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Extraction of Bioactive Compounds from Longan Peel by Using Solvent Extraction Method and its Antioxidant Activity

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Bioactive compounds from local longan (*Dimocarpus longan* Lour.) peels waste were extracted. Different temperatures and solvent systems were used to investigate the effects of extracting bioactive compound by using solvent extraction method. The temperature was varied from 30°C to 70°C with 10°C interval. Three different polarities of solvent were chosen, which were distilled water, ethanol and hexane. Total phenolic content and antioxidant activity for all extracted samples were performed by using Folin-Ciocalteu assay and free radical scavenging activity with 2,2-diphenyl-1-picrilhydrazil (DPPH) method, respectively. Ultraviolet-visible (UV-Vis) spectrometer was used to analyse all extracted samples. Total phenolic content for distilled water was the highest at 70°C (24.03 ± 4.15 mg GAE/g), ethanol at 60°C (68.63 ± 0.27 mg GAE/g) and hexane at 60°C (26.10 ± 0.14 mg GAE/g). Among the solvents, ethanol was found to be the best solvent for extracting phenolic compound from longan peels at 60°C and showed the higher antioxidant inhibition, which was 94.36%.

Keywords: antioxidant activity; total phenolic compound; longan peels

I. INTRODUCTION

Longan fruit is from a subtropical evergreen tree that belongs to the family Sapindaceae. This fruit has a thin, leathery and indehiscent pericarp that surrounds a succulent edible aril with a large dark brown seed and yellow brownish peel. It is rich in carbohydrates, protein, fibre, fat, vitamin C, amino acids and minerals (Liao *et al.*, 2017). Extractions from longan fruit such as aril, pericarp and seed produce excellent antioxidant ability, good anti-tyrosinase and anticancer activities (Chung *et al.*, 2010; Park *et al.*, 2010). Longan peel is usually regarded as a waste material. However, in recent years scientist discovered some applications of bioactive compound inside longan peels extract to act as an antioxidant, antibacterial and anticancer (He *et al.*, 2016).

In human, the production of free radical involves an oxidation process which will form a dangerous chain reaction that can cause metabolic impairment and cell death (Pizzino *et al.*, 2017). Consequently, the production of synthetic antioxidant that is chemically synthesised to reduce the number of free radicals is increasing. Unfortunately, this synthetic antioxidant was found to have genotoxic effect. Bioactive compound such as phenolic compound that can be extracted from fruit has strong in-vitro and in-vivo antioxidant activities. The phenolic compound can scavenge free radicals by breaking the radical chain reaction and chelate metal (Norshazila *et al.*, 2010).

Therefore, to minimise the risk of free radicals in the human body and minimise the usage of synthetic antioxidant, extracting phenolic compound as a natural antioxidant from longan peels was studied. Figure 1 gives the basic structure of the phenolic compound which has different hydroxyl groups that can conjugated into sugar, acids or alkyl group (Ahmad and Abdullah, 2013). It is difficult to possess a single technique for the efficient extraction of all phenolic compounds. To achieve an accurate assessment of phenolic compound through different food matrices it requires an extraction process

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optimisation (Garcia *et al.*, 2010). The solvent systems and temperatures of the phenolic compound extraction process from longan peels were optimised. Total phenolic content and antioxidant activities were determined by using Folin-Ciocalteu's method and DPPH assay, respectively.

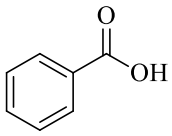

Figure 1. The chemical structure of phenolic acids

II. MATERIALS AND METHOD

A. Materials

5 kg of longan was purchase from fruit stall in Kuala Pilah, Negeri Sembilan. Chemicals used in the research were ethanol (HmbG Chemicals), hexane (HmbG Chemicals), methanol (HmbG Chemicals), 10 % of sodium carbonate anhydrous (QREC), gallic acid (Sigma-Aldrich), Folin-Ciocalteu reagent (Sigma-Aldrich) and 2,2-diphenyl-1-picrylhydrazyl (DPPH)(Merck) were purchased and used without auxiliary purification.

B. Methods

1. Sample preparation

The peels were washed with distilled water and oven dried at 50°C for 24 h. Then the dried peels were ground into powder and keep dried in the oven at 50°C for 72 h to remove excess moisture.

2. Extraction of peels

Extraction of powdered peels was performed according to Yang, *et al.* (2011). 8 g of powdered peels were added to 100 ml of ethanol and stirred for 2 h at various temperatures, which were from 30°C to 70°C, with 10°C interval. The crude extraction solution was filtered to remove the solid residue. Then the filtrate was quickly covered with aluminium foil to avoid direct light exposure. The steps were repeated by using distilled water and hexane as a solvent. The phenolic compound in the peel extracts were determined by using UV-Vis spectrophotometer.

3. Determination of total phenolic content

The Folin-Ciocalteu's (FC) assay was conducted to determine the total phenolic contents (TPC) of the peels extracts as described by Singleton and Rossi (1965). A standard calibration curve was prepared by using gallic acid with concentrations of 2, 4, 6, 8 and 10 μg/mL in methanol. Each concentration was mixed with 1580 μg/mL of distilled water and 100 μL of Folin reagent in a test tube. The mixture was shaken and left for 2 min to react. Sodium carbonate solution (300 μL) was added to the mixture and vigorously shaken for 15 s. The mixture was allowed to stand in dark for 30 min at room temperature before the construction of a standard calibration curve of gallic acid by using UV-visible spectrophotometer at 765 nm absorbance wavelength was conducted.

The procedure was repeated by using 20 μL peels extract solution. Unknown concentration of gallic acid from peels extract was determined by using a constructed standard calibration curve. The total phenolic content was then calculated by using the equation below, where C is concentration of gallic acid obtained from the calibration curve (Alias and Abbas, 2017).

$$\text{Total phenolic content} = \frac{\text{C} \times \text{volume extract (mL)}}{\text{mass of extract (g)}}$$

4. Antioxidant activity assay

Antioxidant activity of peels extract was determined through the evaluation of free radical-scavenging effect on the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical by using a method established by Gulcin (2006). The DPPH (0.3 mg) was prepared as a blank or standard by dissolving in methanol (1500 mL) to produce a concentration of 2.0 x 10^{-4} mg/mL and analysed by using UV-Vis spectrophotometer at wavelength of 517 nm. Other samples were prepared by adding 2 ml of the peels extract sample to 3.8 ml of DPPH solution followed by a vigorous shake and was then left in the dark for 30 min before being analysed. Changes in colour was observed from violet to yellow. All absorbance values were recorded, and the antioxidant activity of crude extract was calculated by using the following formula.

$$\text{Percentage inhibition (\%)} = \frac{\text{Absorbance of (standard} - \text{sample)}}{\text{Absorbance of standard}} \times 100\%$$

III. RESULT AND DISCUSSION

A. Bioactive Compound in Longan Peels Extract

UV-visible spectrophotometer was used to determine the bioactive compound present in the peels extract solution at a wavelength of 200 nm to 700 nm. The extraction was conducted in water, ethanol and hexane at a temperature that ranged from 30°C to 70°C. The results are summarised in Table 1.

Table 1. The bioactive compound in the peels extract solution

Solvent	T (°C)	λ_{max} (nm)	A	Bioactive compound
Water	30	250	3.095	Ellagic acid
		295	2.916	gallic acid
		370	2.277	Epicatechin
	40	250	3.126	Ellagic acid
		295	2.935	gallic acid
		370	2.320	Epicatechin
	50	250	3.160	Ellagic acid
		295	2.938	gallic acid
		370	3.007	Epicatechin
	60	250	3.160	Ellagic acid
		295	2.969	gallic acid
		370	3.009	Epicatechin
	70	250	3.034	Ellagic acid
		295	2.828	gallic acid
		375	2.621	Epicatechin
Ethanol	30	295	0.040	gallic acid
		370	0.155	Epicatechin
		665	0.507	Carotenoid
	40	295	0.050	gallic acid
		370	1.815	Epicatechin
		665	0.517	Carotenoid
	50	250	2.868	Ellagic acid
		295	2.632	gallic acid
		370	1.815	Epicatechin
		665	0.377	Carotenoid
	60	250	2.888	Ellagic acid
		290	2.697	gallic acid
		370	1.756	Epicatechin
		665	0.366	Carotenoid
	70	250	2.716	Ellagic acid
		295	2.526	gallic acid
		370	1.547	Epicatechin
		665	0.370	Carotenoid
Hexane	30	250	2.182	Ellagic acid
		415	0.267	Epicatechin
		670	0.068	Carotenoid
	40	250	2.309	Ellagic acid
		435	0.705	Epicatechin
		670	0.188	Carotenoid
	50	250	2.509	Ellagic acid
		435	0.809	Epicatechin
		670	0.210	Carotenoid
	60	250	2.619	Ellagic acid
		410	0.550	Epicatechin
		670	0.168	Carotenoid
	70	ND	ND	ND

ND= not determine; T= Temperature; λ = wavelength; A=Absorbance

From Table 1 it was observed that peels extract in ethanol at temperature of 60°C was the best condition to extract all bioactive compounds with the highest concentration. The UV-Vis spectrum of peels extracts in ethanol at 60°C is shown in Figure 2. Three phenolic compounds were observed, such as ellagic acid (250 nm), gallic acid (290 nm) and epicatechin (370-435 nm). Another bioactive compound that can be observed was carotenoid at 665-670 nm (Lim, 2012).

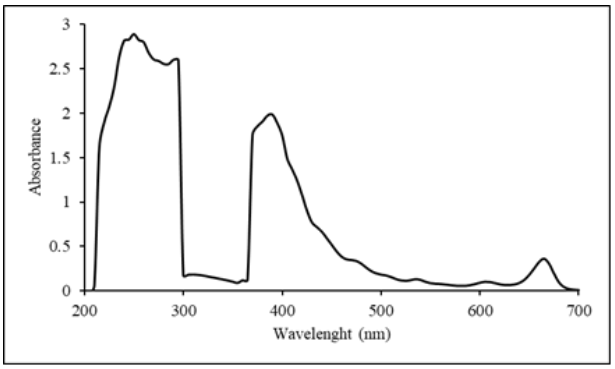

Figure 2. The UV-Vis spectrum of peels extracts in ethanol at 60°C

B. Total Phenolic Content

The phenolic contents in peels extract at different temperatures and solvent systems were determined by Folin-Ciocalteu (FC) assay. The FC assay consisted of gallic acid, distilled water, and Folin reagent. Figure 3 shows the change in FC assays from green to dark blue-green when reacted with sodium carbonate (Na_2CO_3) (Blainski *et al.*, 2013). This occurred due to the reduction of Folin reagent in the reaction mixture when phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$) were donated electrons through the phenol oxidation in alkaline condition as shown in the reaction below (Geremu *et al.*, 2016).

$$ArOH + Mo^{6+} + \bar{e} \longrightarrow ArO\cdot + Mo^{5+} + H^{+}$$

The presence of gallic acid in the FC assay was used as reference. Gallic acid standard calibration curve of absorbance at 765 nm against the concentration was plotted in Figure 4. The standard calibration curve showed linearity in the range of 2 until 10 mg/ml with correlation coefficient R^2 of 0.9965. The unknown concentration of gallic acid present in the peels extract at different temperatures and solvent systems was determined by using this standard calibration curve. The total phenolic content (TPC) of peels extract was determined by gallic acid equivalent (GAE). The

result of TPC at different temperatures and solvent systems was done in three replicates and the data are recorded in Figure 5.

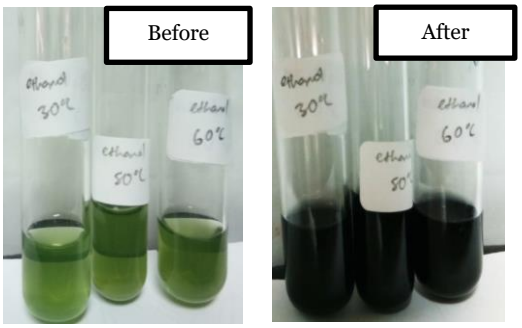

Figure 3. The colour changes observation before and after adding sodium carbonate in FC assay

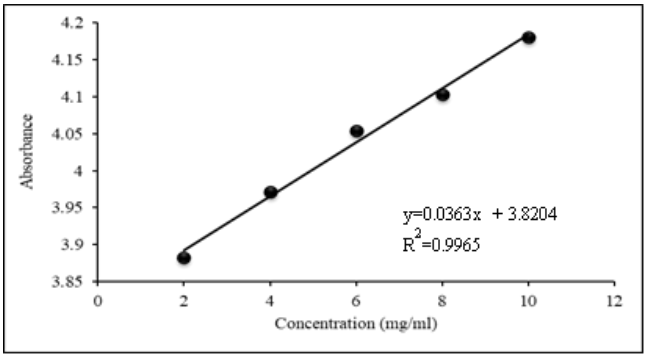

Figure 4. Gallic acid calibration curve

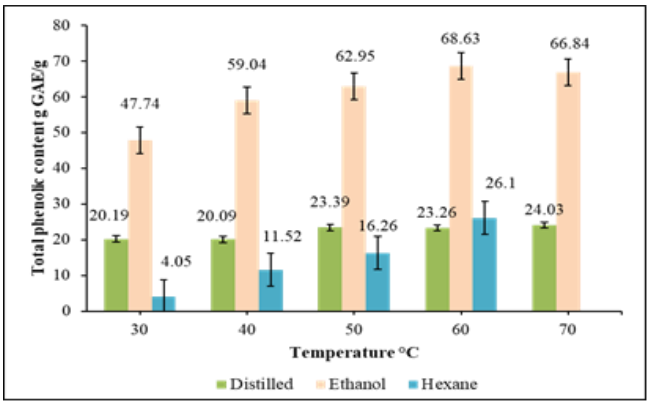

Figure 5. Total phenol content for distilled water, ethanol and hexane at different temperatures

Difference in solvent polarities is the main factor in extracting phenolic compound, which is a polar compound. Based on the tabulated data in Figure 5, ethanol solvent system at 60°C extracted the highest of total phenolic compound of about 68.63 mg GAE/g as compared to hexane and distilled water. These results showed that ethanol polarity is the most suitable for extraction all phenolic compound as compared to distilled water which is more polar. Furthermore, the low TPC detected in distilled water solvent system could be related to the non-polar character of the plant material cell wall. This characteristic allows solvents that are less polar than water like methanol, ethanol and acetone to extract the phenolic compounds from cells more easily (Dorta *et al.*, 2012).Meanwhile hexane is not suitable due to its non-polar solvent character.

Heating process during extraction is also a factor that needs consideration. The presence of heat will soften plant tissue and decrease the interaction between phenol protein and phenol-polysaccharide, hence will enhance the diffusion rate and solubility of phenolic compound. Figure 5 shows that the TPC is increased as the temperature increased for all solvent system. However, at temperature of 70°C, the TPC in ethanol was decrease due to chemical and enzymatic decomposition which caused the phenolic compound to decompose (Minatel *et al.*, 2017). Meanwhile in hexane the TPC was 0 because hexane was evaporated at that temperature.

C. Antioxidant Activity

The free radical scavenging capacity of antioxidant in peels extract was measured in terms of the ability to donate hydrogen by using DPPH scavenging method. DPPH is a compound that has a delocalisation of extra an electron over the molecule and this characterize it as a stable free radical. The transfer of electron or hydrogen from phenolic compound in peels extract to DPPH has neutralised its free radical character and this reaction is known as antioxidant activity (Ighodaro and Akinloye, 2018). The antioxidant activity caused the colour change from violet to yellow.

Figure 6 shows that colour was changed after the peels extract was added into the DPPH solution. This observation indicated that the peels extract contains an antioxidant compound. However, the peels extract in hexane showed a dense colour of violet which proved that the solution contained small amount of antioxidant compound. The observation was proven by the calculation of % inhibition of antioxidant and the data are tabulated in Table 2. From the result, peels extract in ethanol had highest percentage of antioxidant activity of about 94.36 % at 60°C. Therefore, the results showed an agreement with TPC data whereby the amount of TPC is higher in ethanol solvent system at 60°C.

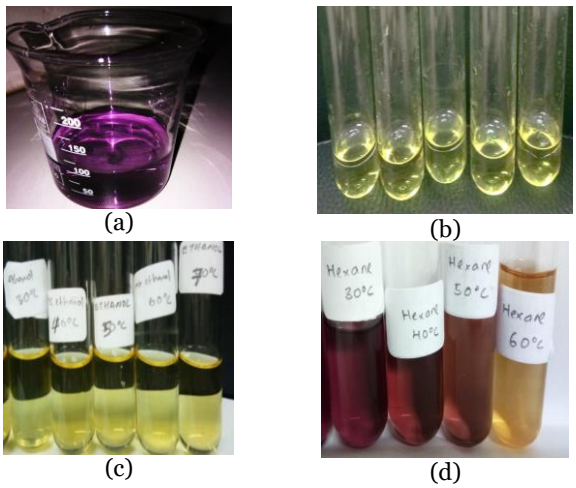

Figure 6. Colour change of DPPH (a) after additional of peels extract in distill water (b), in ethanol (c) and in hexane (d). Temperature increase from left to right

Table 2. The antioxidant activity of longan peel extract at different temperature and solvent system

solvent	T (°C)	Absorbance			inhibition (%)
		1st	2nd	3rd	
water	30	0.124	0.123	0.123	92.84
	40	0.133	0.113	0.113	93.43
	50	0.143	0.143	0.143	91.68
	60	0.146	0.145	0.146	91.59
	70	0.168	0.167	0.168	90.25
Ethanol	30	0.123	0.123	0.123	93.31
	40	0.137	0.137	0.137	93.83
	50	0.143	0.143	0.143	94.01
	60	0.146	0.145	0.146	94.36
	70	0.168	0.167	0.168	93.43
Hexane	30	1.025	1.024	1.024	40.45
	40	0.863	0.862	0.862	49.86
	50	0.770	0.770	0.770	55.23
	60	0.340	0.340	0.340	80.23
	70	ND	ND	ND	ND

ND= not determine; T= Temperature

A previous study on longan peels extraction in ethanol by using soxhlet and microwave-assisted method conducted by Parashar *et al.*, (2014) had reached about 90.35 % and 96.78 % of antioxidant activity, respectively. Meanwhile, in this study the longan peels extract in ethanol had achieved 94.36% of antioxidant activity. The results, showed no significant different and consequently, proved that the solvent extraction method is a comparable method with other extraction methods for phenolic compound extraction.

IV. CONCLUSION

The total phenolic compound (TPC) was determined by using FC assay and showed that ethanol had the highest yield of TPC of about 68.63 mg GAE/g and 94.36 % of antioxidant activity at 60°C extraction temperature. This finding confirmed that the optimum temperature and solvent polarities are major factors that affect the solubility of phenolic compound during the extraction process as compared to extraction method used.

V. ACKNOWLEDGEMENTS

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