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Evaluation of Extraction Solvent on Antioxidant Activity, Total Phenolic and Total Flavonoid Content in *Elaeis Guineensis*

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ABSTRACT

Oil palm or Elaeis guineens is a rich natural source of phenolic with flavonoid as the main constituents. These phenolics are potent antioxidants that can be used in the food industry, cosmetics and others. Therefore, the study was aimed to determine the effect of solvents which were methanol, ethyl acetate and hexane also different plant parts which were leaves, frond and fresh fruit bunch toward antioxidant activity (AOA), total phenolic content (TPC) and total flavonoid content (TFC). The antioxidant was analysed using the DPPH method, TPC by Ciocalteu assay and TFC by aluminium chloride colorimetric assay. The result from ANOVA indicated that there was a difference ($P < 0.05$) in the extracting ability of each solvent and different plant parts for AOA, TPC and TFC. Generally, the result suggested that methanol give the highest antioxidant activity, TPC and TFC compared to ethyl acetate and hexane. Therefore, the solvent used should be selected properly to allow for a high level of extraction efficiency.

Keywords: *antioxidant activity, Elaeis guineensis, extraction, total flavonoid content, total phenolic content*

1. INTRODUCTION

There have been a lot of demands in these recent years on the use of natural antioxidant instead of synthetic antioxidant. A natural antioxidant is known to be safe and possesses a role in protecting the human body against free radicals and any progress of chronic diseases. *E.guineensis* has been claimed for treatment of cancer, rheumatism, headaches, and as an aphrodisiac, liniment and diuretic by the folklore medicinal in ethnobotanical studies [1]. There are abundant of naturally occurring antioxidant that present which includes the waterdashsoluble antioxidant as well as the lipid-soluble antioxidant in fats and oils. The antioxidant component that contributed to the antioxidant activities in palm oil is water soluble antioxidant. Flavonoids and phenolic acids are commonly found rich in palm oil that has the capacity to function as antioxidants [2]. There are many phytochemicals that can be derived and obtained from various kind of plants including oil palm. Antioxidants are one of these phytochemicals in plants that have an ability to deal with oxidative stress that is commonly associated with various kind of disease including cardiovascular disease and cancer [3].

Biologically active compounds which are phenolic compounds, flavonoids, and many others, with known antioxidant, can be of great significance in therapeutic treatments.

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Therefore, the present research will aim to investigate the total antioxidant, phenolic and flavonoid content from oil palm extract along with its antioxidant.

2.0 MATERIALS AND METHODS

2.1 Chemicals and apparatus

Methanol, hexane, ethyl acetate, Folin–Ciocalteu's phenol reagent, the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH), Sodium chloride (NaCl) catechin, aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_2$) and sodium hydroxide (NaOH) were used. Spectrophotometric analyses were performed using a UV- 2450 spectrophotometer from MARDI Serdang, Selangor.

2.2 Sample preparation

The sample for the present research was obtained from the oil palm plantation area in Kampong Seri Mendapat Jasin, Melaka. The plant parts that were collected include the leaves, frond, empty fruit bunch and fresh fruit bunch. The fine powder of each sample was weighed for 20 gram and was soaked in hexane, methanol and ethyl acetate.

2.3 Determination of antioxidant content

This test was measured following the method of Blois[4]. The presence of antioxidants was measured from the ability of the oil palm extracts donate the electrons or hydrogen and that were determined from the bleaching of a purple methanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical. The reagent used for this assay was DPPH reagent. DPPH absorbs at 517 nm, and as its concentration was reduced by the existence of an antioxidant, the absorption gradually disappears with time. A 100 µl of a suspension of the methanol, hexane and ethyl acetate extracts of oil palm was mixed with 0.1 ml of 0.1 mM DPPH solution. The mixture was incubated in the dark for about 30 minutes at ambient temperature. The absorbance of the following mixtures were measured by using a UV- 2450 spectrophotometer. Each solvent which was methanol, hexane and ethyl acetate was used as a negative control for the antioxidant content in each solvent. Radical scavenging activity is often expressed as percentage inhibition and was often calculated using the formula.

$$\% \text{ scavenging activity} = 1 - (\text{Abs sample} / \text{Abs control}) \times 100$$

where Abs control is the absorbance of DPPH solution without extracts.

2.4. Determination of total phenolic contents (TPC)

The TPC of each extract was determined spectrophotometrically using the Folin–Ciocalteu assay [5]. Briefly, 0.5 ml of the crude extract was mixed with 8 ml of double-distilled water and Folin–Ciocalteu reagent (0.5 mL) and followed by the vortex. After 5 minutes at room temperature, 1 mL of 200 g/L sodium carbonate was added. The absorbance was measured at 725 nm using a UV/Visible spectrophotometer (U-2001, Hitachi Instruments Inc., and Tokyo, Japan).). The TPC of the extracts were expressed as gallic acid equivalent (GAE) g/20 g dry weight.

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2.5. Determination of total flavonoid content (TFC)

UV – visible spectrophotometer was used to determine the TPC. Briefly, 1 ml extract was mixed with 4 ml of double distilled water in the test tube and then followed by adding 0.3 ml of 5% $NaNO_2$ solution. After 5 minute, 0.3 ml 10% $AlCl_3$ was added. After another 1 minute, 2 ml of 1 M NaOH was added and the solution was marked up to 10 ml with distilled water solution then was vortexed and measured immediately at 510nm. Sample blank was prepared in the same way by replacing aluminium chloride with distilled water [6]. The TFC was calculated using a similar equation for TPC [7].

2.6 Statistical analysis

The results were replicated two times and expressed as mean values ± SD. The data were analysed and compared by using one - way analysis of variance (ANOVA). The relationship degree of statistic between the related linear was analysed through Pearson's linear correlations with a significance level ($P < 0.01$) using Statistical Package for the Social Science (SPSS). The average values were compared by Tukey's test.

4.0 RESULT AND DISCUSSION

Based on previous studies, it has been widely reported that polarity of the solvents used for yield extraction, as well as for the AOA, TPC and TFC analysis had given a significant effect toward the results. The polarity of the solvents could be classified into polar, semi-polar and non-polar solvents based on their dielectric constant. Polar solvents have large dipole moments or partial charges and they contain bonds between the atoms with very different electronegativities such as hydrogen and oxygen. On the other hand, the non-polar solvents contain bonds between atoms with similar electronegativities such as carbon and hydrogen. The bonds between the atoms with similar electronegativities will lack partial charges, thus making these molecules as non-polar. As for this study, methanol had been used for polar solvent, ethyl acetate as semi-polar solvent and hexane as the non-polar solvent.

4.1 Extraction yield

There are many steps involved before phytochemicals in plants are able to be obtained such as milling, grinding, homogenization and extraction [8]. Among the steps, extraction is the crucial parts for recovering the antioxidant, total phenolic and flavonoid content in oil palm and other plants. The chemical nature of the phytochemicals, the extraction methods that were used, the size of the sample particles, types of solvent used as well as the presence of interfering substances highly affect the extraction efficiency. The yield of extraction depends on the solvent with varying polarity, pH, temperature, extraction time, and composition of the samples [8]. In this paper, the yield of oil palm was obtained by using three different solvents which are methanol, ethyl acetate and hexane. In general, the yield of extraction from various solvents decrease in the following order: methanol < ethyl acetate < hexane. Figure 1 showed that the methanol extract from leaves parts had given the highest yield (15.20 %), followed by methanol from fresh fruit bunch extract (13.80 %) and ethyl acetate from empty fruit bunch extract (6.3 %).

It clearly indicated that the polar solvent is able to extract a higher yield than the non-polar solvent. It can be seen that the methanol that has the highest polarity had given the highest

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yield extract compared to ethyl acetate and hexane. Methanol is also found more effective in extracting the solute as it has the shorter chain. Other variation in yield for the solvents may due to other related factors such as phytochemicals in plants, extraction temperature, extraction time and solvent to solid ratio.

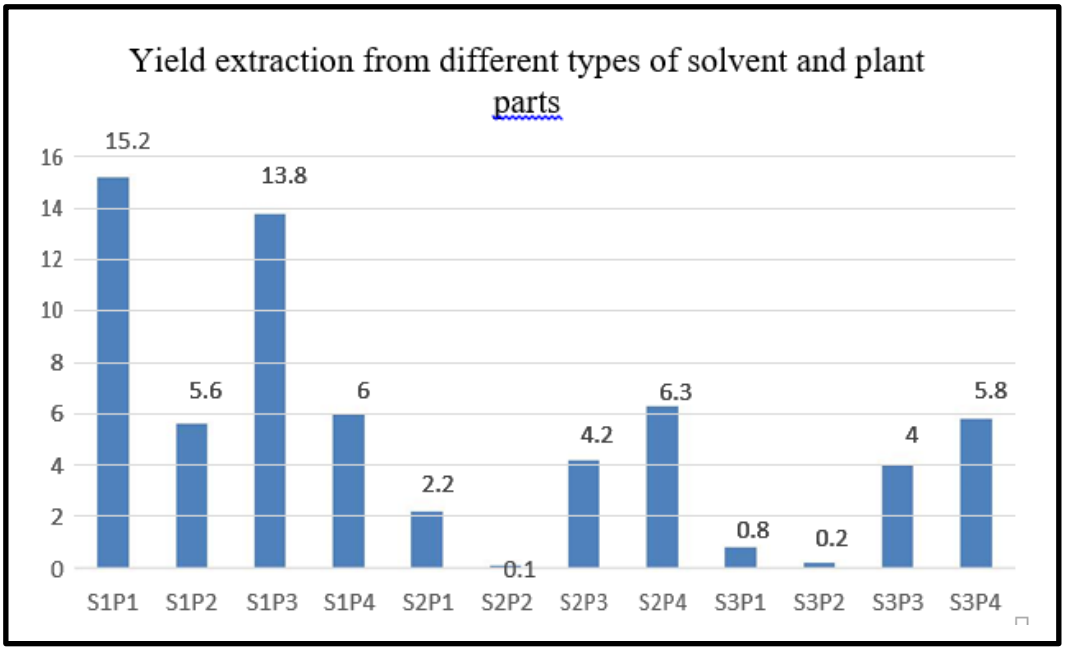

Figure 1 Yield extraction from different types of solvent and plant

Table 1: Antioxidant activity, Total phenolic content and Total flavonoid content

	P1 (Leaves)			P2 (Frond)			P3 (Fresh fruit bunch)			P4 (Empty fruit bunch)		
	AO	**TPC**	**TFC**	**AO**	**TPC**	**TFC**	**AO**	**TPC**	**TFC**	**AO**	**TPC**	**TFC**
S1	67.13 ±3.08	5.40± 0.063	0.39± 0.01	87.72 ±0.35	1.11± 0.06	0.11± 0.00	90.23 ±3.04	2.15± 1.00	0.26± 0.04	34.48 ±0.79	0.16± 0.04	0.11± 0.00
S2	36.47 ±0.69	0.63± 0.041	0.01± 0.00	51.88 ±2.07	0.01± 0.00	0.01± 0.00	90.49 ±0.72	1.78± 0.05	0.25± 0.05	37.46 ±0.46	0.71± 0.07	0.13± 0.00
S3	31.03 ±2.32	0.02± 0.006	0.02± 0.00	15.46 ±0.86	0.00± 0.00	0.01± 0.00	29.99 ±0.40	1.39± 0.05	0.07± 0.00	20.79 ±0.12	0.07± 0.00	0.13± 0.00

Values are expressed as mean ± standard deviation (n = 2)
Note S1: methanol S2: ethyl acetate S3: hexane

Table 1 showed the DPPH free radical scavenging (% inhibition) for types of solvent and plant parts. The analysis of the crude extract revealed the highest antioxidant properties in S2P3 (90.49 %) followed by S1P3 (90.23%), S1P2 (87.72 %/) and S1P1 (67.13 %). The lowest antioxidant content was found to be in S3P2 (15.46 %), followed by S3P4 (20.79 %), S3P3 (29.99 %) and S3P1 (31.03%). This indicates that the highest antioxidant was found in FFB along with the use of ethyl acetate as the solvent. Consistently, S1P3 also showed that FFB had given the highest antioxidant although the solvent used was methanol. On the other hand, the use of hexane from all parts which include the leaves, frond, FFB and EFB had given the lowest

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percentage of antioxidant. Therefore, it can be concluded that the use of hexane was less effective in extracting the antioxidant in oil palm.

AOA of the present study showed that ethyl acetate from the FFB parts gave the highest result instead of methanol and decreased with the order ethyl acetate > methanol > hexane. The results were not in agreement with the previous study by Hui [9] that has conducted a research on antioxidant activity for leaves extract of oil palm by using different polarity of solvent which is methanol, ethyl acetate, hexane, water and insoluble residue. The results of that study indicated that the result for DPPH free radical scavenging decrease as the polarity decrease, insoluble residue > methanol > ethyl acetate > water > hexane. This variability could be explained by a lack of method that was used to analyse the antioxidant activity in this study. It has been reported that a single procedure may not be able to represent all possible mechanism characterizing an antioxidant sufficiently [9]. There were many other previous studies that agree a single procedure is not sufficient to indicate the exact value of the antioxidant activity of any plants. Therefore, another method should be used other than DPPH assay to evaluate the antioxidant activity such as Lipid Peroxidation (LPO) inhibition assay. Past research by [8] reported that LPO had given the highest percentage of antioxidant activity (93.20 %) compared to DPPH assay.

Table 1 presented that S1P1 give the highest TFC which is 0.39 mg/g followed by S1P3 (0.26 mg/g) and S2P3 (0.25 mg/g). The lowest phenolic content was specified by S3P3 (0.07 mg/g) and followed by S1P2 (0.11 mg/g). Other extracts with the combination of solvent and plant parts had given negative and zero values which are S1P4, S2P1, S2P2, S2P4, S3P1 and S3P2 and S3P4. These results indicate that there was no flavonoid compound found in that particular crude extracts. From the result that was obtained, it clearly showed that using hexane as a solvent also had given the lowest TFC in all plant parts.

Table 1 also showed S1P1 dominated the highest TPC (5.40 mg/g), followed by S1P3 (2.15mg/g), S3P3 (1.78 mg/g) and S2P3 (1.39 mg/g). Based on the result in this study, the highest TPC were extracted from S1P1 which is methanol from leaves parts. The same trend was recorded for S1P3 which showed methanol as an extracting solvent giving the highest TPC. S1P3, S3P3 and S2P3 indicated that FFB had given the highest TPC compared to other plant parts. In this study, S3P2 had given zero value for total phenolic content in oil palm. This as well indicated that there was no TPC found in that particular extract. The lowest TPC were recorded in S2P2 (0.01 mg/g), followed by S3P1 (0.02 mg/g), S3P4 (0.07 mg/g) and S1P4 (0.16 mg/g). In general, the same tendency was observed in TPC where most plant parts showing the lowest value in hexane based on Table 1 above. Results indicated that TPC produced the highest value by using methanol as the solvent from leaves parts. The same tendency was observed for TFC when using leaves with methanol as the solvent had given the highest amount. However, the result for AOA was not in agreement as TPC and TFC.

In the present study, the result from TPC and TFC showed that methanol had given the highest value compared to ethyl acetate and hexane. The phenomena of TPC was similar to total TPC as flavonoid was major phenolic compounds in plants. The result from both TPC and TFC also suggested that the order of the value was methanol > ethyl acetate > hexane. The results were consistent with most of the studies that had supported the higher polarity of the solvent will lead to a higher result of TPC and TFC. The study by [10] revealed that the highest TPC and TFC were obtained by using the methanol as the solvent, followed by ethyl acetate and hexane in *Pluchea indicia plant.* The result was obtained due to the polarity of the solvents

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used [11] reported that as polarity increased, TPC would increase. Methanol also was indicated to be the most suitable solvent in the extraction of TPC due to its ability to inhibit the reaction of polyphenol oxidase (prevent food browning). Furthermore, it was reported that the least polar solvents are typically considered suitable for extracting lipophilic phenols while the polar solvent is suitable for extracting the hydrophilic phenols. Thus, the composition of the TPC in the parts of the oil palm plant must consist of hydrophilic phenols as it is able to generate a higher content of phenolic by using more polar solvent.

In addition, the TPC also seems to be low and not detected in the other samples of oil palm where it only ranges from 0.00±0.00 to 5.40±0.063 gallic acid equivalent. This could be due to other extrinsic factors. The content of phenol in plants was affected by the intrinsic (solvents, etc.) and extrinsic (environment, handling and development stage of the plant [12]. Additionally, during the plant development, the secondary metabolites of a plant such as the phenolic might be changed as it might occur due to the harsh climatic condition that was varied from the plant's usual habitat. Other than that, there were also several factors during the sample preparation that will affect the TPC such as the time of extraction, time of heating and others. Heating could cause significant losses of antioxidant, TPC and TFC, thus a low value of TPC and TFC might be caused by a longer heating time prior to grinding during the sample preparation.

3.2 ANOVA analysis of AOA, TPC and TFC based on types of solvents and plant parts.

The result showed a significant difference ($p < 0.05$) between TPC, TFC and AOA with the solvents and plant parts that were used. TPCs of the samples. For antioxidant capacity, it also showed a significant difference with solvents used even though the highest amount of AOA were obtained by using ethyl acetate as a solvent. Based on Table 1, it was clearly shown that the result for ethyl acetate (90.49 %) was only slightly higher than methanol (90.23 %). Table 1 also showed the lowest AOA, TPC and TFC were obtained from the hexane extract. Based on previous research by [13], it was reported that polar compound such as sugar, amino acid, phenolic compound with low and medium molecular weight and medium polarity, flavonoid aglycones and others can be dissolved in methanol. Besides, alkaloid and glycoside compounds also can be effectively extracted by ethyl acetate. [14] informed that lignin, wax, lipid and terpenoid which are non- polar compounds can be dissolved by hexane.

In another previous study by [15], the strongest scavenging activity was observed in ethyl acetate extract on the *Thapsia garganica*. The results were in line with the present result for the antioxidant that showed the highest AOA from ethyl acetate extract. The variation again might due to the phytochemical properties of the particular extract were less polar. Therefore, ethyl acetate works well as the solvent compared to methanol and hexane. In most studies before, it was reported that usually extract that contains high TPC would produce high AOA [16,17]. A strong positive correlation between TPC and antioxidant activity was found by few researchers for different plants [18].

CONCLUSION

The result from ANOVA suggested that there was a significant difference between the types of solvent used and different plant parts on the total antioxidant, total phenolic and flavonoid content. It has been proven that the solvent highly influenced the amount of antioxidant activity, total phenolic and flavonoid content in oil palm. It showed that the best

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solvent to be used for determining the total phenolic content and flavonoid content were methanol which is a polar solvent, followed by the intermediate polar solvent which is ethyl acetate and hexane, the non-polar solvent. It suggested that as the polarity increases, the amount of TPC and TFC will increase. The result also recommended that the best parts of oil palm that contain high antioxidant activity were leaves and FFB compared to other parts.

In conclusion, the ethyl acetate and methanol extract had given the highest AOA, TPC and TFC. The differences in phenolic content and antioxidant capacity were due to the different polarities. Therefore, several parameters such as the analysis method and solvent used need to be considered carefully. Additionally, the parameters required during the sample preparation also need to be optimized such as the extraction technique, extraction time and temperature, as well as solvent because the composition of antioxidant was also influenced by those factors.

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