Innovative Romanian Food Biotechnology *Vol. 14, Issue of March, 2014*

Received November 25, 2013 / Accepted January 10, 2014

RESEARCH ARTICLE

HPLC ANALYSIS OF ANTIOXIDANT COMPOUNDS IN SOME SELECTED TROPICAL FRUITS' PEEL

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Abstract

The objective of this study was to identify the major antioxidative components present in the peel of rambutan (*Nephelium lappaceum*), mangosteen (*Garcinia mangostana*) and langsat (*Lansium domesticum*). The identification and quantification of the major antioxidative components were performed using high performance liquid chromatography at 254 nm. The results showed that ellagic acid and corilagin were the major antioxidants in rambutan and langsat peel extracts. Both rambutan and langsat peel extracts had ellagic acid content (0.47 and 0.23 mg/g, respectively) higher than corilagin (0.14 and 0.11 mg/g, respectively). These two antioxidants were not detected in the mangosteen peel, but α-mangostin (0.89 mg/g) as one of the major xanthones was detected in the mangosteen peel extract. All these detected compounds are potentially strong antioxidants and known to have functional values, which suggest the potential use of these tropical fruit peels as sources of nutraceutical and pharmaceutical ingredients.

Keywords: *Nephelium lappaceum*, *Garcinia mangostana*, *Lansium domesticum*, ellagic acid, corilagin, α-mangostin

Introduction

Malaysia is well-known for its plant biodiversity and a variety of edible tropical fruit. Tropical fruits are renowned for their diversity and high content of antioxidants (Williams, 2001). Tropical fruits are also known as good sources of natural antioxidants that protect humans against oxidative stress, thus playing an important role in prevention of various life threatening diseases (Kuate *et al*., 2011). Epidemiological studies have shown that consumption of fruits prevented oxidative damages by quenching free radicals and reactive oxygen species as well as inhibition of lipid peroxidation (Huang *et al*., 2009; Kuate *et al*., 2011). Among the Malaysian tropical fruits, rambutan, langsat and mangosteen are some of the famous and seasonal tropical fruits. In recent years, Malaysian tropical

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fruits have been shown to possess valuable antioxidants and therapeutic values (Thitilertdecha *et al.*, 2008; Ikram *et al*., 2009). The beneficial effects are believed to be attributed to the phenolic compounds in the fruits (Huang *et* al., 2009).

To date, some phenolic compounds have been detected in rambutan and mangosteen (Thitilertdecha *et al*., 2010; Aisha *et al.*, 2012; Khaomek *et al*., 2012). The major phytochemicals identified in rambutan and langsat fruits were ellagic acid and corilagin (Thitilertdecha *et al*., 2010).

Ellagic acid has shown to prevent the formation of various tumors by explicitly interacting with the cell walls or sites with the ability to complex proteins, and preventing the proliferation of metastatic cells (Sepulveda *et al*., 2011).

These compounds have been previously isolated from the other plants and have been reported to exhibit various antiviral, anti-inflammatory, apoptotic, cytotoxic, cytoprotective, antimicrobial and antioxidant properties (Kashiwada, 1992; Rogerio *et al*., 2008). Studies have also shown that the main bioactive component found in mangosteen is xanthone (Aisha *et al*., 2012; Walker, 2007).

Xanthones are active and natural substances rarely found in other fruits besides mangosteen, and are extremely powerful antioxidants (Zareena and Sankar, 2009).

Xanthones, particularly α-mangostin exhibits significant biological properties, such as antioxidant, anti-allergy, anti-tumor, anti-inflammatory, anti-fungal, anti-bacterial and anti-viral activities (Pothitirat and Gritsanapan, 2009; Shan *et al*., 2011).

Based on previous literature, high antioxidant properties are expected to be presented in the peel extracts of rambutan, mangosteen, and langsat due to the high phenolics determined in these peels.

Therefore, this study aimed to confirm the presence of ellagic acid, corilagin and α-mangostin in the selected tropical fruits peel using high performance liquid chromatography.

Materials and Methods

Chemicals and reagents

HPLC grade ellagic acid and corilagin were purchased from Sigma-Aldrich (Malaysia), and α-mangostin (HPLC grade) was obtained from Acros Organics (Belgium). HPLC grade methanol and acetonitrile were supplied from Merck (Germany), while HPLC grade acetic acid (glacial) and formic acid were purchased from Fisher Scientific (UK). HPLC column used was Purospher STAR RP-18 endcapped (Merck, Germany).

Preparation of samples

The fruits of rambutan (*Nephelium lappaceum*), mangosteen (*Garcinia mangostana*) and langsat (*Lansium domesticum*) were purchased from a fruit farm in Selangor. The selected fruits have been registered with the Department of Agriculture (DOA), Malaysia (No. R161: Rambutan; No. GA2: Manggis; No. DL2: Langsat). The fruit peel was separated from the edible part, cut into pieces, and dried in a hot air oven (Memmert, Schwabach, Germany) at 45°C for 24 h. The oven-dried samples were ground into powder using a grinder.

Extraction of antioxidants

The oven-dried peels samples were extracted with aqueous ethanol using a modified method of Thoo *et al.* (2010) and Chew *et al.* (2011). The rambutan samples was extracted using 80% ethanol for 2 h and at 50°C, whereas mangosteen was extracted with 60% ethanol for 1 h at 25°C, and langsat was extracted using 80% ethanol for 2 h at 25°C based from the optimized results obtained previously (Samuagam *et al*., 2013). The extracts were then concentrated using a rotary evaporator (Rotavapor R-200, Buchi, Switzerland) applied 40°C of heat. The water content of the extracts was removed using a freeze dryer (CHRIST, Germany) and stored at -20°C until further analysis.

HPLC analysis

Crude extracts of rambutan, mangosteen, and langsat peels were prepared at extract concentration of 500 µg/ml by dissolving the extracts with HPLC grade methanol. The diluted extracts were filtered through a 0.45 µm syringe filter prior to HPLC analysis. Stock solutions of

the reference standards (ellagic acid, corilagin and α-mangostin) were prepared at concentration of 1.0 mg/ml. Standard calibration of the reference standard was prepared by diluting the stock solution to obtain final concentrations of 10, 30, 50, 70 and 100 µg/ml. The prepared standards were filtered through a 0.45 µm syringe filter prior to HPLC analysis.

Procedure for HPLC analysis was based on the method described by Wang *et al.* (2008) with some modifications. Separation of antioxidants in rambutan and langsat peel extracts was done using an Agilent 1200 series HPLC (Agilent Technologies, USA) equipped with G1311A quaternary pumps with G1322A degrasser, and G1315D diode array detector (DAD). A Purospher STAR RP-18 endcapped column (250 mm × 4.6 mm, i.d. 5 µm) was used. The elution was carried out using a gradient solvent system with flow rate of 1 ml/min at temperature of 30°C. The mobile phases were composed of 2% aqueous acetic acid (solvent A) and 0.5% aqueous acetic acid - acetonitrile (50:50, v/v) (solvent B). The gradient elution was performed as follows: 0-10 min, 5-10% B; 10-40 min, 10-40% B; 40-60 min, 40-55% B, 55-60 min, 55-80% B; 60-5 min, 80-100% B. The mobile phases were filtered under vacuum through a 0.45 µm membrane filter before use. The sample injection volume was 10 µl while the detection was set at 254 nm.

Separation of antioxidants in mangosteen extract was based on the method described by Pothitirat and Gritsanapan (2009) with slight modification. The elution was carried out using a gradient solvent system with flow rate of 1 ml/min at 30°C. The mobile phases consisted of 0.1% aqueous orthophosphoric acid (solvent A) and acetonitrile (solvent B). The gradient elution was as follows: 0-15 min, 70% B; 15-18 min, 70-75% B; 18-19 min, 75-80% B; 19-25 min, 80% B; 25-26 min, 80-70% B; 26-35 min, 70% B. The mobile phases were filtered through a 0.45 µm nylon membrane filter before use. The sample injection volume was 10 µl, while the detected was set at 254 nm.

Results and Discussion

HPLC is one of the reliable methods for detection and quantification of antioxidants in fruit extract. In this study, two different mobile phase systems were used. Separation of phenolic compounds and xanthones needs different combination of solvent systems. HPLC chromatograms for the rambutan, langsat and mangosteen peel extracts were shown in Figures 1. Based on the HPLC chromatograms obtained, ellagic acid and corilagin were detected only in the rambutan and langsat peel extracts, while α-mangostin was detected in mangosteen. As shown in Table 1, the rambutan peel extract had the highest ellagic acid content (0.47 mg/g dried weight, dw), followed by the langsat peel extract (0.23 mg/g dw). Higher amount of corilagin (0.14 mg/g dw) was determined in the rambutan peel extract compared to the langsat peel extract (0.11 mg/g dw). The major compound detected in the mangosteen peel extract was α-mangostin. High amount of α-mangostin was determined in mangosteen peel because α-mangostin is one of the xanthone specifically occurred naturally in some of the *Garcinia* species.

Table 1. *Selected antioxidants content in the tropical fruits peels*

Sample	mg/g dw		
	Ellagic acid	**Corilagin**	**α-mangostin**
Rambutan	0.47 ± 0.03	0.14 ± 0.05	ND
Langsat	0.23 ± 0.08	0.11 ± 0.03	ND
Mangosteen	ND	ND	0.89± 0.45

Values are the mean ± SD (n=3). ND: Not detected

The confirmation of these compounds detected in the fruit peel extracts was done based on the spiking tests and by comparing with the UV absorption spectra for the phenolic and xanthone standards. The result showed all the peaks identified had similar absorption spectra as found in the standards. As shown in Figure 1 (A), a total of three major peaks were observed in the chromatogram and some minor peaks were eluted. These minor peaks were not the compounds of

interest except the peak for ellagic acid at retention time of 44.01 min. At retention time of 45.4 min, the peak was not identified based on standard, but it was tentatively identified as geraniin. The peak of corilagin was detected at 34.34 min, while another major peak eluted at 41.7 min was determined as unknown. As one of the major phenolic compounds in rambutan peel, geraniin (568.0 mg/g extract) was the highest in rambutan peel extract, followed by corilagin (71.9 mg/g extract) and ellagic acid (53.5 mg/g extract) (Thitilertdecha *et al*., 2010). Conversely, corilagin determined in the rambutan peel extract was lower than ellagic acid. The lower amount of corilagin determined in the rambutan peel extract may due to different varieties of rambutan used as compared to what have found from the previous study.

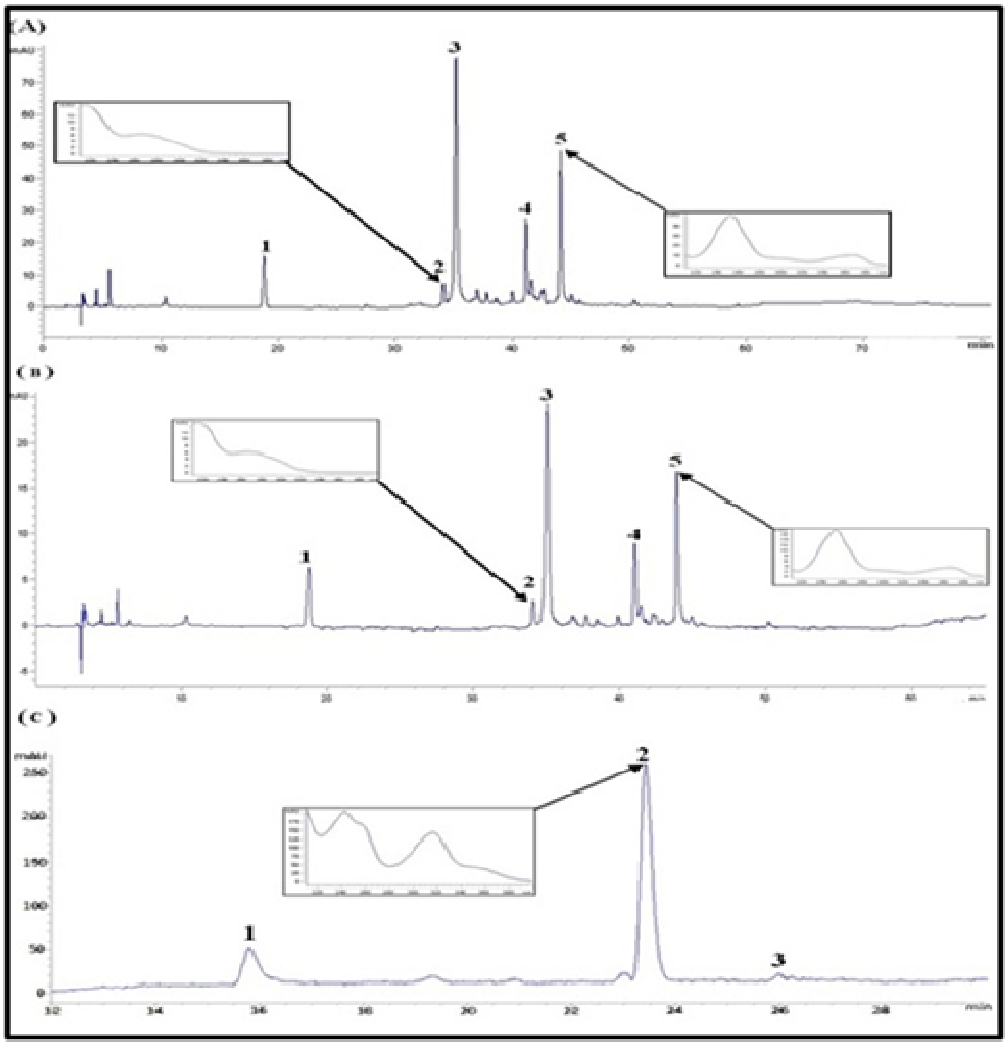

Figure 1. *HPLC chromatographic profiles of (A) rambutan peel extract and (B) langsat peel extract: peak 2 as corilagin; peak 5 as ellagic acid, respectively; peak 3 is tentatively identified as geraniin (Thitilertdecha et al. 2010), while peaks 1 and 4 were unknown; (C) mangosteen peel extract: peaks 1and 3 were unknown and peak 2 as α-mangostin*

The identification of these compounds was done by comparing the retention time and UV absorption spectra of sample peak with the reference peak (Figure 2). The peak of corilagin eluted at retention time of 34.1 min, while ellagic acid was detected at retention time of 43.9 min, which has a 2.2 min shift in the retention time. The langsat peel extract (Figure 1 (B)) had similar trend

for the eluted peaks shown in the chromatogram as for the rambutan peel extract. Although no previous study has been done to determine the contents of ellagic acid and corilagin in langsat peel, these compounds were detected in other fruits, where Rangkadilok *et al.* (2005) reported that ellagic acid (0.10 µg/ml) and corilagin (0.34 µg/ml) were detected in longan fruit.

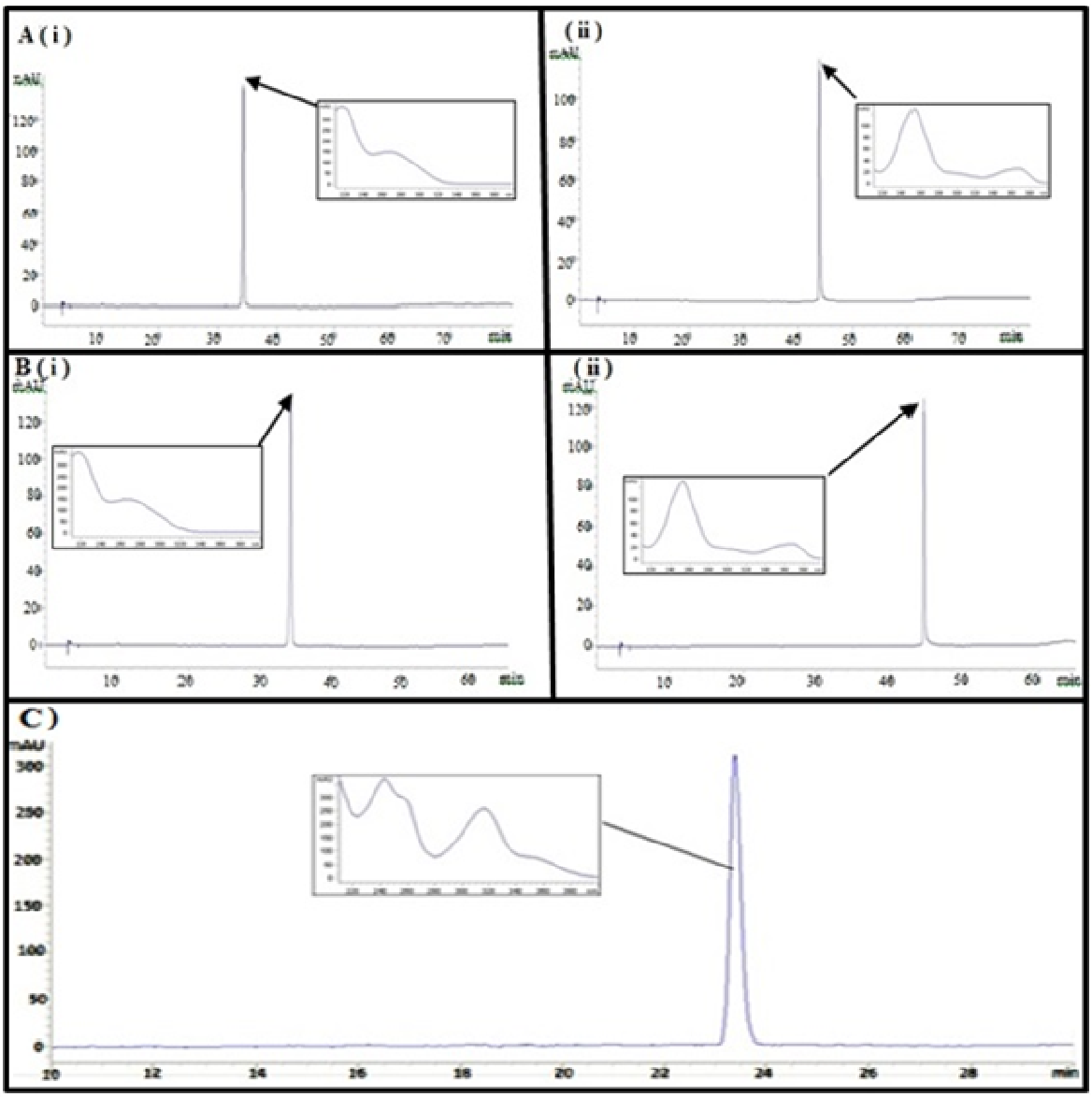

Figure 2. *HPLC chromatographic profiles of standards A (i) corilagin and (ii) ellagic acid for rambutan peel extract; B (i) corilagin and (ii) ellagic acid for langsat peel extract; C) α-mangostin for mangosteen peel extract*

Figure 1 (C) shows the chromatographic profile of the mangosteen fruit peel extract and its matched standard (α-mangostin). The result showed that α-mangostin was one of the major compounds detected in mangosteen peel extract. The retention time of the peak was 23.4 min and it was identified as α-mangostin with a concentration of 0.89±0.45 mg/g dw. The identification of the peak was confirmed by spiking with α-mangostin standard (Figure 3). Walker (2007) supported our finding that α-mangostin was present at the highest concentration (5510 µg/g) in the dried mangosteen peel. The other peaks eluted were not the compounds of our interest which remaining unknown.

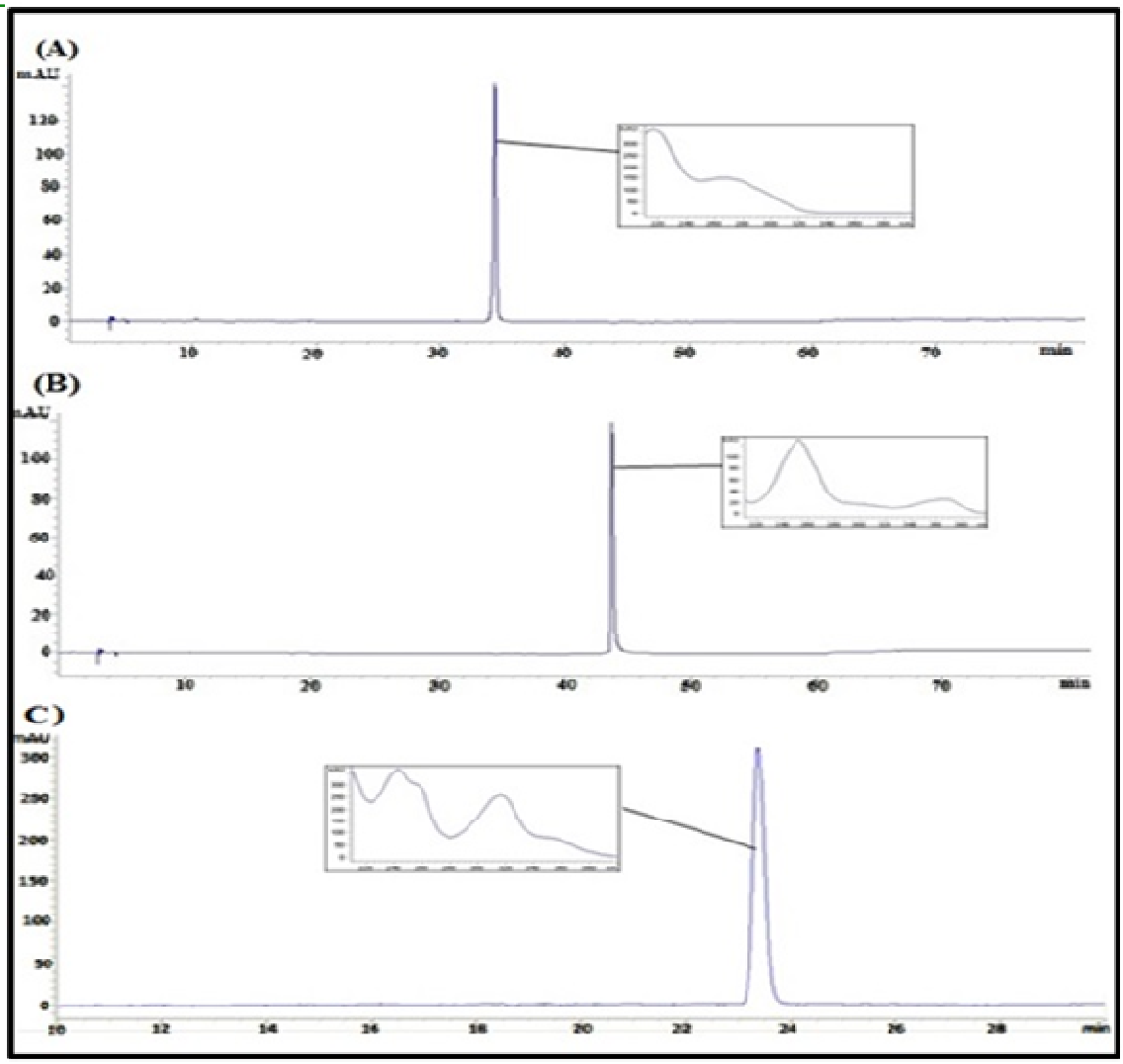

Figure 3. *HPLC chromatographic profiles of standards (A) corilagin, (B) ellagic acid and (C) α-mangostin*

Conclusion

Based on the results obtained, it can be concluded that the major phenolic compound detected in rambutan and langsat peel extracts were ellagic acid and corilagin, whereas α-mangostin was the major xanthone found in mangosteen peel. High ellagic acid content was determined in the rambutan peel extract, as well as high amount of α-mangostin was found in the mangosteen peel extract. Further identification of the unknown phenolic compounds in these fruits' peels is necessary for a complete determination of potent antioxidative compounds. These fruit peels have great nutritional and functional values for potential nutraceutical and pharmaceutical applications due to their potent antioxidative properties.

Acknowledgements

The authors wish to acknowledge UCSI University Research Grant Scheme (Proj-In-FAS-006) for the financial support.

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