The Effect of Extraction Conditions on Total Phenolic Content and Free Radical Scavenging Capacity of Selected Tropical Fruits' Peel

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ABSTRACT: The present study aimed to investigate the effects of ethanol concentration (0-100%, v/v), extraction time (60-300 min), and extraction temperature (25-60°C) on total phenolic content (TPC) and free radical-scavenging capacity of rambutan *(Nephelium lappaceum)*, mangosteen *(Garcinia mangostana)*, and langsat *(Lansium domesticum)* peels using single-factor experiments. Folin-Ciocalteu's method was used for the determination of TPC, while the antioxidant capacity was determined by 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. All the extraction conditions showed significant effect (p<0.05) on TPC and DPPH radical-scavenging capacity. The best extraction conditions determined for rambutan peel were 80% ethanol concentration for 120 min at 50°C; for mangosteen peel were 60% ethanol concentration for 60 min at 25°C; and 80% ethanol concentration for 120 min at 25°C were the best extractions for langsat peel. Extracts were then subjected to various antioxidant assays such as DPPH assay, TPC, nitric oxide scavenging activity (NO) and β-carotene bleaching (BCB) assay. Based on these optimized extraction conditions, high antioxidant capacity was obtained with EC_{50} value of 8.87 µg/mL (DPPH radical scavenging activity), 64.88% for NO activity, and 98.19% for BCB activity; as well as 53.94 mg GAE/g (TPC) for rambutan peel. While mangosteen peel showed a EC_{50} value of 19.75 µg/mL (DPPH), 54.66% (NO), 79.94% (BCB), and TPC value of 42.25 mg GAE/g; and langsat peel with EC_{50} value of 85.28 µg/mL (DPPH), 50.42% (NO), 23.95% (BCB) and TPC value of 22.04 mg GAE/g. With these extraction

conditions, maximum antioxidant capacity could be obtained for future studies of all the selected fruits peel and it will be useful for nutraceutical development.

Keywords: Extraction condition, antioxidant activity, *Lansium domesticum*, *Garcinia mangostana*, *Nephelium lappaceu)*, total phenolic content

Introduction

Malaysia is a tropical country with a large diversity of fruits which is high in antioxidant properties. Guo *et al*., (2003) claimed that high fruit intakes were associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer. One of possible mechanisms was attributed to the antioxidant activity presented by the fruits. Interest has been expressed in fruits as natural sources of antioxidants in recent years, as most of the bioactive compounds from fruit extracts have been discovered to exhibit antioxidant activity. The most abundant antioxidants in fruits are vitamin C, A, B and E, and carotenoids are present to a lesser extent in some fruits. Besides classical antioxidants, phenolic compounds had been identified as important antioxidants contained in fruits (Norshazila *et al.,* 2010).

Phenolics and their derivatives are the largest group of plant secondary metabolites that are produced to aid plant survival in natural. Phenolic compounds are effective antioxidants which possess good redox properties to scavenge free radicals and form stable end products. Some phenolic compounds are more powerful as antioxidants than vitamin C and vitamin E in vitro (Guo *et al.,* 2003). However, fruits are diverse in antioxidant composition and antioxidant activity and those with high antioxidant activity generally contain more antioxidants. Interestingly, the peel fractions of some fruits possess higher antioxidant activity than the pulp fractions. Arazo *et al.,* (2011) reported that mangosteen peel exhibited higher antioxidant activity compared to its pulp. Therefore, the peel fractions of fruits may potentially contain more antioxidants quantitatively or qualitatively than the pulp fractions.

Extraction process is widely known as a separation process of a crude extract of phytochemicals from the raw materials (Chew *et al*, 2011a) However, there is no single universal extraction

method applicable for all food matrices because of the complexity of phenolic compounds and its interaction with other bioactive compounds presented in the food matrices. There are few factors would contribute in influencing the rate of extraction and quality of extracted bioactive phenolic compounds, including type of extraction solvent, solvent concentration, temperature and pH of extraction and extraction time (Chew *et al.,* 2011a; Ng *et al*., 2012).

In the present study, single factor experiment approach was used to determine the optimization of the extraction process on the selected tropical fruits peel. Single factor experiment is a classical method in which only one factor is variable at one time while all others are kept constant (Zhang *et al*., 2007). The famous seasonal fruits being investigated in this study are rambutan *(Nephelium lappaceum)*, mangosteen *(Garcinia mangostana)*, and langsat *(Lansium domesticum)*. Since numerous studies conducted previously have proven that these fruits possess high antioxidant properties (Arazo *et al*., 2011; Khaomek *et al*., 2012), thus the extraction of these selected tropical fruits waste using different extraction parameters is crucial. Therefore, this study aims to determine the total phenolics and antioxidant activities of the selected tropical fruit's peel. The best extraction condition was determined by studying the effects of ethanol concentration, extraction time and extraction temperature using single factor experiment.

Materials and Methods

Chemical and reagents

Analytical grade ethanol and sodium carbonate anhydrous were obtained from Fisher Scientific (Loughborough, Leicestershire, UK). Folin-Ciocalteu's reagent was purchased from Merck (Darmstadt, Hesse, Germany). 2,2-Diphenyl-1-picrylhydrazyl (DPPH, 95% purity) and other chemicals were purchased from Sigma Chemicals (St. Louis, Missouri, USA).

Sample preparation

The samples (rambutan, mangosteen and langsat fruits) were purchased from a fruit farm in Selangor. The selected fruits have been registered with the Department of Agriculture, Malaysia (No. R161: Rambutan; No. GA2: Manggis; No. DL2: Langsat) (Plant Variety Protection Malaysia, DOA).The fruits were washed under tap water and the peels were separated from the pulp. The peels were cut into small pieces of about 1 cm^2 before drying in a convection oven (Memmert, Schwabach, Germany) at 45°C. The initial drying and the final drying weights of the each sample were recorded separately. The dried samples were grinded into powdered form using grinder. All samples were vacuum-packed using vacuum packager Model DZQ400/500 (Zhejian, China) into small packets of about 5 g each. The vacuum packaged powder was wrapped with aluminum foil to prevent light exposure and stored in the dark at room temperature for further analysis.

Sample extraction

Five grams of each dried powder sample was accurately weighed into 100 mL conical flask and mixed with a 1:10 sample solvent ratio of ethanol and water. Sterilisation of the samples was not performed with the purpose of determining the antioxidant activity at their raw state.The conical flask was then sealed with parafilm (Pechiney plastic packaging, USA) and wrapped with aluminium foil (Diamond, USA) to prevent spilling of mixture and light exposure. Subsequently, the mixture was shaken at 150 rpm by using an orbital incubator for a particular duration at a specific temperature and time. The extract was then centrifuged at 3000rpm for 10 minutes. The mixture of supernatants was filtered through a filter paper (Whatman No.1) and the first filter were collected and stored in 4°C while the residues were re-extracted. Pooled extracts were concentrated using rotary evaporator at 40°C and was stored in the -20°C until further test. The yield (%, w/w) of crude extract was determined using the Eq. 1 below:

$$\text{Yield } (\%) = \frac{\text{Mass of extract}}{\text{Mass of sample}} \times 100\% \qquad (1)$$

Experimental design

In this study single factor experiment was used to determine the optimum condition of the selected fruit's peel. A total of three parameters namely ethanol concentration, extraction time and temperature were studied of which one parameter was varied at a time while the other parameters were chosen based on the values of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total phenolic (TPC) assays. The optimal extraction conditions were selected based on the values of DPPH and TPC assays; the crude extract of each sample was then subjected to antioxidant assays.

a) Ethanol concentration

By fixing extraction time and extraction temperature at 60 min and 25°C, respectively, samples were extracted using binary solvent of ethanol and water. The optimal ethanol concentration was selected based on the values of DPPH and TPC assays with ethanol concentrations ranging from 0% to 100% (Thoo *et al*., 2010).

b) Extraction time

In this parameter, the samples were extracted using the optimum ethanol concentration in stage one. Extraction time was varied from 60 to 300minutes while extraction temperatures were kept constant at 25°C. The optimal extraction time was again chosen based on the values of DPPH and TPC assays. (Chew *et al*., 2011b)

c) Extraction temperature

By using the optimal ethanol concentration and extraction time as determined in first step and second step, respectively, samples were extracted at different temperatures, ranging from 25°C to 60 °C. The best extraction temperature was again selected according to the values of DPPH and TPC of the samples (Chew *et al*., 2011b).

DPPH radical scavenging assay

Free radical scavenging activity of the extracts will be determined by the method of Xu and Chang (2007) with slight modification. Briefly, 1 ml of DPPH solution (7.8mg, in 95% ethanol) was incubated with varying concentration of the extract. The reaction mixture was shaken well and incubated for 30 min at room temperature and the absorbance of resulting solution was read at 517 nm against a blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following Eq. 2:

$$\text{Scavenging effect (\%)} = [1- (\text{Abs}_{\text{sample}} / \text{Abs}_{\text{control}})] \times 100 \quad (2)$$

Nitric oxide scavenging activity

Nitric oxide radical scavenging was estimated on the basis of Griess Illosvoy reaction using a modified method of Govindarajan *et al*. (2003). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and fruit peel extract (10 to 200 µg/ml) or standard solution (ascorbic acid, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylene diamine dihydrochloride (NADH) was added, mixed and allowed to stand for 30 min at 25°C. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions.

$$\text{Scavenging effect (\%)} = [(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})/ \text{Abs}_{\text{control}}] \times 100 \quad (3)$$

β-carotene bleaching assay

The β-carotene bleaching assay was determined using the method of Nsimba *et al.* (2008) with slight modification. About 2 mg of β-Carotene powder in 1 ml of chloroform was added into a flask containing 0.02 ml of linoleic acid and 0.2 ml of Tween 40. The chloroform is removed

under vacuum at 40°C for 10 minutes using rotary evaporator. Distilled water (100 ml) was added to the mixture and 1 ml of the mixture was then mixed with 0.1 ml of the sample extract with different concentrations. The mixture was later incubated at 45°C and the absorbance (470 nm) was read at every 15 minutes for 120 minutes. Degradation rate (DR) was calculated according to first order kinetics, using the following equation based on Al-Saikhan *et al*. (1995):

$$DR_{sample} = \ln (a/b) \times 1/t \quad (4)$$

Where, ln is natural log, a is the initial absorbance at time 0, b is the absorbance at t = 20, 40, 60, 80, 100 or 120 minutes.Antioxidant activity (AA) was expressed as percent of inhibition relative to the control, using the following formula where A_A is the absorbance of the sample and A_C is the absorbance of the control:

$$AA\ (\%) = [(A_{A(120)} - A_{C(120)})/ (A_{C(0)} - A_{C(120)})] \quad (5)$$

Total phenolic content

The amount of total phenolics in the selected fruit extracts was determined with the Folin-Ciocalteu's reagent using the method of Ferreira *et al.* (2007) with slight modification. To 1 ml of each sample, 4 ml of 1/10 dilution of Folin-Ciocalteau's reagent is added and after 3 min, 5 ml of Na_2CO_3 (7.5%, w/v) were added and incubated for 30 min at room temperature. The absorbance of all samples was measured at 765 nm. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g).

Statistical Analysis

All the experiments were conducted in triplicates. Statistical analyses were conducted using SPSS (Statistical Package for the Social Sciences) version 19. One-way analysis of variance (ANOVA) with Tukey's multiple comparisons was performed and significant level was defined using $p<0.05$.

Results and Discussion

Extraction yield

The yields of the selected tropical fruits peel are presented in Table 1. Mangosteen *(Garcinia mangostana)* contributed to the highest yield, amounting 38.31%, followed by langsat *(Lansium domesticum)* with the total yield of 28.58%, while rambutan has the lowest yield of 28.23%.

Table 1: Yields (%) of rambutan, mangosteen and langsat peel

Species	Weight of sample (g)		Yield[a] (%)
	Before drying	After drying	
Rambutan *(Nephelium lappaceum)*	1693.68	478.11	28.23
Mangosteen *(Garcinia mangostana)*	1637.6	627.44	38.31
Langsat *(Lansium domesticum)*	1558.18	443.75	28.58

(%)Yield = Weight $_{\text{after drying}}$ / Weight $_{\text{before drying}}$ x 100%.

Effect of extraction solvent concentration

Antioxidant and phenolic compounds of rambutan, mangosteen, and langsat peel were extracted using different concentration of ethanol ranging from 0% (v/v) to 100% (v/v). A suitable solvent to ratio was able to further improve the extracting efficiency (Yu *et al*., 2002). Ethanol and water were used as the extraction solvents in this study due to that they are safer in handling as compared to other organic solvents, such as methanol and acetone and more importantly, they are acceptable for human consumption. In this study, six different concentrations were used to extract total phenolics and antioxidant compounds from the selected fruits peel. TPC and antioxidant activity of rambutan, mangosteen, and langsat peel were shown in Figure 1a-1c. The results for DPPH assay were calculated as EC_{50}, where a lower EC_{50} value is associated with a stronger DPPH scavenging capacity. The results for TPC were expressed as gallic acid equivalents (mg GAE/100 g).

As observed in Figure 1a, rambutan peel showed the highest extraction capability for DPPH assay at 40% followed by 100%, and then 80% where their EC_{50} values were 8.57±0.44 µg/ml, 8.41±0.16 µg/ml and 9.36±0.39 µg/ml respectively. However, there were no significant differences among (p<0.05) the three concentrations. It was also shown that all ethanol concentration were capable of extracting phenolics but however ethanol concentration at 80% was more effective than 40% and 100% for extracting the rambutan peel sample. The highest value of TP was recorded for 80% (397.06±27.79 mg GAE/g) and it was significantly different (p<0.05) from all other concentration studied. Thus, a concentration of 80% of ethanol was chosen for the determination of the effects of extraction time and extraction temperature for rambutan peel.

The result of antioxidant activity (DPPH) and TPC of mangosteen are presented in Figure 1b, respectively. The highest DPPH scavenging activity was observed at 80% with EC_{50} value of 7.98±0.23 µg/ml followed by 60% with EC_{50} value of 9.87±0.78 µg/ml and it was significantly different(p<0.05) from all other concentration studied. The highest TPC value was observed at 80% (233.25±27.23 mg GAE/g) followed by 100% (204.15±20.57 mg GAE/g) and 60% (187.61±20.65 mg GAE/g). However, there was no significant difference (p<0.05) observed from the three concentration. From the economical point of view, 60% ethanol was selected instead of 80% ethanol for the determination of the effects of extraction time and extraction temperature for mangosteen peel. It was because there was no significant difference (p<0.05) in TPC between these two ethanol concentrations and also it has high scavenging activity as depicted in Figure 1b.

For langsat peel, the extraction concentration selected was of 80% which has the lowest EC_{50} value (24.35±0.31 µg/ml) and higher TPC value (131.33±6.69 µg/ml) when compare to all other concentration used as shown in Figure 1c. For DPPH assay, the highest scavenging activity was observed at a concentration of 80 % and followed by 60% of ethanol, however no significant difference (p<0.05) was observed. Highest TPC value was observed at a concentration of 80% and followed by 60%. Thus 80% of ethanol concentration was chosen for the determination of extraction time and temperature since it gives the highest antioxidant and TPC in both assays.

Similar finding was reported for extraction of dried sage using 80% ethanol, which resulted in highest TPC (Durling *et al*., 2007).

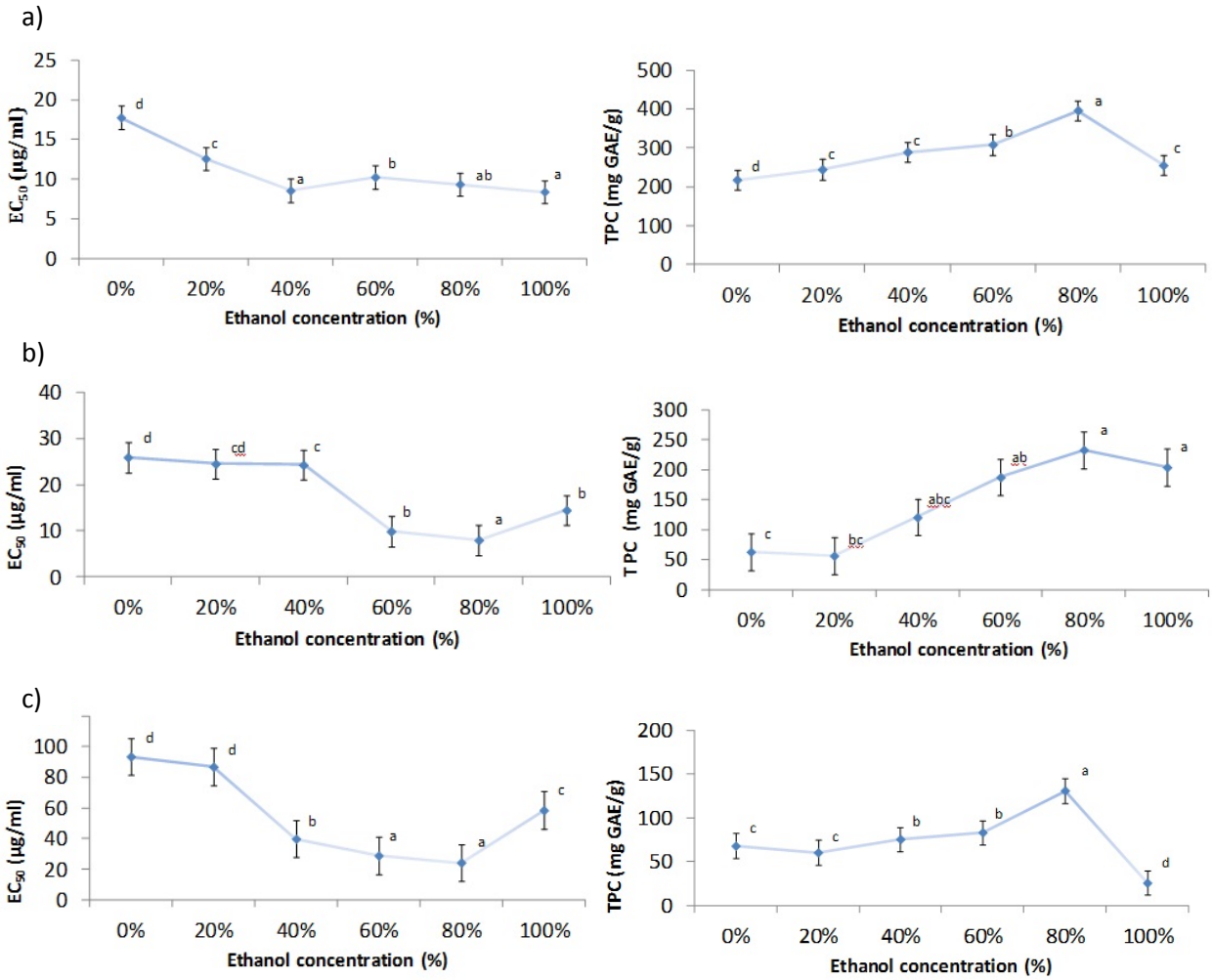

Figure 1: Effect of the ethanol concentration on the scavenging activity (EC_{50}) and TPC from (a) rambutan *(Nephelium lappaceum), (*b) mangosteen *(Garcinia mangostana)* and (c) langsat *(Lansium domesticum)* peels. Values are presented as means ± standard deviation of six measurements. Values marked by different lower case letters (a-d) are significantly different (p<0.05).

According to Spigno *et al*. (2007), alcoholic solvents have been commonly employed to extract phenolics from natural sources where they gave quite a high yield of total extract even though they are not highly selective for phenols. Mixtures of alcohols and water have revealed to be more efficient in extracting phenolic constituents than compared to mono-component solvent system. Addition of small quantity of water to organic solvent usually creates a more polar medium which facilitate the extraction of

polyphenols as suggested by Spigno *et al*. (2007). Thus, ethanol concentration of 60% for mangosteen and 80% for both rambutan and langsat were selected for the determination of the effects of extraction time and extraction temperature.

Effect of extraction time

Extraction time is crucial in minimizing energy and cost of the extraction process. Figure 2a-c describes the antioxidant activity and TPC extracted from rambutan, mangosteen and langsat peel samples using various range of extraction time. As observed in Figure 2a, the highest DPPH scavenging activity for extraction time of rambutan peel was observed at 2 h and began to decrease at 3 h. The EC_{50} obtained at extraction of 2 h was 7.48±0.51 µg/ml. As observed in Figure 2a, the highest TPC value obtained from the rambutan peel was at 1 h (308.36±39.35 mg GAE/g) and followed 3 h (294.56±6.70 mg GAE/g). However, there was no significant difference (p<0.05) existed at extraction time between 1 to 3 h. Thus based on the values of DPPH assay and TPC assay, extraction time for rambutan peel was finalized at 2 h.

Figure 2b describes the DPPH scavenging activity of mangosteen. It is shown that the highest scavenging activity was observed at 1 h and decreased at the following hour. EC_{50} value obtained at extraction time of 1 h was 8.97±0.40 µg/ml. The highest TPC value observed is at extraction time of 5 h (235.41±6.97 mg GAE/g) compare to lower extraction temperature. However, there was no significant difference (p<0.05) observed among all the five extraction times. Thus, the extraction time of 1 h was selected for the determination of the extraction temperature for mangosteen since the antioxidant capacity is higher at that respective time. The range of time for mangosteen was basically determined based on the practical and economical aspects (Chew *et al*., 2011a). It was probably because longer time will increase cost. However, even at longer time, there was not much difference in extraction of phenolic compounds when compared to shorter time. Time does have a significant effect (p<0.05) on extraction of phenolic compound as shown in Figure 2b. It was obvious that a shorter time, 1h, will extract the same amount of phenolic extracts as longer time, 5hr, while saving cost and is more practical (Silva *et al*., 2007). Extraction time of 2 h was selected for langsat peel based on the highest DPPH scavenging

activity and highest TPC extracted at that duration based on Figure 2c. The EC_{50} value at extraction time of 2 h was 21.48±0.14 µg/ml and decreased at the subsequent hours, there was no significant difference (p<0.05) observed among all the extraction times.

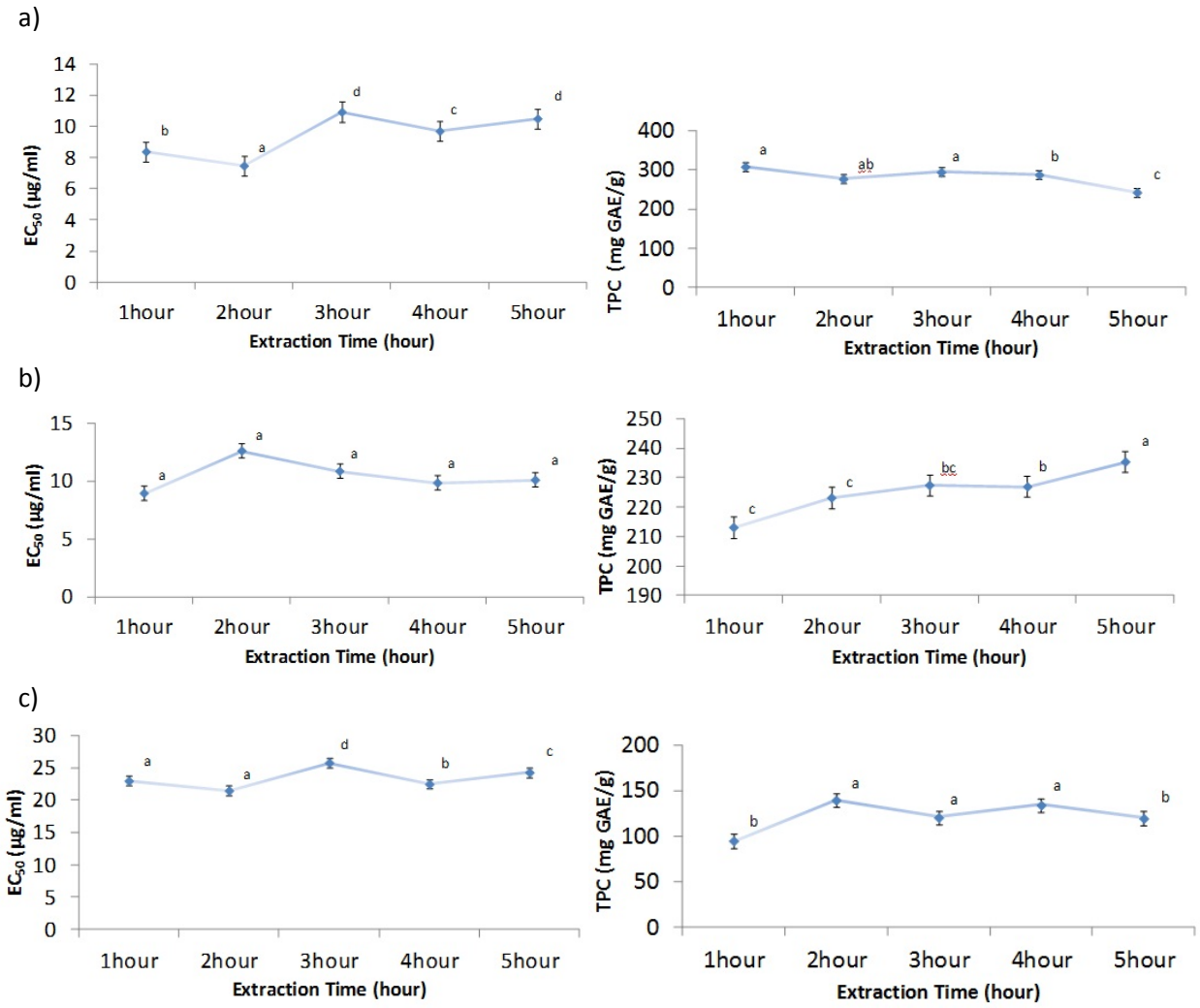

Figure 2: Effect of the extraction time on the scavenging activity (EC_{50}) and TPC from from (a) rambutan *(Nephelium lappaceum), (*b) mangosteen *(Garcinia mangostana)* and (c) langsat *(Lansium domesticum)* peels. Values are presented as means ± standard deviation of five measurements. Values marked by different lower case letters (a-d) are significantly different (p<0.05).

In general, it was believed that prolonged extraction time (> 2h) would lead to exposure of more oxygen and thus increase the chances for occurrence of oxidation on phenolic compounds (Naczk and Shahidi, 2004). In term of antioxidant capacity, the DPPH free radical scavenging ability will decreased after reaching the maximum extraction time as the prolonged extraction time would increase the chance for occurrence of oxidation of phenolic compounds (Chew *et al*., 2011b).

Extraction temperature evaluation

The effects of extraction temperature on antioxidant capacity and TPC of all the selected tropical fruit peel extract are showed in Figure 3a-c. Based on the Figure 3a, the yield of the scavenging activity of rambutan peel was increased proportionally with the increasing of extraction temperature, reaching maximum values at 50°C with EC_{50} of 9.02±0.14 µg/ml. However, TPC showed different tendency with antioxidant activity with increasing of extraction temperature. The highest TPC value was achieved at extraction temperatures of 50°C (290.98±16.31 mg GAE/g) and 60°C (294.31±22.30 mg GAE/g), respectively. Since there is no significant difference (p<0.05) observed in both the extraction temperature, 50°C was selected as the best extraction temperature as this temperature exhibit the highest antioxidant activity. According to Spigno *et al*. (2007), when there is an increase in temperature, it favours extraction thus enhancing both solubility of solute and diffusion coefficient. However, beyond a certain temperature, phenolic compounds can be denatured as thermal destruction of phenolic compounds can also be taken place and caused a reduction in the antioxidant capacities of crude extract (Chew *et al.,* 2011a).

The highest scavenging activity for mangosteen peel was observed at the temperature of 25°C and 30°C with the EC_{50} value of 9.83±0.35 µg/ml and 9.14±0.41 µg/ml, respectively. No significant difference (p<0.05) was observed in these two temperatures as shown in Figure 3b. Since the highest TPC value was extracted at the temperature of 25°C with the GAE value of 218.39±12.99 mg GAE/g, the extraction temperature for mangosteen peel was fixed at 25°C. For langsat peel as shown in Figure 3c, the highest scavenging activity was observed at 25°C with the EC_{50} value of 27.60±0.11 µg/ml. The scavenging activity decreases with increasing temperature

and all the values are significantly different (p<0.05) from each other. The highest yield of TPC was observed at 25°C (104.70±6.85 mg GAE/g) with the rest decreasing with the increasing of extraction temperature. Thus, the best extraction temperature chosen for langsat peel was of 25°C.

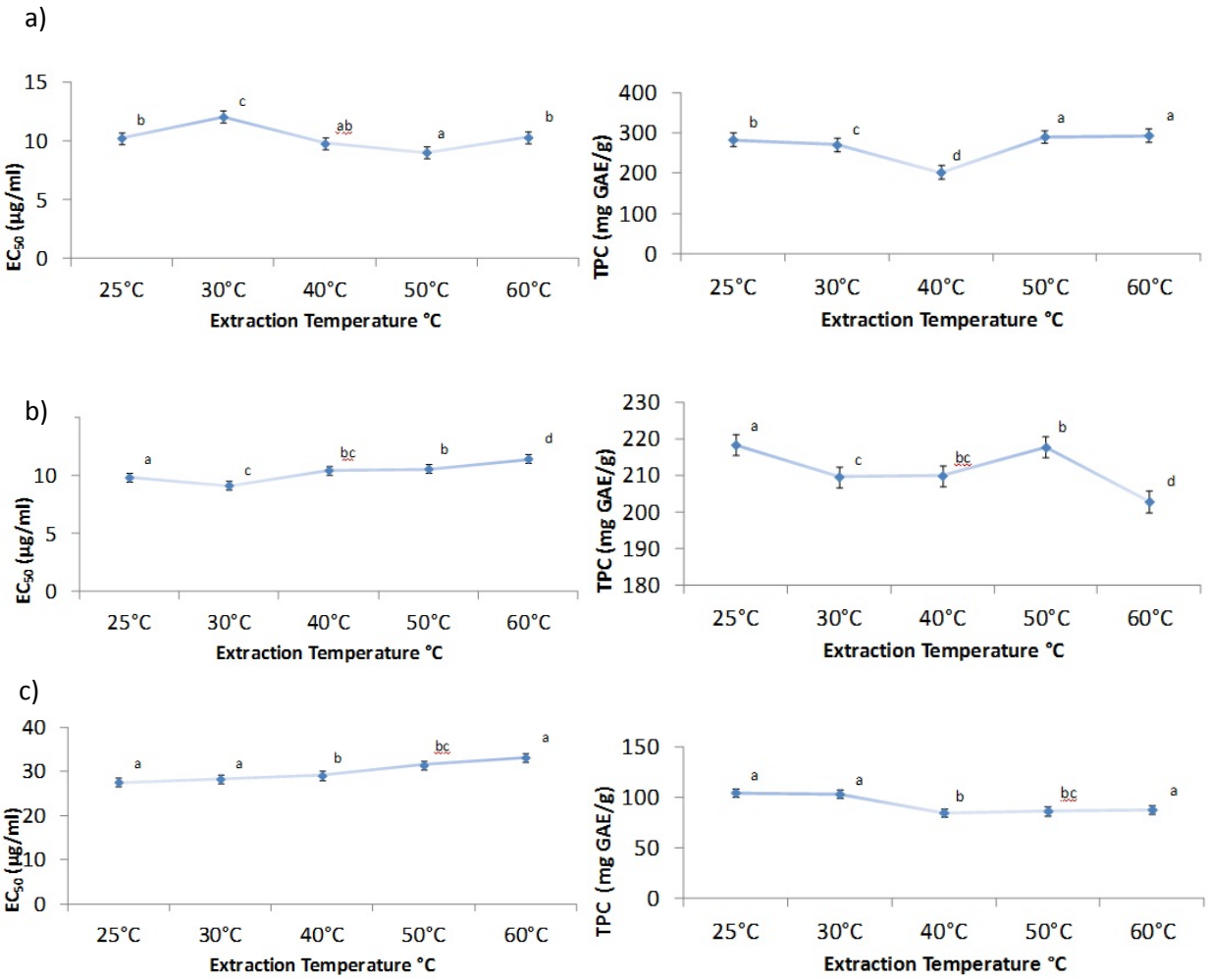

Figure 3: Effect of the extraction temperature on the scavenging activity (EC_{50}) and TPC from a) rambutan *(Nephelium lappaceum), (*b) mangosteen *(Garcinia mangostana)* and (c) langsat *(Lansium domesticum)* peels. Values are presented as means ± standard deviation of five measurements. Values marked by different lower case letters (a-d) are significantly different (p<0.05).

It was observed that high temperature may encourage solvent loss since boiling point of ethanol is very close to 78.5°C through vapourisation and hence increase the cost for extraction process from the industry point of view (Chew et al., 2011a). Vapourisation will create a more concentrated solvent extraction system where high concentration will increase high organic solvent content which reduces polarity and so eventually disturbs the phenolic extraction process as high concentration yield a lower extraction of phenolic compound (Liyana-Pathirana and Shahidi, 2005; Chan *et al*., 2009). Therefore, moderate extraction temperatures of 25°C (mangosteen and langsat) and 50°C (rambutan) were chosen respectively, for the optimization of the selected tropical fruit peels.

Evaluation of antioxidant activity at optimized extraction conditions

The optimized condition for rambutan, mangosteen, and langsat peels were 80% of ethanol, 2 h and 50°C; 60% of ethanol, 1 h and 25°C; and 80% of ethanol, 2 h and 25°C, respectively. Figure 4a shows the dose-response curve of DPPH scavenging activities of the respective extracts. BHA and tocopherol was used as positive controls. The results indicated that the peel extracts exhibited a potential free radical scavenging activity. The EC_{50} of the radical scavenging activity of the peel extracts were illustrated in Figure 4b. The results revealed that rambutan peel possessed highest radical scavenging activity (EC_{50} of 8.87 μg/mL), comparable to that of BHA (9.66 μg/mL). Many studies reported that high polyphenols content of fruit peels contributes towards high radical scavenging activity (Lim and Murtijaya, 2007). Previous study done by Thitilertdecha *et al*. (2008) confirmed that the rambutan peel exhibited higher antioxidant activity that may be attributed to its phenolic components.

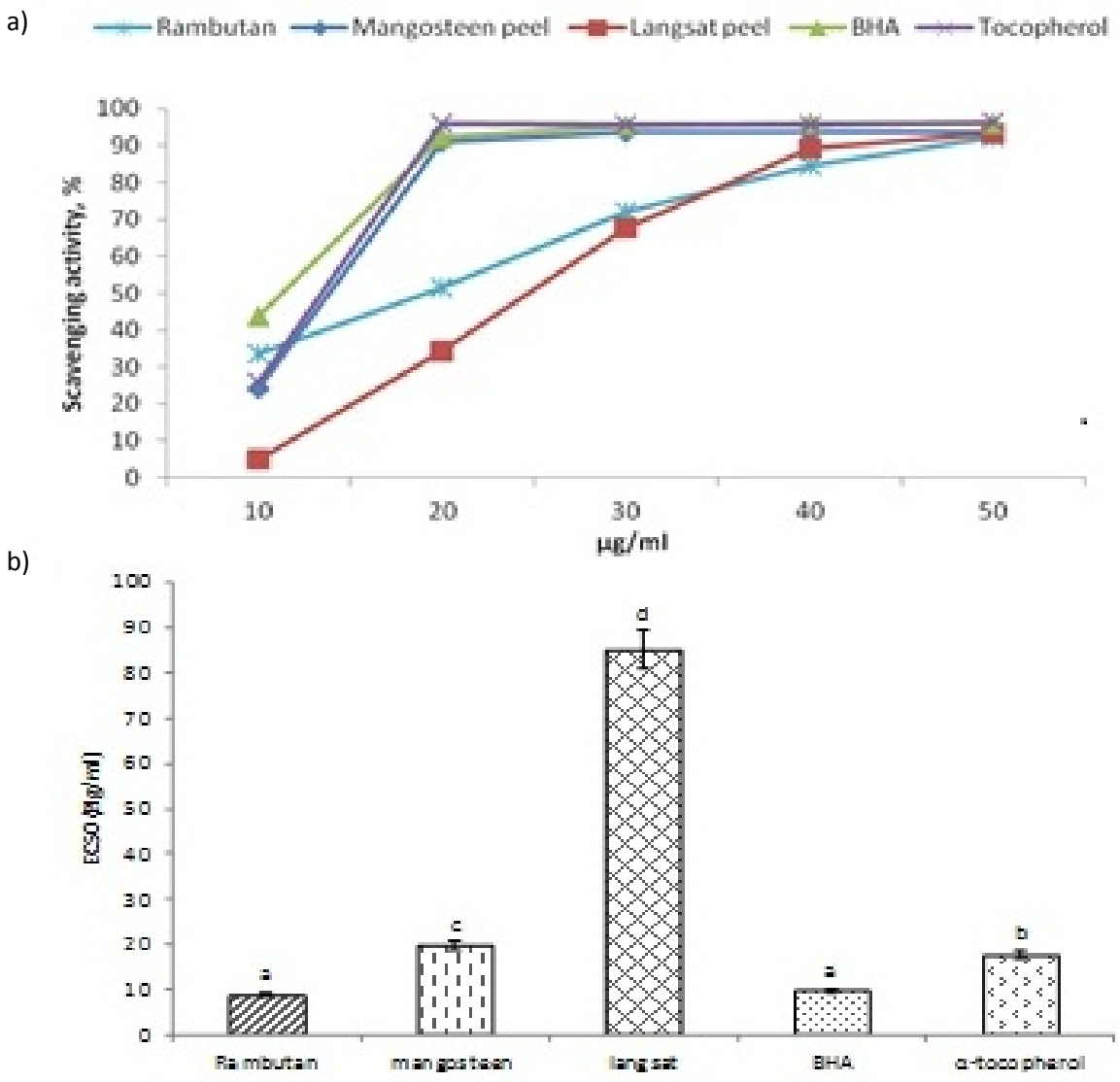

Figure 4: Evaluation on antioxidant activity on (a) DPPH scavenging activity and EC_{50} of rambutan *(Nephelium lappaceum),* mangosteen *(Garcinia mangostana)* and langsat *(Lansium domesticum)* peels' extract. Values are presented as means ± standard deviation. Values marked by different lower case letters (a-d) are significantly different (p<0.05).

Scavenging activity of nitric oxide (NO) was also found to rise with increasing concentration of the extract and the scavenging activity was better than ascorbic acid, the positive control used. Scavenging of nitric oxide radical is based on the generation of nitric oxide from sodium nitroprusside in buffered saline, which reacts with oxygen to produce nitrite ions that can be

measured by using Griess reagent (Lobo *et al*. 2010). Based from the result shown in Figure 5, the selected tropical fruit's peel potentially decreased the amount of nitrite generated from the decomposition of sodium nitroprusside *in vitro* which may be due to the presence of antioxidant principles in the extract. The result revealed that at the maximum concentration, 200µg/ml, rambutan peel possessed the highest scavenging activity (64.88%) followed by mangosteen peel (54.66%) and langsat peel (50.42%). The scavenging activity found in these three fruit extract was better than the scavenging activity found in the positive control used (49.87%).

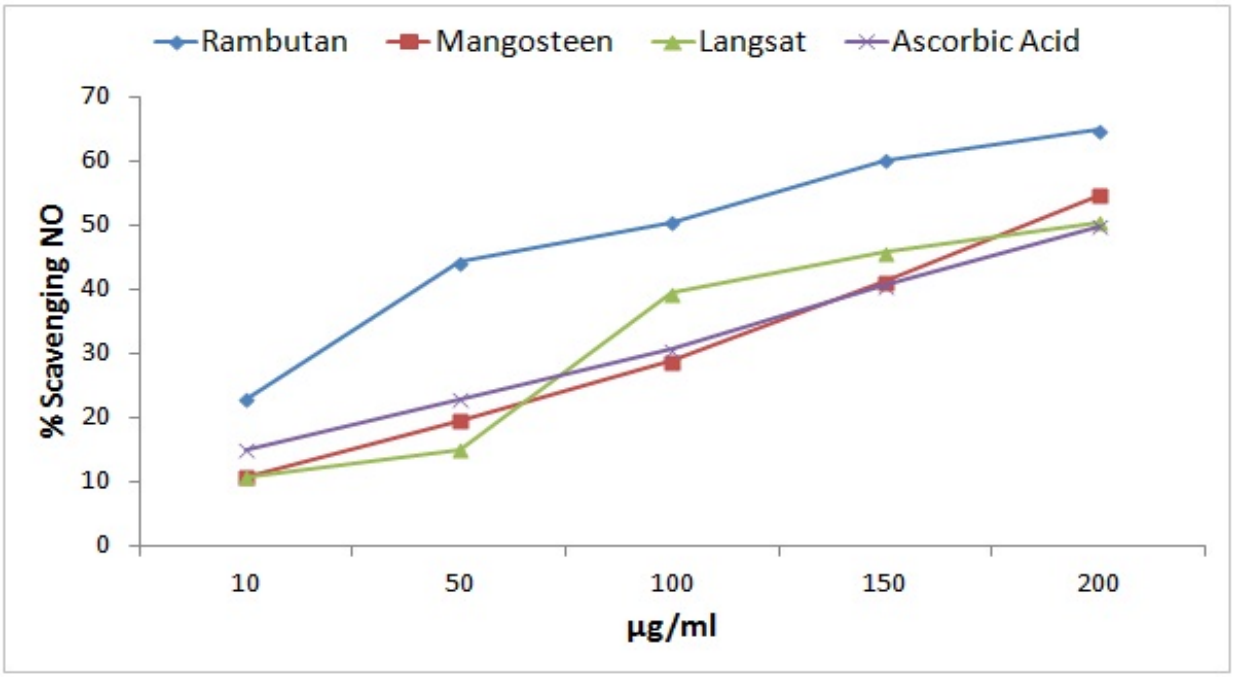

Figure 5: Evaluation of nitric oxide scavenging activity of rambutan *(Nephelium lappaceum),* mangosteen *(Garcinia mangostana),* and langsat *(Lansium domesticum)* peels' extract. Values are presented as means ± standard deviation.

Figure 6a and 6b shows the antioxidant activity and the degradation rate of the β-carotene bleaching method (BCB) for the respective fruit peel extract in comparison with the well known, synthetic antioxidant, namely BHT, used as the positive control. Based from the result shown, the antioxidant power decreased in the order rambutan peel > BHT > mangosteen peel > langsat peel. It can be seen that at concentration of 200 µg/ml, rambutan peel extract shows the highest

antioxidant activity (98.19%) and also a relatively lower degradation rate followed by BHT and mangosteen peel, 79.99% and 79.94% respectively. However langsat peel extract showed a very low antioxidant activity (23.94%) and possesses a very high degradation rate.

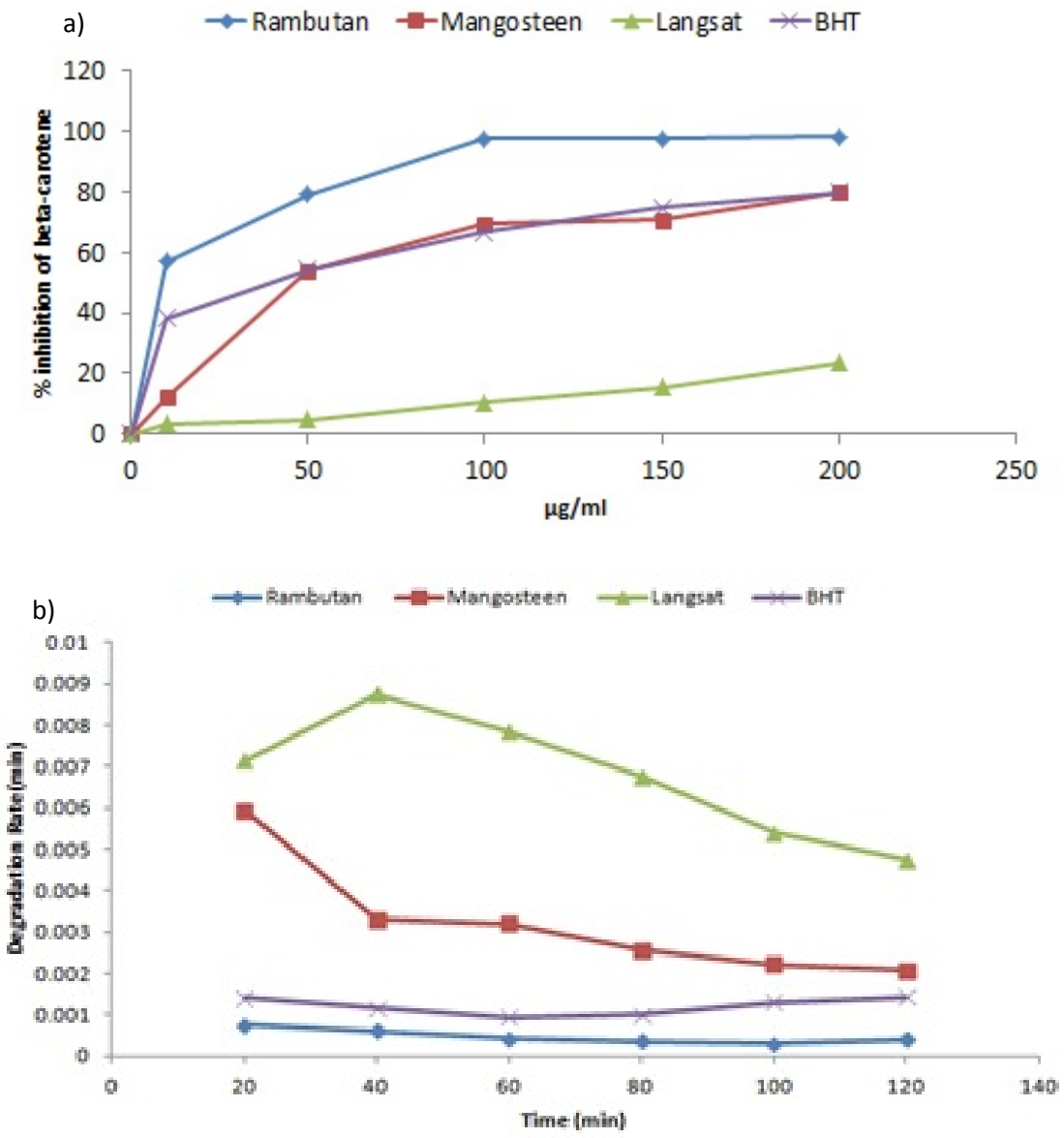

Figure 6: Evaluation of antioxidant activity on the (a) percentage of inhibition (b) degradation rate as measured by β-carotene bleaching method of rambutan *(Nephelium lappaceum)*, mangosteen *(Garcinia mangostana)*, and langsat *(Lansium domesticum)* peels' extract. BHT was used as standard. Values are presented as means ± standard deviation.

Generally in the β-carotene bleaching assay, linoleic acid produces hydroperoxides as free radicals during incubation and with presence of antioxidants in the extract; it will minimize the oxidation of β-carotene. Hydroperoxides formed in this system will be neutralized by the antioxidants from the peel extracts (Othman *et al*., 2007). Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts. There was a correlation between degradation rate and the bleaching of β-carotene; where the extract with the lowest b-carotene degradation rate exhibited the highest antioxidant activity (Kulisic *et al*., 2004).

Figure 7 shows the dose-response bar chart of TPC for the respective fruit extracts. Based from the results, it can be seen that at 200 µg/ml, rambutan peel extract shows the highest TPC (53.94 mg GAE/g) followed by mangosteen peel (42.25 mg GAE/g), while langsat peel exhibiting the lowest phenolic content (22.04 mg GAE/g).

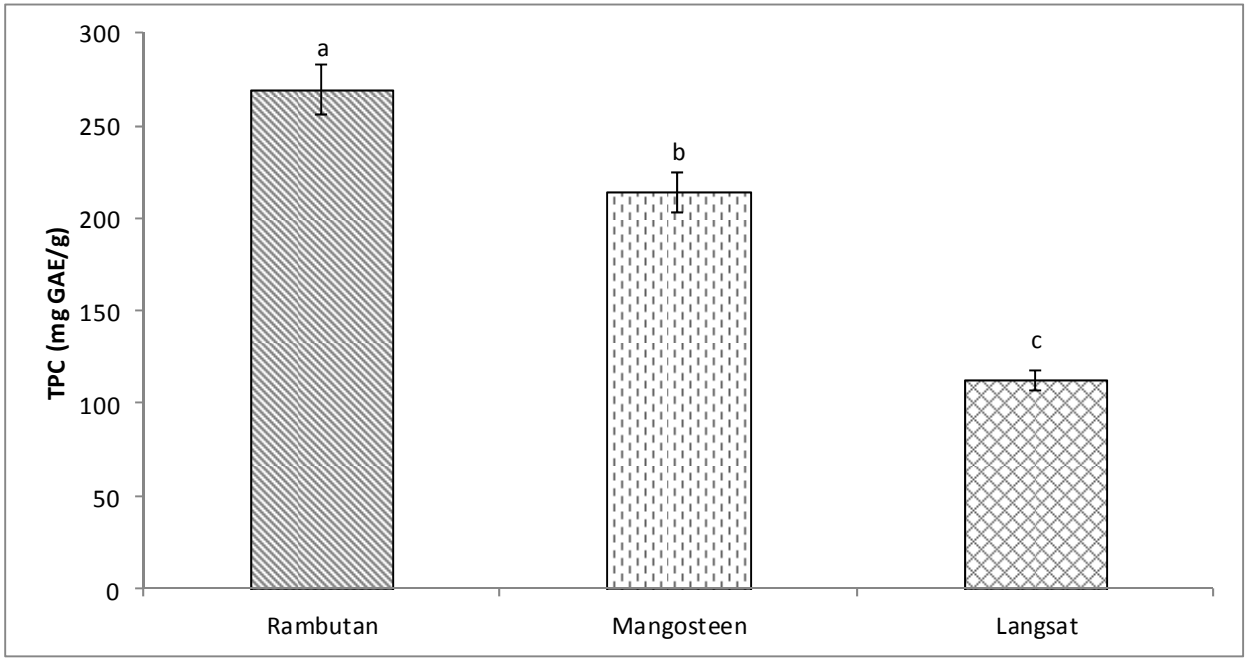

Figure 7: Evaluation of total phenolics content for rambutan *(Nephelium lappaceum),* mangosteen *(Garcinia mangostana),* and langsat *(Lansium domesticum)* peel extract. Values are presented as means ± standard deviation. Values marked by different lower case letters (a-c) are significantly different ($p<0.05$).

Plant phenolics have multiple biological effects as they constitute one of the major groups of compounds acting as primary antioxidant or free radical terminator (Thitilertdecha *et al*., 2008). Several studies have demonstrated that the antioxidant activity of plant extract is strongly correlated with the total content of phenolic compounds (Lim and Murtijaya, 2007). There are many types of compounds possessing antioxidant activity in higher plants (Larson, 1988) and the phenolic compounds were highlighted to be the potential antioxidants .The fact that phenolic compounds possess a high potential to scavenge radicals can be explained by their ability to donate a hydrogen atom from their phenolic hydroxyl groups (Yu *et al.*, 2002).

Conclusions

All the extraction parameters (ethanol concentration, extraction time and extraction temperature) showed significant effect on the extraction efficiency of antioxidant capacity and total phenolic content of rambutan, mangosteen and langsat peel extracts. The optimized condition for rambutan, mangosteen, and langsat peels were 80% of ethanol, 2 h and 50°C; 60% of ethanol, 1 h and 25°C; and 80% of ethanol, 2 h and 25°C, respectively. Based on these extraction conditions, maximum antioxidant activities could be obtained for future studies of all the selected fruits peel and it will be useful for potential nutraceutical development. In the future, investigation of the activity associated with further purification, identification and quantification of each phenolic compound are necessary to provide useful comparative information on the antioxidant level and activities

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