coli,* and *Pseudomonas aeruginosa.*

INTRODUCTION

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tert-Butylhydroquinone (TBHQ) are unsafe to be added into cosmetics that are designated for topical applications. This is due to concerns over their detrimental side effects on human skin health, as well as claims of these being carcinogenic and allergic [1]. Henceforth, there is a great demand for plant-derived natural antioxidants to replace these synthetic antioxidants. Natural antioxidants are abundant in plants, and these compounds can be further divided into water soluble and oil soluble antioxidants [2], as the differential solubility of the different types of antioxidants in plant extracts also dictates their subsequent potential use. Naturally occurring antioxidants are vital in the prevention of chronic ailments such as cancer and cardiovascular complications, as well as neurodegenerative diseases triggered by harmful free radicals [3].

Reactive oxygen species (ROS) are highly reactive free radicals or chemical species which include species of the hydroxyl radicals (OH·), superoxide anion radicals (O_2·-) and non-free radical species, hydrogen peroxide (H_2O_2), as well as singlet oxygen (O_2). These harmful and highly reactive chemical species are produced in the human body as products from different metabolic pathways [4]. Fortunately, these harmful species may be removed from the human body by consumption or applications of certain compounds with good antioxidant

Proceedings of the 2nd International Conference on Biosciences and Medical Engineering (ICBME2019)
AIP Conf. Proc. 2155, 020001-1–020001-7; https://doi.org/10.1063/1.5125505
Published by AIP Publishing. 978-0-7354-1900-1/$30.00

capacity. Antioxidants scavenge for these ROS through three different mechanisms, namely hydrogen atom transfer (HAT) which involves the i) transferring of a hydrogen atom to the free radical, ii) single electron transfer (SET) in which an electron is donated to the highly reactive species and by iii) transition metal chelation (TMC) where the metal ions are chelated to form stable complexes [5].

In this study, the leaves of *Elaeis guineensis* was the targeted source of antioxidants. This oil-producing crop belongs to the family Arecaceae, native to West Africa [6]. It grows well in tropical countries like Malaysia and Indonesia due to the large rainfall throughout the year [7]. The study was focused on oil palm leaves as this part of the tree constitutes the largest produced biomass from the oil palm industry. Moreover, extracts of the leaves of *Elaeis guineensis* may have wide applications in pharmaceuticals and food industries due to its higher content of antioxidative phenolic phytochemicals. The compounds reported to exist in the leaves extract is mainly glycosylated flavonoids, epigallocatechin gallate, and epicatechin gallate. Other studies reported that oil palm leaves contained a myriad of other beneficial phenolic compounds, for instance, gallic acid, ferulic acid, catechins and its derivatives, protocatechuic acid which is mostly water-soluble antioxidants. These hydrophilic phenolic compounds have remarkable bioactivity properties such as antimicrobial and antioxidant activity, as an antimelanogenic agent, tyrosinase inhibition, and so forth [8, 9]. Therefore, the current study set about to investigate the bioactivity of the oil palm leaves extract previously acoustically isolated and gave the highest total phenolic content of 209.70 mg GAE/g. The oil palm leave extract was subjected to ferric, reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) tests. This study also reports the proximate analysis as well as the antimicrobial activity of the extracts.

MATERIALS AND METHODS

Chemicals and Instrumentations

2,2-diphenyl-1-picrylhydrazyl and 2, 4, 6- tri(2-pyridyl)-s-triazine) were purchased from Sigma-Aldrich (St. Louis, USA). Analytical grade ethanol (purity>99.8%) was purchased from Hayman Limited (Essex, England). Analytical grade methanol (purity > 99.8%), hydrochloric acid (37%), ferric chloride, ferrous sulfate, acetic acid glacial, and sodium acetate were purchased from Merck (Darmstadt, Germany). Streptomycin was purchased from Sigma-Aldrich (St. Louis, USA). Millipore Milli−Q water purification system was used to produce 18 mΩ deionized water that was used in all analyses. UV-visible spectrophotometer (UV-1601PC, Shimadzu) and analytical balance Shimadzu Philippines Manufacturing Inc. (Philippines) was used for measuring wavelength and weighing, respectively. *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC11775) and *Bacillus subtilis* (ATCC21332) were obtained from the American Type Culture Collection (ATCC).

Collection and Preparation of Plant Material

Fresh *E. guineensis* leaves were collected from a plantation belonging to Universiti Teknologi Malaysia, Johor in January 2019. The leaves were air-dried, cut into smaller pieces, and subsequently ground into powder. The powdered oil palm leaves were then kept in zip-locked plastic bags and stored at room temperature until further use. One gram of powdered sample was transferred into a 50 mL falcon tube containing 25 mL of 50% (v/v) ethanol: water and acoustically extracted using a probe ultrasonicator (Sonics Vibra Cell, America) for 30 min at an amplitude of 60%. Next, the suspension was centrifuged for 15 min at 4,000 rpm. The supernatant was decanted into an Erlenmeyer flask and lyophilized to yield the crude extract of *E. guineensis*. The extract was stored in a chiller at 4°C for further analysis.

Proximate Analysis

Proximate analysis to determine the percentage of moisture, ash, and protein, fat, carbohydrate as well as energy value of powdered *E. guineensis* leaves was performed according to the AOAC protocol (2007) and in−house methods, respectively.

Ferric Reducing Antioxidant Power Assay (FRAP)

The ferric reducing ability was determined by FRAP assay, in accordance with a protocol described by Benzie and Strain [10] with slight modifications. A 100 µL of sample solution of powdered *E. guineensis* (1 mg/mL) was added to a 1.8 mL of FRAP reagent. The standard calibration curve was then plotted using ferrous sulfate ($FeSO_4.7H_2O$) ranged from 0.2 to 2.0 mM. The absorbance was measured at 593 nm after 15 mins of incubation

at 37°C. The reducing ability was expressed as micromolar of Fe^{2+} equivalent to 1 mg of the highest TPC crude extract of *E. guineensis leaves* (µM/mg).

2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

Radical scavenging activity of *E. guineensis* leaves crude extract was performed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) using a method by Fu et al. [11] with minor modifications. Briefly, 4.0 mg of DPPH was dissolved in a 250 mL beaker containing 100 mL methanol to form the 100 mM DPPH methanolic solution. The *E. guineensis* leaves crude extract of the highest TPC of a concentration of 1 mg/mL was prepared in DMSO, and two-fold serial dilution was carried out from 1000 µg/mL to 7.8125 µg/mL. After that, an aliquot of 100 µL of crude *E. guineensis* leaves extract of each concentration was reacted with 100 µL DPPH methanolic solution in a 96 well microplate. After 5 min of incubation, the absorbance was measured at 517 nm, and Trolox and BHT were used as reference standards. The sample was then replaced with methanol as the blank, and all analysis was triplicated. The percentage of DPPH scavenging was determined using the following equation (1):

$$\%DPPHScavenging = [(A_{BlankDPPH} - A_{Sample}) / A_{BlankDPPH}] \times 100 \quad (1)$$

Where $A_{Sample} = (A_{Sample\ -\ Blank\ Sample})$. The IC_{50} value of DPPH free radical scavenging activity was obtained using GraphPad software.

Antibacterial Activity

A 50 µL bacterial suspension consisting of Gram-positive and two types of Gram-negative bacteria were spread evenly over nutrient agar using a sterile cotton swab. Equidistant wells each 5 mm in diameter were punched aseptically onto the nutrient broth agar using the end of a sterile P1000 tip. *E. guineensis* leaves crude extracts were prepared at three different concentrations (1 mg/mL, 50 mg/mL and 100 mg/mL), filtered sterilized (0.2 µm) and pipetted into the test wells. Sterile distilled water and 50% (v/v) ethanol: water were used as the controls for diluting the Streptomycin and samples, respectively. Streptomycin (1 mg/mL) prepared in sterile distilled water was used to test for reproducibility and to illustrate the bacterial resistant profiles. Each bacterial susceptibility test was carried out in triplicate. The plates were incubated at 37°C for 24 h, and the produced inhibitory zones were measured in millimeter using a ruler and a Vernier caliper.

RESULTS AND DISCUSSION

Proximate Analysis

Proximate composition (moisture content, ash, protein, fat, carbohydrate, the energy value of food) of the *E. guineensis* leaves extract is tabulated in Table 1. Ash content in the *E. guineensis* leaves was moderately low at 5.2% insinuating that there is a minimal presence of inorganic nutrients in the sample [12]. The high moisture content at 18.8% was suggestive of the high humidity of the leaves [13] while the fat content was at 7.1%. The fat content demonstrated the potential of oil palm leaves as a dietary supplement with promising nutritional properties. The protein was 11.2% in the oil palm leaves, which conveyed that the leaves were a potential source of antioxidants. This fact is supported by the high energy value corresponding to 339.5 kcal/100g and a carbohydrate amount of 57.7%.

TABLE 1. Proximate composition of *E. guineensis* leaves extract

Parameters	Unit	Results
Moisture	%	18.8
Ash	%	5.2
Protein	%	11.2
Fat	%	7.1
Carbohydrate	%	57.7
Energy value of food	kcal/100g	339.5

FRAP Assay

The reducing potential of *E. guineensis* leaves from Fe^{3+} to Fe^{2+} was assessed, and the data are tabulated in Table 2. The highest ferric reducing power was determined to be 1,417.40 µM Fe^{2+}/mg when reacted with the FRAP reagent consisting of acetate buffer, TPTZ solution, and ferric chloride solution. The outcome seen here in the FRAP data was due to electron transfer by the phenolics compound when Fe^{3+} was reduced to Fe^{2+} occurs in the presence of 2,4,6-tripyridyl-s-triazine. This reduction reaction is materialized and can be monitored by the formation of a colored complex with Fe^{2+} absorption at 593 nm [14]. The standard calibration curve is shown in Figure 1.

TABLE 2. FRAP of *E. guineensis* leaves extracts

Sample	Absorbance (nm)	Results (micromolar Fe^{2+}/mg)
Crude extract with highest TPC	1.569	1417.40

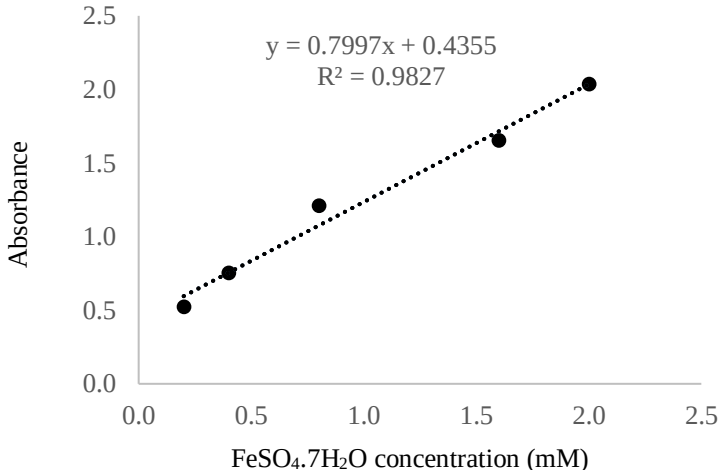

FIGURE 1. Standard calibration curve of ferrous sulfate ($FeSO_4.7H_2O$)

DPPH Free Radical Scavenging Activity

In this study, DPPH free radical scavenging capacity of the crude *E. guineensis* leaves extract was carried out using two-fold serial dilution from concentrations 7.8125 to 1000 ppm in a 96 well microplate (Figure 2). The antioxidant activity of the *E. guineensis* leaves extract scavenged the methanolic DPPH solution at a very low concentration, 7.8125 ppm to give a corresponding inhibition of 49.074 ± 0.047% (Table 3). It was noted that inhibition imparted by the crude leaves extract was relatively comparable to the antioxidant capacity of the standards, BHT, and Trolox, as depicted in Figure 3. The lower IC_{50} value at 9.899 ± 0.050 µg/mL seen in this study thus indicated a stronger antioxidant activity [9]. Hence, it was shown that the crude ethanolic: water extract of *E. guineensis* leaves possessed a promising antioxidant activity, thus indicating its suitability as a bioactive compound for topically applied nanoemulsions.

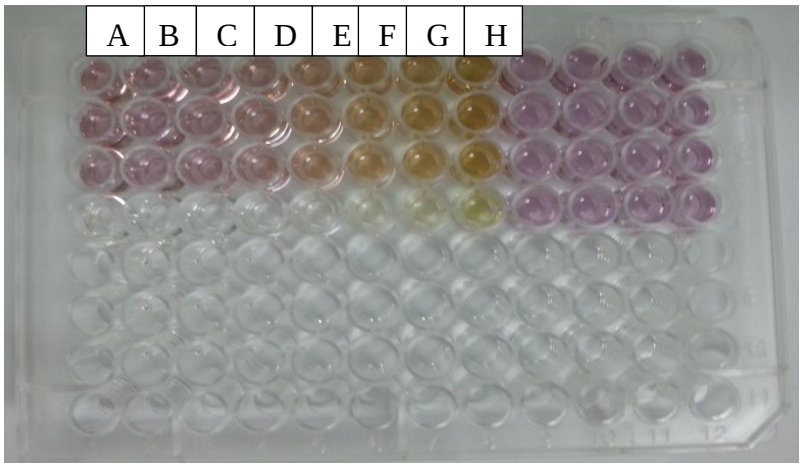

FIGURE 2. The 96 well microplate containing two-fold serial dilution of the different concentrations of the *E. guineensis* leaves crude extract after reaction with 100 mM of DPPH methanolic solution

TABLE 3. DPPH scavenging activity of *E. guineensis* leaves crude extract

Sample/Standard	**Concentration (µg/mL) and percentage of inhibition (%)**								**IC_{50} (µg/mL)**
	7.8125 (A)	**15.625 (B)**	**31.25 (C)**	**62.5 (D)**	**125 (E)**	**250 (F)**	**500 (G)**	**1000 (H)**	
DPPH assay for trolox	54.686 ± 0.244	57.575 ± 1.586	58.210 ± 0.532	60.535 ± 0.999	79.633 ± 2.468	81.677 ± 1.760	87.174 ± 0.440	96.405 ± 0.366	8.875 ± 0.087
DPPH assay for BHT	51.374 ± 1.730	55.743 ± 0.244	59.478 ± 1.164	62.649 ± 0.645	69.837 ± 0.244	72.797 ± 0.440	81.888 ± 0.976	89.855 ± 0.005	9.260 ± 0.062
DPPH assay for crude with highest TPC	49.074 ± 0.047	52.713 ± 0.440	53.981 ± 1.164	57.857 ± 1.586	61.099 ± 1.057	63.777 ± 1.407	66.596 ± 2.796	69.274 ± 2.328	9.899 ± 0.050

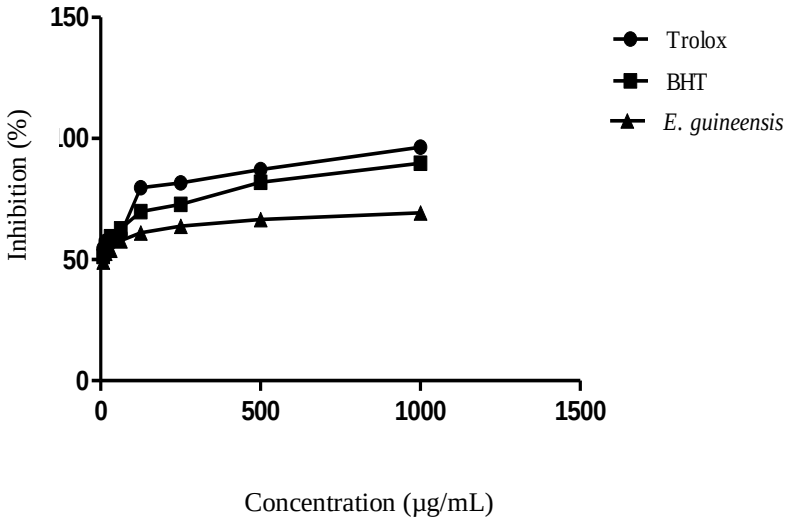

FIGURE 3. Percentage of inhibition of DPPH scavenging activity of Trolox, BHT, and the highest TPC crude extract of *E. guineensis* leaves.

Antibacterial

Antibacterial activity of the crude *E. guineensis* leaves extract was tested against the Gram-positive, *Bacillus subtilis.* The study found to be inhibited the bacterium when the concentrations 50 mg/mL and 100 mg/mL were used, to yield inhibition zones of 4.7 ± 3.1 mm and 4.7 ± 1.2 mm. Whereas, both species of Gram-negative bacteria were not inhibited by all three concentrations of the crude extract, as shown in Table 4. The antibiotic s*treptomycin* moderately inhibited the bacteria at 10.7 ± 2.9 mm for both types of bacteria in all test concentrations of the crude extract. The results seen here are consistent with a previous report that described the solvent extract of oil palm possessed a certain degree of antimicrobial activity against Gram-positive bacteria but was ineffective against Gram-negative ones [9]. The result of the antibacterial study is illustrated in Figure 6.

TABLE 4. Antibacterial assay of *E. guineensis* leaves extracts at three different concentrations

Bacteria	**Inhibition zone (mm)**		
	1 mg/mL	**50 mg/mL**	**100 mg/mL**
Gram-positive			
Bacillus subtilis ATCC 21332	ND	4.7 ± 3.1	4.7 ± 1.2
Gram-negative			
Escherichia coli ATCC 11775	ND	ND	ND
Pseudomonas aeruginosa ATCC 27853	ND	ND	ND
Antibiotic			
Streptomycin	10.7 ± 2.9 mm	10.7 ± 2.9 mm	10.7 ± 2.9 mm

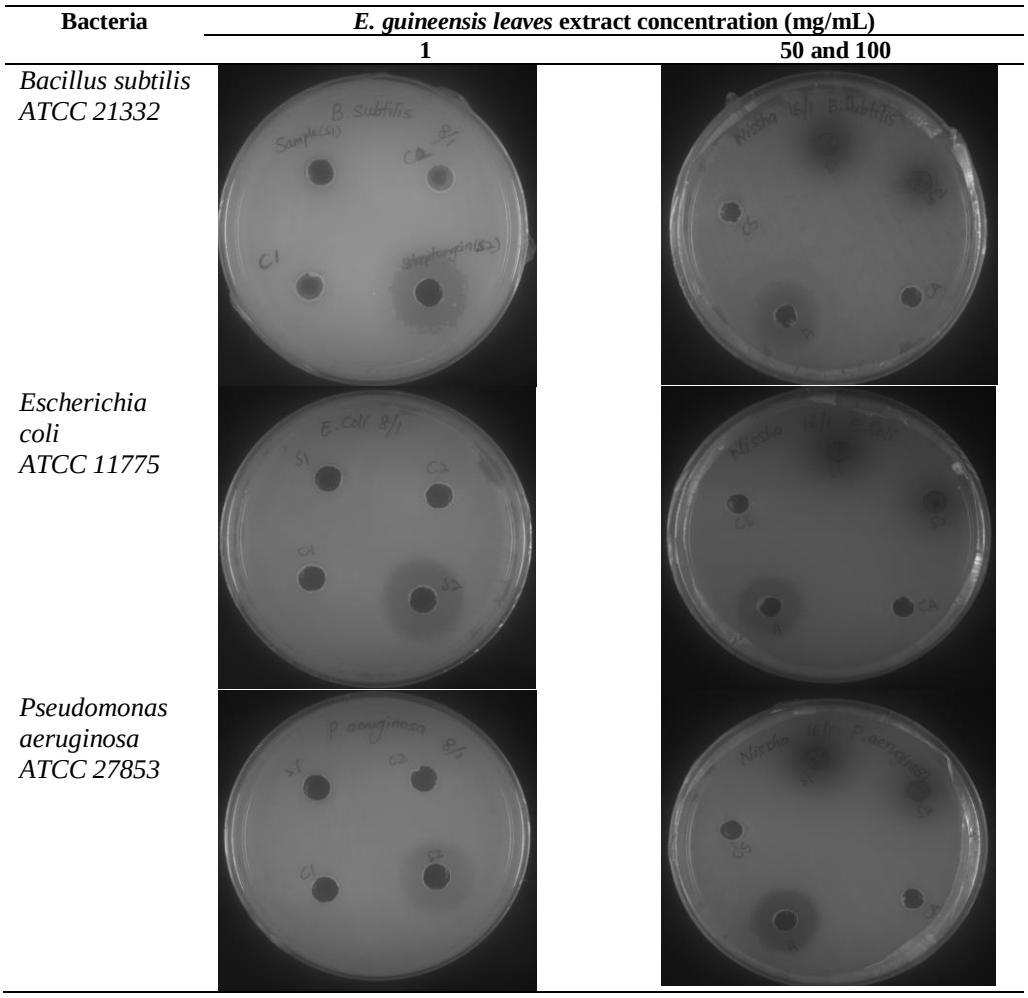

FIGURE 4. Petri dish containing nutrient broth and inhibition zone of the sample, streptomycin, control sample (solvent), and control sample (distilled water).

CONCLUSION

The findings of this study, therefore, indicated that the crude 50% (v/v) ethanol: water extract of *E. guineensis* leaves possessed an appreciable antioxidant capacity, as indicated by the results of FRAP, DPPH alongside antibacterial activity against Gram-positive bacteria but inactive against Gram-negative bacteria. FRAP assay of the crude extract showed a moderate value of 1,417.40 µM Fe^{2+}/mg. Apart from this, the crude *E. guineensis* extract exhibited strong DPPH free radical scavenging activity with a corresponding IC_{50} value of 9.899 ± 0.050 µg/mL. The assessed extract is a promising component of natural antioxidant that may be useful as a substitute over the synthetic antioxidant, BHT. This is based on the estimated IC_{50} value that is relatively close to BHT at 9.260 ± 0.062 µg/mL. In a nutshell, crude *E. guineensis* leaves extract can be further employed as a bioactive component to be added into nanoemulsions.

ACKNOWLEDGMENTS

This work was supported by the Fundamental Research Grant Scheme (QJ130000.2526.17H48) from the Ministry of Higher Education. The author is grateful to Universiti Teknologi Malaysia for funding her Zamalah Scholarship.

REFERENCES

1. N. Ahmad, Z. A. A. Hasan, H. Muhamad, S. H. Bilal, N. Z. Yusof, Z. Idris, J. Oil Palm Res. **30**(2), 315-325 (2018).
2. M. H. Maisarah, A. Noriham, M. Zainon, Int. J. Biosci Biochem Bioinforma., **3**(4), 349 (2013)
3. D. Ahmed, M. Khan, R. Saeed, Antioxidants, **4**(2), 394-409 (2015)
4. T. Ak, İ. Gülçin, Chem.-Biol. Interact., **174**(1), 27-37 (2008)
5. M. Leopoldini, N. Russo, M. Toscano, Food Chem., **125**(2), 288-306 (2011)
6. A. C. Hui, C. S. Foon, C. C. Hock, J. Oil Palm Res., **29**(3), 343-351 (2017)
7. E. Onoja, S. Chandren, F. I. A. Razak, N. A. Mahat, R. A. Wahab, Waste Biomass Valorization, 1-19 (2018)
8. S. Mohamed, Int. J. Food Processing Techno., **5**(2), 1 (2014)
9. N. Z. Yusof, S. S. A. Gani, Y. Siddiqui, N. Fadzillah, M. Mokhtar, Z. A. A. Hasan, J. Oil Palm Res. **28**(4), 520-530 (2016)
10. I. F. Benzie, Y. Szeto, J. Agric. Food Chem., **47**(2), 633-636 (1999)
11. R. Fu, Y. Zhang, Y. Guo, F. Liu, F. Chen, Ind Crop Prod, **58**, 265-270 (2014)
12. N. H. C. Marzuki, M. A. Hamid, R. A. Wahab, Mal. J. Fund. Appl. Sci. **14**(2) (2018)
13. S. K. Loh, Energy Convers. Manag., **141**, 285-298 (2017)
14. L. Cerretani, A. Bendini, "Rapid assays to evaluate the antioxidant capacity of phenols in virgin olive oil" , in *Olives and Olive Oil in Health and Disease Prevention*, edited by V.R. Preedy and R. Ross-Watson (Academic Press, Cambridge, 2010), pp. 625-635