Comparison of antioxidant effect and phenolic compounds in tropical fruits

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Abstract

There is a growing interest in consumption of tropical fruits because of their higher degree of antioxidant effects. In present study, we assessed the antioxidant potential as a functional food using popular three tropical fruits, such as litchi (*Litchi chinensis* Sonn.), rambutan (*Nephelium lappaceum* L.) and mangosteen (*Garcinia mangostana* L.). Tropical fruits were extracted in water, 70% ethanol and 70% methanol for 48 h. Then, we analyzed the antioxidant effects of tropical fruit extracts using α , α -diphenyl- β -picrylhydrazyl (DPPH) radical scavenging, thiobarbituric acid reactive substance (TBARS) and reducing power assay. The DPPH radical scavenging and TBARS activities showed the highest degree in rambutan 70% ethanol extract compared with other tropical fruit extracts, as well as total phenolic and flavonoid contents were also highly identified in rambutan 70% ethanol extract. Furthermore, DPPH radical scavenging activity and reducing power was higher in the EtOAc fraction and the F-2 sub-fraction of rambutan 70% ethanol extract as compared with other fractions at the same concentrations. The EtOAc fraction and the F-2 sub-fraction had a large amount of phenols and flavonoids. The antioxidant effects of rambutan extract were closely associated with phenolic compound such as (–)-epicatechin. In conclusion, our results indicate that tropical fruits containing natural antioxidants might be used as supplements for human health, but this deserves further studies.

Keywords Tropical fruits · Antioxidant effects · Polyphenols · Flavonoids

1 Introduction

Recently, there is a growing interest in consumption of tropical fruits including litchi, mangosteen, rambutan, guava, mango, pineapple, wax apple and persimmon. Their sweet flavor can make consumer easy to eat every day, and abundant presence of dietary polyphenols such as, phenolic acids, flavonoids, tannins, coumarins, epicatechin and curcuminoids is anticipated to induce positive biological activity [1]. With recent commercial availability of tropical fruits in Korea, Japan and Europe, biological actions and therapeutic effects of tropical fruits have been studying by many researchers. Furthermore, phytochemicals in tropical fruits commonly comprise of flavonoids,

phenolic acids, stilbenes and lignans [2]. Since they may have a broad-spectrum of biological effects arising from antioxidant and anti-inflammatory properties against obesity-induced oxidative stress and chronic inflammation, rich contents of phenolic compounds have been of extensive interest among researchers [3, 4].

Litchi is a tropical or subtropical fruit with a sweet flavor [5], and its pericarp (peel, rind, hull or ripe) contains a considerable amount of phenols, flavonoids, proanthocyanidin and procyanidin [6]. Rambutan, also known as rongroen, is a tropical fruit that is unique to the Southeast Asia [7]. In addition, it has polyphenols at a mean concentration of 1.64 mg/100 g (range 1.57–1.77 mg/100 g) [1]. Mangosteen, known as “the queen of fruits”, is also cultivated in

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the tropical rainforest of Southeast Asian countries, such as Indonesia, Malaysia, Sri Lanka, Philippines and Thailand [8]. Moreover, its pericarp has been used as a traditional medicine for the treatment of abdominal pain, skin infections and inflammations in Asian countries over the past centuries [9]. Numerous studies have reported that consumption of mangosteen is involved in the inhibition of oxidative damages caused by low-density lipoprotein (LDL) [5], anti-tumor, anti-bacterial and anti-oxidant effects [10]. Although these tropical fruits have been widely consumed as a functional food, the antioxidant capabilities have been reported using different analytical methods. Therefore, it is difficult to estimate the antioxidant activities of tropical fruits based on the comparison of literature studies.

Radical oxygen species (ROS) including superoxide (O_2^-), hydroxyl ($HO^\bullet$), peroxy ($ROO^\bullet$), alkoxyl ($RO^\bullet$) and nitric oxide ($NO^\bullet$) are a natural by-product during biochemical metabolism in every aerobic organism [11]. However, development of chronic diseases is closely involved with unremoved ROS in molecular levels [11]. Especially, mammalian cell membrane lipids are vulnerable to oxidative reactions, and increase of ROS links to formation of malondialdehyde (MDA) which continuously causes oxidative stress and tissue damage. Once MDA is initiating to accumulate in tissues or biological fluids, it contributes to occur chronic diseases [12]. Although the antioxidant enzymes, such as superoxide dismutase, catalase and glutathione peroxidase can modulate the level of ROS in our body, consumption of natural antioxidant derived from food materials positively affect to reduce excessive ROS level [13–15]. Thus, in vitro and in vivo antioxidant studies on bioactive compounds and food ingredients to scavenge reactive oxygen can be helpful to improve human health [16].

The present study compared an anti-oxidant activity of water, ethanol and methanol extract of litchi, rambutan and mangosteen using different radical scavenging assay, and identified phenolic and flavonoid compounds in each extract, respectively. Compared to butylated hydroxytoluene (BHT), tropical fruit extracts efficiently induced antioxidant activity through various radical scavenging mechanisms. Our findings objectively elucidate the antioxidant potential of popular tropical fruit extracts in different extract condition, and could help facilitate the development of promising functional food material to reduce oxidative stress.

2 Materials and methods

2.1 Chemicals

α,α -Diphenyl- β -picrylhydrazyl (DPPH), thiobarbituric acid (TBA), BHT, (+) catechin, (–)-epicatechin, (–)-catechin

gallate and Folin–Ciocalteu-s reagent were purchased from Sigma-Aldrich Chemical (St. Louis, MO). Other chemicals were of analytical grade.

2.2 Preparation of tropical fruit extracts

For the current experiment, we purchased commercially-available fruits; these include litchi (*Litchi chinensis* Sonn.), rambutan (*Nephelium lappaceum* L.) and mangosteen (*Garcinia mangostana* L.). The pulp of tropical fruit was dried using dry incubator at 60 °C. Dried fruit samples were prepared into a powder with a cyclotec mill (Tecator, Hoganas, Sweden). A 100 g of powder samples were obtained from each fruit 3 times and then sequentially placed with 900 mL of distilled water, 70% ethanol (EtOH) and 70% methanol (MtOH) at room temperature for 48 h. This was followed by a continuous mixing of the sample using a magnetic force stirrer. The mixture was filtered using a vacuum-assisted filter (Whatman No. 1 filter paper) (Toyo 2A; Toyo Roshi, Tokyo, Japan), which was followed by a vacuum-assisted evaporation of the solvent using a rotary evaporator at 40 °C for 4 h. Finally, the dried sample extract was obtained and then freeze-dried. Its yield was expressed as the percentage (%). Dried tropical extracts were resolved by water, ethanol and methanol, respectively, and an in vitro antioxidant assay was performed using resolved extracts.

2.3 Preparation rambutan fractions

The rambutan powders were extracted using 70% EtOH. Dried EtOH extract of rambutan was dissolved in 200 mL of ethyl acetate (EtOAc), then which was mixed with 200 mL of distilled water. The mixture was partitioned onto EtOAc and aqueous layers. The EtOAc and water layers were separated from each other and then evaporated to dryness again. The resulting sample was weighed and the yield of each extract was determined accordingly.

The dried EtOAc extract 3.7 g was applied to open-column chromatogram (35 mm $\times$ 100 mm) packed with a silica gel (Kiesel gel 60, 70–230 mesh; Merck, Dramstadt, Germany). The resulting sample was eluted using 100% of dichloromethane, 100% of EtOAc, a mixture of EtOAc and MtOH (1:1), 100% of MtOH and a mixture of MtOH and water (1:1) at a flow rate of 10 mL/min. Based on the retention factor (R_f) on thin layer chromatography (TLC), fractions were divided into 5 sub-fractions: fraction-1, -2, -3, -4 and -5 (F-1, -2, -3, -4 and -5). Silica gel 60 F_{254} 25 aluminum sheets (Merck) were used as TLC plates. The chromatograms were developed up to 10 cm for 25 min using a mixture of chloroform and MtOH (5:1, v/v) at 25 °C. The 5 fractions were concentrated using a rotary vacuum

evaporator and then used to analyze the antioxidant effects and reducing power of each fraction.

2.4 Quantification of polyphenols

The total amount of polyphenols was determined as previously described [17]. Briefly, 0.1% sample solution 10 mL was treated with Folin–Ciocalteu phenol reagent 2 mL. After 5 min, it was also treated with saturated sodium carbonate (Na_2CO_3) solution 2 mL, and placed on shaking incubator for 60 min. Optimal density was measured at a wavelength of 725 nm using a UV mini 1240 UV–VIS spectrophotometer (Shimadzu, Kyoto, Japan). Then, samples and standard compound (chlorogenic acid) were treated to quantify polyphenols in tropical extracts. Phenolic contents were calculated by the standard curve plotted versus the concentration of the sample. The amount of polyphenols was expressed as the ratio of the amount of chlorogenic acid (mg) to that of the sample (100 g).

2.5 Quantification of flavonoids

Flavonoid was quantified using the colorimeter method [18]. Briefly, 0.1% (w/v) sample solution (0.25 mL) was mixed with a distilled water (1.25 mL), and 5% NaNO_2 solution (0.75 mL) in test tube. After 5 min, the mixture was incubated with 10% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (0.15 mL) for 6 min. Sequentially, 1 M NaOH (0.5 mL) and 0.275 mL of distilled water were added to test tube. This was followed by the measurement of the absorbance of the mixture as compared with the blank at a wavelength of 510 nm using a UV mini 1240 UV–VIS spectrophotometer (Shimadzu, Tokyo, Japan). The amount of flavonoid was calculated using the

light scattering detector (ELSD) at a wavelength of 280 nm, and a flow rate of 1.0 mL/min and a temperature of 30 °C as previously described [19]. The temperature of evaporation using the ELSD was set at 40 °C. In addition, the pressure of nebulizing gas (N_2) was set at 3.5 bar. The mobile phase was (A) water (0.1% trifluoroacetic acid) and (B) acetonitrile (0.1% trifluoroacetic acid). The gradient elution was as follows: 0 min, 80% A; 10 min, 80% A; 20 min, 50% A; 25 min, 0% A; 35 min, 0% A; 38 min, 100% A; 48 min and 80% A (initial conditions). Then, it was held for 5 min before next injection. Individual compounds were identified through a comparison of the retention time (Rts) and UV spectral shapes of standard. Moreover, (+)-catechin, (–)-epicatechin and (–) catechin gallate were used as a standard. The UV spectrum was scanned between 190 and 600 nm. All the samples were prepared in methanol.

2.7 DPPH radical scavenging assay

The degree of antioxidant effects was determined based on the radical scavenging activity of the sample solution, for which 16 mg of DPPH was dissolved in 100 mL of EtOH as a concentration of 0.4 mM. The sample was filtered through the Whatman filter paper No. 2 (Toyo Roshi, Tokyo, Japan). This was followed by the addition of 2 mL of DPPH to the 1 mL of samples at varying concentrations of 0, 250 and 500 $\mu\text{g/mL}$ (tropical fruit extracts) and 100 $\mu\text{g/mL}$ (rambutan fractions). The mixture was incubated at room temperature for 30 min, which was followed by the measurement of the absorbance a wavelength of 528 nm using the UV mini 1240 UV–VIS spectrophotometer (Shimadzu). In the current experiment, BHT 100 or 500 $\mu\text{g/mL}$ was used as a positive control. The percentage of the antioxidant activity was expressed based on the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \left[1 - \left(\text{sample absorbance}_{528 \text{ nm}} / \text{control absorbance}_{528 \text{ nm}} \right) \right] \times 100$$

calibration curve ($R_2 = 0.999$) obtained using (+)-catechin hydrate as a standard (2–200 $\mu\text{g/mL}$). It was expressed as catechin equivalents (mg)/sample (100 g). All the extracts were analyzed in triplicate.

2.6 Identification of bioactive compounds

To identify bioactive compounds from the EtOH and EtOAc extracts, each extract and its fractions were filtered through a 0.2- μm membrane filter (Millipore, Nylon, 170 mm). This was followed by the analysis of the filtrate using the Agilent 1200 Series high-performance liquid chromatogram (HPLC) (Agilent Technologies, USA), the Zorbax eclipse plus C_{18} column (5 $\mu\text{m} \times 4.5 \text{ mm} \times 250 \text{ mm}$) and the gradient solvent system. Thus, it was detected using the diode array detector (DAD) and the evaporating

2.8 Thiobarbituric acid reactive substance (TBARS) assay

Inhibitory effect of tropical fruit extracts on linoleic acid (LA) peroxidation was determined by TBARS assay [20]. Two mL of extracts (0.1%, w/v) was mixed with 2 mL of LA (2.5 mg/mL in EtOH), 4 mL of 0.2 M phosphate buffer (pH 7.0) and 2 mL of deionized distilled water. This was followed by the incubation at 40 °C in the dark place for 7 days. Then, 0.5 mL of solution was mixed with 0.25 mL of 35% trichloroacetic acid and 0.5 mL of 0.75% aqueous TBA. Thus, the mixture was heated in a boiling water bath for 15 min to develop color, and then treated with 0.5 mL of 70% trichloroacetic acid. After 20 min, it was centrifuged at 3000 rpm for 15 min to isolate

micro-particles. This was followed by the measurement of the absorbance of the supernatant at a wavelength of 532 nm using the UV mini 1240 UV–VIS spectrophotometer (Shimadzu). Deionized distilled water was substituted for the extract in the blank sample. The inhibitory effects against the lipid peroxidation in LA was calculated based on the following formula:

$$\text{Inhibition ratio (\%)} = \left[1 - \frac{(\text{sample absorbance} - \text{blank absorbance})}{(\text{control absorbance} - \text{blank absorbance})} \right] \times 100$$

2.9 Reducing power assay

The reducing power was measured with the slightly modified method of Oyaizu [21]. The fractions or extracts (0.75 mL) were mixed with 0.2 M sodium phosphate buffer (pH 6.6) 1.25 mL and 1% (w/v) potassium ferricyanide ($\text{K}_3\text{Fe}(\text{CN})_6$) 1.25 mL at varying concentrations ranging from 10 to 1000 $\mu\text{g/mL}$. The mixture was shaken vigorously at 50 °C for 20 min. Following this, it was treated with trichloroacetic acid (10%, w/v) 1.25 mL and then centrifuged at 3000 rpm for 20 min. The supernatant (2.5 mL) was collected and then mixed with a distilled water 2.5 mL and 0.5% ferric chloride (FeCl_3) 0.5 mL. Then, the mixture was shaken vigorously at room temperature for 10 min. After the color development, the absorbance was measured using the UV mini 2450 UV–VIS spectrophotometer (Shimadzu) at a wavelength of 700 against reagent blank.

3 Results

3.1 Extraction yield of tropical fruit extracts

To compare the effectiveness of extract condition, extraction yield of tropical fruit extract was identified using various solvent including water, EtOH and MeOH (Table 1). Among litchi extracts, the water extract showed the higher extraction yield (37.58%) compared to MeOH (22.41%) and EtOH (18.37%). In rambutan,

extraction yield was outstanding in water solvent (30.96%) compared with EtOH (22.13%) and MeOH (18.29%). However, mangosteen extracts did not show notable difference in water (30.42%), MeOH (30.08%) and EtOH (27.38%) solvents.

3.2 Comparison of DPPH radical scavenging activity in tropical fruit extracts

Since it is useful in analyzing short-term anti-oxidant effects, DPPH assay is commonly used to determine the antioxidant activity of natural products [22, 23]. To compare antioxidant activity of tropical fruit extracts, DPPH radical scavenging was measured for water, EtOH and MeOH extracts of litchi, rambutan and mangosteen (Fig. 1). Treatment of 500 $\mu\text{g/mL}$ of rambutan EtOH extract (95.46%) and mangosteen MtOH extract (93.24%) had a higher capacity of DPPH radical scavenging activity in comparison with other tropical fruit extracts. Especially, 500 $\mu\text{g/mL}$ of rambutan EtOH extract indicated similar degree of DPPH radical scavenging activity with same concentration of BHT, synthetic antioxidant (92.58%). Mangosteen EtOH (92.79%) and MeOH extract (93.24%) also showed high levels of DPPH radical scavenging activity at the concentration of 500 $\mu\text{g/mL}$. Compared with EtOH and MeOH extracts at the concentration of 250–500 $\mu\text{g/mL}$, antioxidant activities of water extracts were higher in litchi (47.27%), however, the levels were lower in rambutan (59.92%) and mangosteen (33.64%). These findings demonstrate that EtOH extracts of rambutan and mangosteen were potential resource for antioxidant functional food.

3.3 Comparison of linoleic acid (LA) peroxidation inhibitory effect in tropical fruit extracts

Antioxidant activity includes an inhibitory effect against ferrous iron (Fe^{2+})-catalyzed lipid peroxidation due to ferric thiobarbituric acid (TBA) arising from in vivo membrane damage [24]. In present study, we further studied inhibition effect of tropical fruit extracts on LA peroxidation to identify antioxidant effect on lipid peroxidation. After 24 h incubation, a rapid increase of peroxidized LA was observed in both of control and fruit extract treated group, and it was maintaining until 7 days (data not shown). LA peroxidation inhibition rate indicated that EtOH extracts of litchi, rambutan and mangosteen had a higher degree of inhibition rate 39.95%, 52.19% and 41.22%, respectively

Table 1 The percentage yield of water, ethanol and methanol extracts of tropical fruits

Extracts	Percentage yield (%)		
	Litchi	Rambutan	Mangosteen
Water	37.58	30.96	30.42
EtOH	18.37	22.13	27.38
MtOH	22.41	18.29	30.08

EtOH Ethanol, MtOH methanol

Fig. 1 The DPPH radical scavenging activity of water (W), ethanol (E) and methanol (M) extracts of tropical fruits. *BHT* Butylated hydroxytoluene, *DPPH* α,α -diphenyl- β -picrylhydrazyl. Values are mean $\pm$ SD (SD: standard deviation) (n = 3)

Fig. 2 The degree of antioxidant effects of water, ethanol and methanol extracts of tropical fruits based on the inhibition of linoleic acid peroxidation thiobarbituric acid assay. *BHT* Butylated hydroxytoluene. Values are mean $\pm$ SD (SD: standard deviation) (n = 3)

as compared with each water and MeOH extracts (Fig. 2). Moreover, BHT had the highest degree of antioxidant effects (Fig. 2). Although synthetic antioxidant BHT indicated highest LA peroxidation inhibitory effect (83.61%), rambutan EtOH extract were higher antioxidant activity on LA peroxidation than other tropical fruit extracts. This result indicates that EtOH extract of rambutan is outstanding functional food materials to prevent lipid peroxidation.

3.4 Total amount of polyphenols and flavonoids

In order to identify whether or not contents of polyphenols and flavonoids in tropical fruit extracts are related with antioxidant effect, we further analyzed total amount of phenolic and flavonoid compounds in water, EtOH and MeOH extracts of tropical fruit (Tables 2, 3). Litchi, rambutan and mangosteen EtOH extracts showed 9.97, 23.50 and 20.99 mg of total phenolic contents, and 0.64, 3.10 and

Table 2 Total amount of phenols from water, ethanol and methanol extracts of tropical fruits

Extracts	Total amount of phenols (mg/100 g dry wt)		
	Litchi	Rambutan	Mangosteen
Water	2.00 ± 0.01	11.99 ± 0.01	5.99 ± 0.01
EtOH	9.97 ± 0.05	23.50 ± 0.01	20.99 ± 0.01
MtOH	5.98 ± 0.02	19.00 ± 0.01	19.01 ± 0.01

Values are mean ± SD (SD: standard deviation) (n = 3)

EtOH Ethanol, MtOH methanol

Table 3 The amount of flavonoids from water, ethanol and methanol extracts of tropical fruits

Extracts	Total amount of flavonoids (mg/100 g dry wt)		
	Litchi	Rambutan	Mangosteen
Water	0.20 ± 0.01	1.45 ± 0.04	1.98 ± 0.03
EtOH	0.64 ± 0.01	3.10 ± 0.10	6.08 ± 0.14
MtOH	0.42 ± 0.01	2.31 ± 0.08	4.31 ± 0.12

Values are mean ± SD (SD: standard deviation) (n = 3)

EtOH Ethanol, MtOH methanol

6.08 mg of total flavonoid contents in 100 g of dry weight extract, respectively. The MeOH extract of litchi, rambutan and mangosteen indicated 5.98, 19.00 and 19.01 mg of total phenolic contents, and 0.42, 2.31 and 4.31 mg of total flavonoid contents in 100 g of dry weight extract, respectively. The water extracts of tropical fruit had a slightly smaller amount of phenols and flavonoids as compared with the EtOH or MtOH extracts. These results suggest that antioxidant effect of tropical fruit extracts are related with contents of phenolics and flavonoids.

3.5 Identification of bioactive compounds from extracts of rambutan EtOAc fraction and its sub-fractions

In present study, rambutan EtOH extract had outstanding antioxidant potential compared to other tropical fruit extracts. In EtOAc fraction of rambutan and its sub-fractions, the percentage yield was demonstrated by 10.9% (794 mg) in the F-1, 14.0% (1018 mg) in the F-2, 3.4% (246 mg) in the F-3, 33.0% (2398 mg) in the F-4 and 12.0% (868 mg) in the F-5 on silica gel open-column chromatography. The total weight and yield were 5324 mg and 73.3%, respectively (data not shown).

To increase utilization of rambutan extract as a functional food material, we further analyzed bioactive compounds in rambutan EtOH extract, EtOAc fraction and its sub-fractions (Fig. 3, Table 4). The total amount of phenolic and flavonoid contents in EtOAc fraction of rambutan was

Fig. 3 The high-performance liquid chromatograms of the standard (A), ethyl acetate extract (B) and fractions of ethanol extract (C) of rambutan. EtOAc Ethyl acetate, ND not determined. Peaks: ①, (+)-catechin; ②, (–)-epicatechin; ③, (–)-catechin gallate

Table 4 The α,α-diphenyl-β-picrylhydrazyl radical scavenging activity and the total amount of phenols and flavonoids from butylated hydroxytoluene extract, ethyl acetate extract and fractions of ethanol extract of rambutan

	DPPH radical scavenging activity (%) (100 µg/mL)	Phenols (mg/g)	Flavonoids (mg/g)
BHT	78.37 ± 0.11	ND	ND
EtOAc	91.66 ± 0.94	367.8 ± 0.50	24.84 ± 0.14
F-1	7.24 ± 1.19	150.3 ± 1.60	20.8 ± 1.19
F-2	98.49 ± 0.07	661.9 ± 1.10	73.7 ± 1.17
F-3	94.34 ± 0.08	352.3 ± 0.70	31.1 ± 0.15
F-4	86.70 ± 1.27	353.9 ± 0.70	47.0 ± 0.40
F-5	92.25 ± 1.23	357.5 ± 1.50	37.5 ± 1.10

Values are mean ± SD (SD: standard deviation) (n = 3)

DPPH α,α-Diphenyl-β-picrylhydrazyl, BHT butylated hydroxytoluene, EtOAc ethyl acetate, ND not determined

367.8 ± 0.50 and 24.84 ± 0.14 mg/g, respectively (Table 4). In addition, its sub-fractions including F-1, F-2, F-3, F-4 and F-5 showed 150.3 ± 1.60, 661.9 ± 1.10, 352.3 ± 0.70, 353.9 ± 0.70 and 357.5 ± 1.50 mg/g of total phenolic contents, and 20.8 ± 1.19, 73.7 ± 1.17, 31.1 ± 0.15, 47.0 ± 0.40 and 37.5 ± 1.10 of flavonoid contents, respectively (Table 4).

A large amount of phenols was also identified by HPLC–PDA analysis in EtOAc fraction of rambutan and F-2 sub-fraction. Compared to standard peak, content of (–)-epicatechin (retention time [Rt] 4.316 min) was verified in EtOAc fraction (29.2 mg/g), F-2 (70.4 mg/g) and F-3 (10.4 mg/g) sub-fractions, respectively (Fig. 3). However,

Fig. 4 The degree of reducing power of butylated hydroxytoluene, ethanol and ethyl acetate extracts and fractions of rambutan extract. *BHT* Butylated hydroxytoluene, *EtOH* ethanol, *EtOAc* ethyl acetate. Values are mean $\pm$ SD (SD: standard deviation) ($n = 3$)

Fig. 5 The DPPH radical scavenging activity of water and ethyl acetate fractions of 70% ethanol extract of rambutan. *DPPH*, α,α -Diphenyl- β -picrylhydrazyl, *BHT* butylated hydroxytoluene, *EtOH* ethanol, *EtOAc* ethyl acetate. Values are mean $\pm$ SD (SD: standard deviation) ($n = 3$)

(–)-epicatechin analogues, such as (+)-catechin and (–)-catechin gallate, were not identified in EtOH extract, EtOAc fraction and its sub-fractions. These results suggest that (–)-epicatechin is one of the major phenolic compound in rambutan EtOH extract, and F-2 fraction had a highest amount of phenolic and flavonoid contents in rambutan EtOAc sub-fractions.

3.6 Antioxidant activity of rambutan EtOAc fraction and its sub-fractions

There was a significant positive correlation between the reducing power and concentrations of EtOH extract, EtOAc fraction and its sub-fractions (Fig. 4). As shown in Fig. 5, the F-2 fraction of the EtOAc extract had the highest degree

of reducing power. Of note, the degree of DPPH radical scavenging effects of EtOAc fraction, F-2, -3, -4 and -5 sub-fractions were higher as compared with the BHT with no respect to concentrations (Table 4). In addition, EtOAc fraction showed higher DPPH radical scavenging activity as compared with the BHT at the same concentration of 100 $\mu\text{g/mL}$ (Fig. 5). The F-1 fraction showed the lowest degree of DPPH radical scavenging effect compared with the BHT. These results indicate that rambutan EtOAc F-2 sub-fraction is a potential functional food material for antioxidant activity.

4 Discussion

Since tropical fruits are rich source of antioxidant compounds, recent studies have concentrated their outstanding health improving activity. In the industrial view point, extraction yield is an important condition to processing food materials. According to Wang et al., the yield of crude EtOH extract of mature *Baila litchi* exceeded 22.0%, and 60% of component were water-soluble and the remaining 40% of which were EtOH- and acetone-soluble [25]. In addition, Chomnawang et al. reported that the yield (%) of EtOH extract against the pericarps of *Garcinia mangostana* was 15.63% [26]. In current study, although water extract of litchi, rambutan and mangosteen was identified to have highest extraction yield, EtOH extract showed similar tendency with previous studies in terms of antioxidant effect and extraction yield. These data indicate that water extract of litchi, rambutan and mangosteen are advantageous to develop ordinary food or drink, however EtOH extracts are best extract condition to process tropical fruits as a functional food material.

In the current study, the amount of polyphenols and flavonoids were highest in the EtOH extract of litchi, rambutan and mangosteen compared to their water and MeOH extract. The total amount of phenols in litchi, rambutan and mangosteen was reported to contain by 3.35, 1.39 and 1.64 mg/100 g, respectively [1]. As compared with a previous published studies [1], our results showed a significantly larger amount of phenols were contained in litchi, rambutan and mangosteen. There are considerable amounts of polyphenols in the pericarp of litchi; these include condensed tannins (polymeric proanthocyanidins), epicatechin and procyanidin A2 [25, 27]. Litchi and rambutan belong to the *Sapindaceae* family, and both fruits share similar appearances, flavor, pericarp color and juicy aril. It is presumed that the extract of rambutan shared similar chemical properties with the pericarp of litchi. According to Sun et al., the fractions of EtOAc had a significantly larger amount of phenols (1.475 mg GAE/g FW) as compared with those of n-butanol (1.213 mg GAE/g

FW) and water (0.778 mg GAE/g FW) [28]. Furthermore, Sun et al. showed that the pericaps of litchi and rambutan contained (–)-epicatechin at concentrations of 1655 and 75 µg/g FW, respectively. Moreover, these authors also noted that (–)-epicatechin is present in green tea and other tropical plants, and its antioxidant effects have been well described in the literature [28]. In present study, contents of (–)-epicatechin were identified by HPLC in rambutan F-2 and F-4 fractions which showed outstanding antioxidant activity on DPPH radical scavenging and reducing power.

Flavonoids are phenols that are abundantly present in foods. To date, more than 4000 different types of flavonoids have been identified. They are known as one of major constituents of the extract of mature or premature litchi; they have been reported to have a strong antioxidant effect [29]. The amount of flavonoids was the largest in the EtOH extract of litchi, rambutan and mangosteen, and the smallest in the water extracts. Previous studies have reported that mangosteen and Mao Luang had a total amount of flavonoids of 54.1 and 397.9 mg/100 g, respectively [1, 30]. This is also accompanied by a report showing not only that 15 Mao Luang cultivars contained three types of flavonoids (catechin, procyanidin B1 and procyanidin B2) but also that they were composed of such flavonoid compounds. Furthermore, the amount of catechin contained in 15 cultivars varied, ranging from 73.39 to 316.22 mg/100 g (fresh weight) [30]. Therefore, a large amount of phenols and flavonoids from the EtOAc fraction and F-2 sub-fraction of the EtOH extract of rambutan might be closely involved in their antioxidant effects.

The antioxidant effects based on DPPH radical scavenging activity might arise from the electron or hydrogen donating ability [31]. A number of studies have reported that natural antioxidants, such as tocopherol, phenols, glutathione and zinc, have a higher degree of antioxidant effects to indicate strong free radical scavenging activity [32]. It has been also reported that synthetic antioxidant BHT had a higher degree of effects in scavenging DPPH free radicals [33]. This is in agreement with our results showing that its degree reached the highest level in the EtOH extract of rambutan at a concentration of BHT of 500 µg/mL. The degree of the antioxidant effects was the highest in EtOH extract of rambutan compared with other tropical fruit extracts. In addition, we also found that the EtOAc fraction had a higher degree of antioxidant effect as compared with the BHT at the same concentration of 100 µg/mL. Despite the same concentrations, however, the degree of antioxidant effect was lower in the water fraction of EtOH extract of rambutan as compared with BHT or EtOAc one. It has also been previously shown that the EtOAc extract of rambutan peel had the largest amount of polyphenols

and the highest degree of effects in scavenging free radicals [34]. Previous studies have shown that the EtOH extract of mangosteen had the highest degree of antioxidant effect of the four extracts (water, 50% EtOH, 95% EtOH and EtOAc ones) in a DPPH radical scavenging model [26, 35]. These results showed similar tendency in phenolic and flavonoid contents of tropical fruit extracts. Therefore, the DPPH radical scavenging activity in 70% EtOH extract of rambutan mainly arises from the EtOAc fraction and F-2 sub-fraction containing higher (–)-epicatechin, polyphenol and flavonoid contents.

The effects of polyunsaturated fatty acids, such as LA, methyl LA and arachidonic acid, in inhibiting lipid peroxidation have been studied to identify antioxidants [36]. We found that the EtOH extracts of litchi, rambutan and mangosteen had a higher degree of antioxidant effects in inhibiting LA peroxidation as compared with other extracts. According to another study, there was a marked inhibition in the LA oxidation by the EtOH-soluble extracts of all Philippine fruits and vegetables. Of these, mangosteen had the highest degree of antioxidant activity effect [37].

It is generally known that the antioxidant effects of bioactive compounds are closely associated with their reducing power arising from an ability to donate electrons [38]. We found a significant positive correlation between the total reducing power and concentrations of EtOH extract and EtOAc fraction and their sub-fractions. We also showed that the F-2 sub-fraction had the highest degree of reducing power. Of note, it was higher as compared with the BHT with no respect to concentrations. Presumably, this might be related with rich phenolic and flavonoid compounds in F-2 sub-fraction. Furthermore, it followed the same descending order as that of DPPH radical scavenging activity. As compared with the extracts of rich hull and *Sonchus oleraceus* L., however, the water extract had a relatively lower degree of reducing power [39, 40]. This suggests that there is a significant correlation between the degree of antioxidant effect and that of reducing power of sub-fractions of rambutan, which is in agreement with previous published studies showing that the EtOAc extract had a relatively higher degree of reducing power.

To summarize, our results are as follows: (1) DPPH radical scavenging activity and reducing power occurred in a concentration-dependent manner. (2) The degree of DPPH free radical scavenging activity and reducing power was higher in the EtOAc fraction and the F-2 as compared with other fractions at the same concentrations. (3) The EtOAc extract of 70% EtOH one of rambutan had a large amount of phenols and flavonoids. Its antioxidant effects originated from the F-2 fraction. This indicates that the antioxidant effects of rambutan extract are closely associated

with phenols such as (–)-epicatechin. Based on the above results, it can therefore be concluded that tropical fruits containing natural antioxidants might be used as supplements for human health. But this deserves further studies on optical method of extraction.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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