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Fractionation of Potent Antioxidative Components from Langsat (*Lansium domesticum*) Peel

Kee, M. E., Khoo, H. E., Sia, C. M. and Yim, H. S.*

[1]*Department of Food Science and Nutrition, Faculty of Applied Sciences, UCSI University, No. 1, Jalan Menara Gading, UCSI Heights, 56000 Kuala Lumpur, Malaysia*
[2]*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia*

ABSTRACT

Lansium domesticum (langsat) is a popular tropical fruit bearing trees across the Southeast Asia region. It is cultivated mainly for its fruit, while the peel of langsat as wastes of fruit consumption has been traditionally used as medicine. The aim of this study was to evaluate the total phenolic content (TPC) and antioxidant activity of different fractions of ethanolic extract of langsat peel. The extract was fractionated using open column chromatography and the antioxidant components were observed based on high-performance thin layer chromatography (HPTLC). TPC of the extract and extract fractions (FI-FIII) was estimated using Folin-Ciocalteu reagent assay, while their antioxidant activity was evaluated using DPPH radical scavenging assay. Highest TPC was estimated in the ethanolic extract of langsat peel compared to its extract fractions. No significant difference was found for TPC among the extract fractions. The extract also had the highest DPPH radical scavenging activity, followed by FIII and FII, but no radical scavenging activity was observed in FI. The result obtained from HPTLC also showed that no band of antioxidative compounds was observed under UV or white light. Based on the antioxidative components found in the peel extract of langsat, the fruit peel is a potential source of antioxidants for development of nutraceutical. Fractionation of the extract is not an ideal technique for producing nutraceutical.

Keywords: Antioxidant activity, DPPH radical, HPTLC, Lansium domesticum, total phenolic content

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E-mail addresses:
Mayeng77@hotmail.com (Kee, M. E.),
hockeng_khoo@yahoo.com (Khoo, H. E.),
siacm@ucsiuniversity.edu.my (Sia, C. M.),
hsyim@ucsiuniversity.edu.my (Yim, H. S.)
* Corresponding author

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INTRODUCTION

Fruits are rich in antioxidants such as vitamins, phenolic compounds, carotenoids and other phytochemicals that help in reducing risks of chronic diseases (Feskanich *et al.*, 2000; Hamid *et al.*, 2010). Studies have shown that excessive free radicals in the body cause oxidative damage to all the constituents of the body and increase the risks of developing diseases including hypertension, congestive heart failure and other degenerative diseases (Cornelli, 2009; Scheibmeir *et al.*, 2005). Antioxidants are compounds that delay or prevent the oxidation of cellular constituents of the body by scavenging free radicals such as reactive oxygen species (ROS) (Halliwell, 1994).

For more than fifty years, synthetic antioxidants, particularly butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tertiary-butylhydroquinone (TBHQ) have been widely used in the food industry for the prevention of oxidation and off-flavour development in fats and oils. However, it is known that these chemicals could be carcinogenic (Ajila *et al*., 2007; Moure *et al.*, 2001). Recent evidence suggests that the crude extracts or purified constituents from various medicinal plants are stronger antioxidants than the synthetic antioxidants (Shiban *et al*., 2012). Pourmorad *et al*. (2006) also reported that the antioxidant activity of Iranian medicinal plant was four times greater than BHT. Hence, it can be concluded that natural antioxidants could potentially be alternatives for the synthetic ones.

Langsat (*Lansium domesticum*) is a tropical fruit which contains a variety of nutrients especially vitamins and minerals that are health beneficial. Langsat contains several phytochemicals that possess high antioxidant activities. Lim *et al*. (2007) found that among several tropical fruits, langsat exhibited high antioxidant activity as determined based on DPPH assay. Huang *et al*. (2010) reported a higher total antioxidant capacity, total phenolic content, and carotenoids were determined in the peel of langsat as compared to its pulp. Langsat peel also possesses strong antimicrobial activity against *Candida lypolytica* (Mohamed *et al.*, 1994).

In the past, many studies mainly focused on antioxidant activity of extracts from edible portions of fruits, mainly pulps. However, information regarding inedible part of a fruit such as peel of langsat is insufficient. Nonetheless, studies have revealed that the peel extracts of certain fruits possessed higher antioxidant activity than the pulp extracts (Ajila *et al*., 2007; Contreras-Calderon e*t al*., 2011). For example, dragon fruit peel showed higher radical scavenging activities than the pulp (Nurliyana *et al*., 2010). Therefore, this study aimed to determine the total phenolic content and antioxidant activity of langsat peel extract, as well as its extract fractions obtained from open column chromatography. Confirmation of the occurrence of phenolic compounds in the extract and extract fractions was performed based on the HTPLC method.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used were of analytical grade whereas all solvents used for HPTLC were of HPLC grade. Gallic acid, ethanol, ethyl acetate, formic acid, glacial acetic acid, and sodium carbonate anhydrous were purchased from Fisher Scientific (Leicestershire, UK). Folin-Ciocalteu reagent, methanol, chloroform, sulfuric acid, silica gel 60 F_{254} high performance thin-layer chromatography (HPTLC) plates, and silica gel 60 (230–400 meshes) were purchased from Merck (Darmstadt, Germany). BHA and DPPH were purchased from Sigma Chemicals (St. Louis, MO). Water used was Millipore quality (Millipore, Billerica, MA).

Preparation and Extraction of the Sample

Lansium domesticum (langsat) fruit were purchased from a fruit orchard in Selangor, Malaysia. The langsat fruit has been registered with the Department of Agriculture, Malaysia (No. DL2: Langsat) (DOA, 2013). The fruit was washed and the fruit pulp was removed. The thickness of the peel varied from 2mm to 6mm. The fruit peel was air-dried in an oven at 40°C for 24 h. After 24 h, the fully dried langsat peel was collected, while the not fully dried peel further oven-dried for another 5 h. The dried langsat peel was substantially crisp and dry. The air-dried sample was ground into fine powder using a household grinder, and sieved using a laboratory sieve of 0.5 mm mesh size. The sample powder was vacuum-packed and stored at □20°C before further analysis. The sample was extracted by adding 5g of sample powder to 50 ml of 80% ethanol. The mixture was shaken at room temperature (25°C) for 120 min using an orbital shaker as optimized using langsat peel (Chua, 2012). The residue was re-extracted once under similar conditions. The extracts were pooled, followed by filtration and concentration of the supernatant using a rotary evaporator at 45°C to remove the solvent. The concentrated extract was freeze-dried and stored at -20°C. Triplicate extraction was done for the langsat peel.

Extract Fractionation and HPTLC Analysis

Fractionation of the extract was carried out using 23g of silica gel 60 (230–400 meshes) as an adsorbent for the open-column chromatography (25 cm × 2.5 cm). The column was loaded with the extract (equivalent to 0.3 g of sample) and eluted with a linear gradient of chloroform: ethyl acetate: methanol from 100:0:0 to 0:0:100 (*v*/*v*/*v*) as described by Prasad *et al.* (2005). It was achieved by increasing solvent polarity by 5% each time. The obtained sample fractions were subjected to HPTLC analysis using HPTLC silica gel plates, where an aliquot of each fraction (20 µl, 5 mg/ml of sample) was spotted with a developing solvent system of ethyl acetate/ water/ formic acid/ acetic acid (100:26:11:11, *v*/*v*/*v*/*v*) (Reich & Schibli, 2007). The sample fractions that have antioxidative compounds of similar retention factor (R_f) values were pooled into three major fractions (extract fractions: FI, FII, and FIII). The solvents

in each extract fraction were evaporated to dryness using a rotary evaporator. Bioautographic reagent of 0.04% DPPH in methanol was sprayed on the developed HPTLC plates for the three extract fractions. Yellowish bands were visualised in the purplish background and this indicated the presence of reducing agents, the most possibly phenolic compounds. On the other hand, 10% sulfuric acid was sprayed on another two developed plates and the colour bands were visualised under white light and UV 366 nm, indicating the present of antioxidative compounds. Each extract fraction was collected as triplicate analyses.

Estimation of Total Phenolic Content

Total phenolic content (TPC) of langsat peel extract and its extract fractions were estimated based on the method described by Lim e*t al*. (2007). The test tubes were first added with 1 ml of extract/ extract fraction (200 µg/ml), followed by addition of 4 ml Folin-Ciocalteu reagent. The mixture was vortexed and incubated at room temperature for 3 min. After 3 min, 5 ml of sodium carbonate solution was added. The reacting mixtures were vortexed and kept in the dark for 30 min at room temperature. Absorbance of the reaction mixtures was measured at 765nm using a UV-visible spectrophotometer (Secomam, France). TPC of the extract and extract fractions was expressed as gallic acid equivalent (GAE). The standard curve of gallic acid was obtained using concentrations of gallic acid at 0.2-50 µg/ml.

$$y = 0.015x + 0.006 \ (R^2 = 0.996) \qquad [1]$$

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of the extract and extract fractions was determined using DPPH radical scavenging assay as described by Ajila *et al*. (2007) with modifications. Aliquots of 1 ml extract or extract fraction with a range of concentrations from 10 to 300 µg/ml were added to 1 ml of 0.2 mM DPPH reagent and vortexed for few seconds. The reacting mixtures were incubated in dark at room temperature for 30 min. Absorbance of the mixture was measured at 517 nm against blank (ethanol). Distilled water was used as a control with an addition of extract or extract fraction. The radical scavenging activity was calculated using the equation as follows:

$$\text{Scavenging activity (\%)} = \left(1 - \frac{A_{sample}}{A_{control}}\right) \times 100 \qquad [2]$$

where A_{sample} is the absorbance of the extract/ extract fraction, and $A_{control}$ is the absorbance of the control. EC_{50} value, i.e. the amount of antioxidant required to reduce the initial free radicals concentration by 50%, was calculated using the linear regression equation, as follows:

$$y = 0.1299x + 0.1294 \ (R^2 = 0.937) \qquad [3]$$

Statistical Analysis

All data were presented as mean ± standard deviation. The data were statistically

analysed using SPSS software (version 19.0). One-way analysis of variance coupled with Tukey's post-hoc test was used to assess significant differences between the mean values. The significant value was at $p<0.05$.

RESULTS AND DISCUSSION

Sample and Extraction Yield

In the present study, the oven-dried langsat peel was extracted with 80% ethanol for 2 h at 25°C. The oven-dried langsat peel has 70.3% of moisture loss. As mentioned earlier, the extraction condition had been optimised by Chua (2012). The extract yield of the *Lansium domesticum* peel was 66.11%. It was found that binary solvent system offered higher extraction yield compared to the mono-solvent system (Zubair *et al.*, 2012). The finding is also consistent with the result reported by Sultana e*t al*. (2009) that the extraction of medicinal plant using 80% ethanol exhibits higher extracts yields and total phenolic content as compared to 100% ethanol. Besides, ethanol and water were selected as the extraction solvent because ethanol is less toxic than other solvents such as methanol and acetone (Chew e*t al*., 2011).

Extract Fractionation and HPTLC Analysis

The eighty sample fractions collected were pooled as three extract fractions (FI-FIII) based on the R_f values obtained from HPTLC. Fractions 1–28 were obtained as colourless sample fractions, fractions 29–77 as yellow colour sample fractions, while fractions 78–80 were the light yellow colour fractions. HPTLC analysis of all the 80 sample fractions collected from column chromatography was performed in order to detect the antioxidant compounds in the sample fractions. The developed plates of fractions 1–21 and visualised under UV 366 nm showed no bands on the plates. Therefore, these sample fractions were pooled as extract fraction FI. Fractions 22–29 revealed a significant band at R_F = 0.35 under UV 366 nm and were pooled as FII. As for the subsequent fractions 30–80, seven bands (four major bands and three minor bands) at R_F values of 0.85, 0.73, 0.60, 0.50, 0.39, 0.15 and 0.07, respectively were visualised under UV 366 nm as well. Two dark blue bands and five light blue bands were detected under UV 366 nm after derivatisation. These fractions were pooled as FIII. The total yields of FI-FIII are shown in Table 1.

Bioautographic HPTLC assay was performed as a screening tool that provides quick access for detection of active bands on the plate which could be examined through white light, UV 254 nm or UV 366 nm. The specific phenolic compounds were not determined using HPLTC as this study aimed to screen for the occurrence of phenolic compounds using HPTLC. As shown in Fig.1, the plates for FII and FIII that sprayed with DPPH solution were visualised as light yellow bands in the purple colour background under white light. The plate for the langsat peel had the most obvious light yellow bands compared to the extract fractions, followed by four obviously

seen light yellow bands in the plate of FIII and weakly coloured bands in FII. In contrast, no potential reaction of reducing compounds was seen in FI. In FIII, the four strong, active bands (a, b, c, and d) have R_F values of 0.81, 0.69, 0.57, 0.42, respectively. As the plates were dipped in 10% sulfuric acid, similar R_F values (0.82, 0.71, 0.58 and 0.45, respectively) were obtained for the four band when visualised under white light and UV 366 nm demonstrated that four major antioxidative compounds were detected in langsat.

Total Phenolic Content

TPC of the extract and extract fractions each sample was calculated and expressed as mg GAE per gram sample. The results showed that the colour of the mixture of Folin-Ciocalteu reagent and the extract of langsat peel turned blue, suggesting that phenolic compound is present in the langsat peel extract. To the contrary, the reaction mixtures of FI-FIII was light blue in colour. The lighter blue colour indicates that the extract fractions contain a lower amount of total phenolics as compared to the crude extract.

TPC of the extract and extract fractions of langsat peel are shown in Fig.2. In general, TPC of the langsat peel extract was 3-4 times higher than all the extract fractions. while TPC of the extract fractions was not significantly different. Surprisingly, the TPC of the langsat peel extract (140.5 mg GAE/g extract = 9.2 mg GAE/100 dry weight, DW) obtained was 2.5 times higher than TPC of the langsat peel (3.7 g GAE/100 g DW) reported by Huang *et al.* (2010). A possible explanation for the high

TABLE 1
The yields of extract fractions obtained from a pooled of sample fractions

Extract fraction	Sample fraction	Yield (mg)
Fraction 1	1 – 21	81.4
Fraction 2	22 – 29	70.9
Fraction 3	30 – 80	123.5

TABLE 2
EC_{50} values of the langsat peel extract and its extract fractions

Sample	EC_{50} (µg/ml)
Peel extract	47.94 ± 0.46
FI (Fraction 1)	ND
FII (Fraction 2)	ND
FIII (Fraction 3)	251.44 ± 13.13
BHA	9.66 ± 0.59

Each value is expressed as mean ± standard deviation of triplicate measurements. EC_{50} value is the effective concentration value at which the DPPH radical is scavenged by 50%. ND denotes not determined due to the highest concentration of the extract tested could not give a scavenging activity of ≥50%.

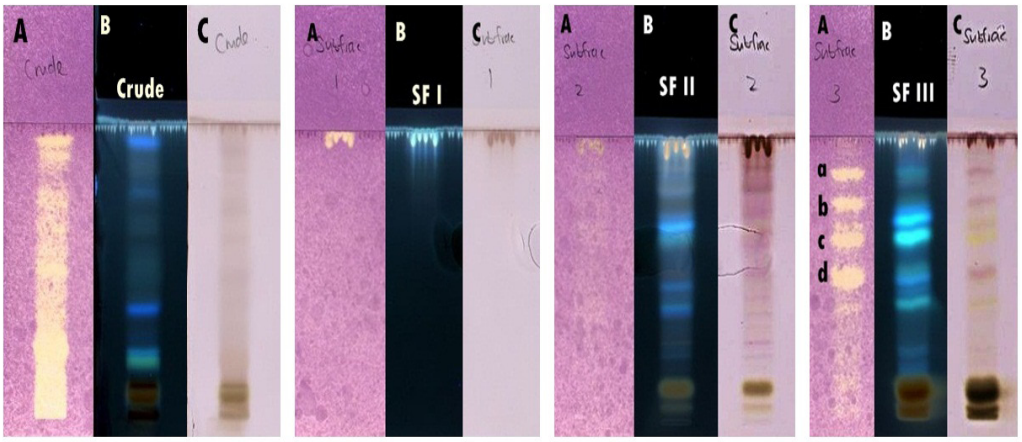

Fig.1: Bioautographic HPTLC analysis for the langsat peel extract and its extract fractions. (A) HPTLC plate sprayed with 0.04% DPPH solution, (B) HPTLC plate dipped in 10% sulfuric acid, under UV 366 nm, (C) HPTLC plate dipped in 10% sulfuric acid, under white light. "Crude", "SF I", "SF II", and "SF III" denote langsat peel extract, extract fraction 1 (FI), extract fraction 2 (FII), and extract fraction 3 (FIII), respectively.

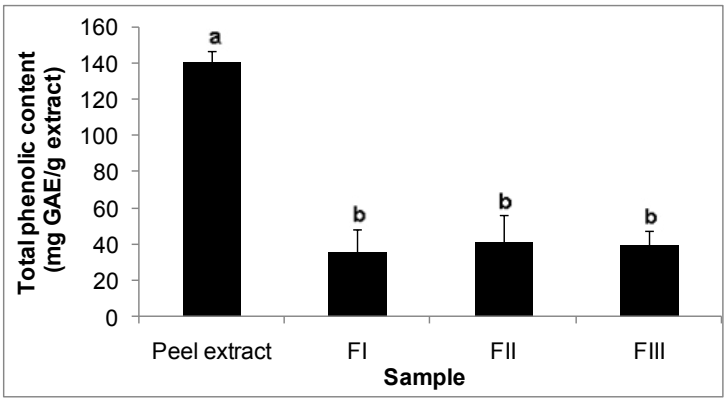

Fig.2: Total phenolic contents of langsat peel extract and its extract fractions estimated using Folin-Ciocalteu reagent assay. Different lowercase letters denote significant difference at $p<0.05$.

TPC in langsat peel is the presence of non-phenolic substances such as vitamin C and other organic acid that might have reacted with Folin-Ciocalteu reagent. Besides, other organic compounds might also interfere with the reaction between phenolic compounds and Folin-Ciocalteu reagent (Yan & Asmah, 2010). Moreover, the lower TPC in langsat peel reported by Huang *et al.* (2010) is due to the fact that the langsat fruit has been bought from the local market in Hong Kong. As langsat is one of the native fruit of Malaysia and Thailand, the fruit sold in the local market in Hong Kong was possibly

imported from Southeast Asia. It could help to explain the low TPC in the langsat peel reported by Huang *et al.* (2010) as the fruit has longer storage duration than the ones purchased in Malaysia. In addition, langsat peel was also reported to have high vitamin C content (22.5 µmol ascorbate/g dry weight) (Huang *et al.*, 2010). Therefore, Folin-Ciocalteu reagent assay seems to overestimate the TPC of the langsat peel extract (Azlan *et al.*, 2011).

DPPH Radical Scavenging Activity

It was observed that, as the concentration of the langsat peel increased, the scavenging activity also increased. At the extract concentration of 300 µg/ml, FIII showed significantly higher scavenging activity (58.34 ± 2.73%) compared to FII (36.27 ± 0.29%). At the extract concentration of 50 µg/ml, the radical scavenging activity of the langsat peel extract was 52.48 ± 0.66% compared to the scavenging activity of the extract fractions (<50%). Thus, crude extract had highest scavenging activity as compared to its extract fractions.

In terms of EC_{50}, the synthetic antioxidant, BHA showed the lowest EC_{50} value (9.66 ± 0.59 µg/ml), followed by the langsat peel extract (47.94 ± 0.46 µg/ml) and FIII (251.44 ± 13.13 µg/ml). EC_{50} for FI and FII could not be determined as the use of 500 µg/ml of the extract fractions could not achieve the DPPH radical scavenging activity of 50% and higher. The results showed that the highest scavenging activity that could be achieved by FI and FII at the concentration of 300 µg/ml was lower than 40%. At a higher concentration of the extract fraction (>500 µg/ml), it could possibly give a scavenging activity of higher than 50%. The EC_{50} values of the langsat peel extract, its extract fractions and BHA are shown in Table 2.

As observed from the experiment, the BHA gave the rapidest colour change from purple to yellow for the DPPH solution. As for the langsat peel extract and FIII, the colour changes of DPPH solution could be obviously seen. However, the experiment carried out for FII showed no obvious colour change. Even worst, no colour change was observed in FI. Therefore, it can be concluded that the antioxidants in FI could not act as reducing agents in scavenge DPPH radical. As reported by Ismail *et al.* (2013), DPPH assay involves in electron-transfer reaction pathway. Based on this mechanism, an antioxidant is known to donate an electron to a DPPH radical molecule as the molecule is unstable under this condition. The presence of antioxidant in the DPPH solution resulted in a decrease of the absorbance that measured at 517 nm (Charhardehi *et al*., 2009).

CONCLUSION

The present study demonstrated that the langsat peel extract contained antioxidant components. The antioxidant components in the extract fractions were also found to vary. Higher TPC and antioxidant activity were determined in FI compared to other extract fractions, but FI had moderately low TPC with undetectable antioxidant activity assessed using DPPH radical scavenging

assay. The highest DPPH radical scavenging activity was determined in the langsat peel extract with the lowest EC_{50} value when compared to its extract fractions. Based on the results obtained, the extract fractions of langsat peel did not have high TPC and antioxidant activity. However, future studies are needed to address some of the factors that caused the discrepancy in TPC and antioxidant activity in Langsat peels in the present study and those reported in the literature. Fractionation of any plant extract is not the ideal way to prepare nutraceutical ingredient unless for purification purpose. Thus, further studies are necessary to identify and elucidate the structure of the phenolic compounds present in langsat peel. The nutraceutical potential of langsat peel extract may also be explored for disease prevention.

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REFERENCES

Ajila, C. M., Naidu, K. A., Bhat, S. G., & Prasada Rao, U. J. S. (2007). Bioactive compounds and antioxidant potential of mango peel extract. *Food Chemistry, 105*, 982-988.

Azlan, A., Ismail, A., Ibrahim, M., Shakirin, F. H., & Khoo, H. E. (2011). Health-promoting properties of selected Malaysian underutilized fruits. II International Symposium on Underutilized Plant Species: Crops for the Future-Beyond Food Security. *ISHS Acta Horticulturae, 979*, 203-210.

Charhardehi, A. M., Ibrahim, D., & Sulaiman, S. F. (2009). Antioxidant activity and total phenolic content of some medicinal plants in Urticaceae family. *Journal of Applied Biological Sciences, 2*, 1-5.

Chew, K. K., Ng, S. Y., Thoo, Y. Y., Khoo, M. Z., Wan Aida, W. M., & Ho, C. W. (2011). Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extracts. *International Food Research Journal, 18*, 571-578.

Chua, C. H. (2012). *Comparative antioxidant activities and total phenolic content of mangosteen and langsat peels*. In Bachelor Thesis. Malaysia: UCSI University.

Contreras-Calderon, J., Calderon-Jaimes, L., Guerra-Hernandez, E., & Garcia-Villanova, B. (2011). Antioxidant capacity, phenolic content and vitamin C in pulp, peel and seed from 24 exotic fruits from Colombia. *Food Research International, 44*, 2047-2053.

Cornelli, U. (2009). Antioxidant use in nutraceuticals. *Clinics in Dermatology, 27*, 175-194.

Department of Agriculture, Malaysia. (2013). *Plant Variety Protection Malaysia*. Retrieved June 21, 2013, from http://pvpbkkt.doa.gov.my/NationalList/Search.php.

Feskanich, D., Ziegler, R. G., Michaud, D. S., Giovannucci, E. L., Speizer, F. E., Willett, W. C., & Colditz, G. A. (2000). Prospective study of fruit and vegetable consumption and risk of lung cancer among men and women. *Journal of the National Cancer Institute, 92*, 1812-1823.

Halliwell, B. (1994). Free radicals and antioxidants: a personal view. *Nutrition Reviews, 52*, 253-265.

Hamid, A. A., Aiyelaagbe, O. O., Usman, L. A., Ameen, O. M., & Lawal, A. (2010). Antioxidants: Its medicinal and pharmacological applications.

African Journal of Pure and Applied Chemistry, 4, 142-151.

Huang, W. Y., Cai, Y. Z., Corke, H., & Sun, M. (2010). Survey of antioxidant capacity and nutritional quality of selected edible and medicinal fruit plants in Hong Kong. *Journal of Food Composition and Analysis, 23*, 510-517.

Ismail, A., Azlan, A., Khoo, H. E., Prasad, K. N., & Kong, K. W. (2013). *Antioxidant Assays: Principles, Methods and Analyses*. Selangor: Universiti Putra Malaysia Press.

Lim, Y. Y., Lim, T. T., & Tee, J. J. (2007). Antioxidant properties of several tropical fruits: A comparative study. *Food Chemistry, 103*, 1003-1008.

Mohamed, S., Hassan, Z., & Abd Hamid, N. (1994). Antimicrobial activity of some tropical fruit wastes (guava, starfruit, banana, papaya, passionfruit, langsat, duku, rambutan and rambai). *Pertanika Journal of Tropical Agricultural Science, 17*, 219-227.

Moure, A., Cruz, J. M., Franco, D., Dominquez, J. M., Sineiro, J., Dominguez, H., Nunez, M. J., & Parajo, J. C. (2001). Natural antioxidants from residual sources. *Food Chemistry, 72*, 145-171.

Nurliyana, R., Syed Zahir, I., Mustapha Suleiman, K., Aisyah, M. R., & Kamarul Rahim, K. (2010). Antioxidant study of pulps and peels of dragon fruits: a comparative study. *International Food Research Journal, 17*, 367-375.

Pourmorad, F., Hosseinimehr, S. J., & Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology, 5*, 1142-1145.

Prasad, K. N., Divakar, S., Shivamurthy, G. R., & Aradhya, S. M. (2005). Isolation of a free radical-scavenging antioxidant from water spinach (*Ipomoea aquatic* Forsk). *Journal of the Science of Food and Agriculture, 85*, 1461-1468.

Reich, E., & Schibli, A. (2007). *High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants*. New York: Thieme Medical Publishers.

Scheibmeir, H. D., Christensen, K., Whitaker, S. H., Jegaethesan, J., Clancy, R., & Pierce, J. D. (2005). A review of free radicals and antixodiants for critical care nurses. *Intensive and Critical Care Nursing, 21*, 24-28.

Shiban, M. S., Al-Otaibi, M. M., & Al-Zoreky, N. S. (2012). Antioxidant Activity of Pomegranate (*Punica granatum* L.) Fruit Peels. *Food and Nutrition Sciences, 3*, 991-996.

Sultana, B., Anwar, F., & Ashraf, M. (2009). Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules, 14*, 2167-2180.

Yan, S. W. & Asmah, R. (2010). Comparison of total phenolic contents and antioxidant activities of turmeric leaf, pandan leaf and torch ginger flower. *International Food Research Journal, 17*, 417-423.

Zubair, M., Anwar, F., & Shahid, S. A. (2012). Effect of extraction solvents on phenolics and antioxidant activity of selected varieties of Pakistani Rice (*Oryza sativa*). *International Journal of Agriculture & Biology, 14*, 935-940.