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Research Article

Influence of Processing in Two Cultivar of *Carica papaya* from West Java-Indonesia to Antioxidant Activities, Total Phenolic, Flavonoid and Carotenoid Content

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ABSTRACT

The aim of this research was to determine antioxidant activity in various flesh extracts from three different processing of two cultivar of papaya using DPPH (2,2-diphenyl-1-picrylhydrazyl) and correlation of total phenolic, flavonoid and carotenoid content in various flesh extracts with their IC_{50} of DPPH antioxidant activities. Extraction was performed by reflux using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH assay, determination of total phenolic, flavonoid and carotenoid content were done by UV-visible spectrophotometry and its correlation with IC_{50} of DPPH scavenging activities were analyzed by Pearson's method. All of ethyl acetate flesh extracts in two cultivar of *C. papaya* were categorized as very strong antioxidant by DPPH method. Phenolic and carotenoid compounds in raw *C. papaya* cv. calina were the major contributor in IC_{50} of DPPH scavenging activities.

Keywords: Processing, *C.papaya*, two cultivar, antioxidant, phenol, flavonoid, carotenoid

INTRODUCTION

Antioxidant can reduce negative effect by inhibiting oxidation reaction of free radical. The excessive amount of free radical related with oxidative stress condition which can cause many diseases. Many plants have active compounds which have antioxidant properties such as flavonoid and phenolic compounds, which have known to multiple biological effects, included antibacterial and antioxidant activity[1-3]. Previous research[4-7] reported that phenolic and flavonoid content could be correlated to their antioxidant activities. Plants including papaya contained phenolic and flavonoid compounds[8-10].

DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) can be used to predict antioxidant activity of vegetables, fruits and food[9,11-13]. The previous researches[7,12,14,15] stated that DPPH, FRAP, CUPRAC and ABTS methods could be used to determine antioxidant activity in many plants extracts. Previous studies[10-16] expressed that papaya had antioxidant activities by using ABTS, DPPH, and FRAP assays.

The aim of this research was to determine antioxidant activities in various flesh extracts (n-hexane, ethyl acetate and ethanol) from three different processing in two cultivar of *C. papaya* using DPPH assay, and correlations of total phenolic, flavonoid and carotenoid content with their antioxidant activities.

MATERIALS AND METHODS

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, quercetin, beta carotene were purchased from Sigma-Aldrich (MO, USA), papaya (*Carica papaya*), ethanol. All other reagents were analytical grades.

Preparation of sample

Flesh of *Carica papaya* which were: *Carica papaya* cv. calina namely as sample CL was collected from Rajamandala, West Java and *Carica papaya* cv. 1x2 as SD collected from Subang, West Java. Each papaya then divided in 3 groups: raw papaya (without treatment papaya) namely as A, pickled papaya as B and candied papaya as C. So there were six samples: CLA, CLB, CLC, SDA, SDB and SDC.

Preparation of raw papaya

Flesh of unripe papaya 1 kg was washed with tap water, sorted while wet, cut, dried and grinded into powder.

Preparation of pickled papaya

Flesh of unripe papaya 1 kg was soaked in calcium hydroxide solution for 30 minutes. Then mixed with salt as needed until soft, washed, soaked with water and squeezed until the water run out. Vinegar sauce was made by grinding 20 red chilies, 4 tablespoons of dried shrimp, salt and 8 tablespoons of sugar, then mixed with 400 ml of water and 2 tablespoons of vinegar, mixed well and boiled. Papaya was soaked in vinegar sauce for 2 nights. Then pickled papaya was dried and grinded.

Preparation of candied papaya

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Flesh of unripe papaya 1 kg was soaked in calcium hydroxide solution for 2 hours. Solution for preparing candied papaya was made using 500 g sugar, a quarter teaspoon of salt and 1 l of water until boiled, then removed and allowed to cool. Papaya was inserted to candied solution and left for 4 hours. Then papaya and the candied solution were separated. The candied solution was cooked until thick, stirred constantly and removed. Papaya was put back into the candied solution and left overnight. Then candied papaya was dried and grinded.

Extraction

Three hundred gram of sample was extracted by reflux using different polarity solvents. Extraction using n-hexane was repeated three times. The remaining residue was then extracted three times by using ethyl acetate. Finally the remaining residue was extracted three times using ethanol. Therefore, totally there were eighteen extracts: six n-hexane extracts (namely CLA1, CLB1, CLC1, SDA1, SDB1 and SDC1), six ethyl acetate extracts (CLA2, CLB2, CLC2, SDA2, SDB2 and SDC2) and six ethanolic extracts (CLA3, CLB3, CLC3, SDA3, SDB3 and SDC3).

Determination of total phenolic content (TPC)

Total phenolic content was done by using the modified Folin-Ciolcalteu[17]. The absorbance was read at wavelength 765 nm. Analysis was carried out in triplicate for each extract. Gallic acid standard solution (40-165 µg/ml) was used to obtain a calibration curve. Total phenolic content was reported as percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

Determination of total flavonoid content (TFC)

Total flavonoid content was using Chang *et al*[18] method with minor modification. The absorbance was observed at wavelength 415 nm. Analysis was done in triplicate for each extract. Quercetin standard solution (36-120 µg/ml) was used to obtain a calibration curve. The total flavonoid content was stated as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Determination of total carotenoid content (TCC)

Total carotenoid content was adapted from Thaipong *et al*[12] method. Each extract was diluted in n-hexane. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extract. Beta carotene standard solution (15-55 µg/ml) was used to obtain a calibration curve. The total carotenoid content was exposed as percentage of total beta carotene equivalent per 100 g extract (g BE/100 g).

Antioxidant activity by DPPH method

Preparation of DPPH solution was performed using Blois's method with minor modification[19]. Various concentration of each extract were pipetted into DPPH solution 50 µg/ml (volume 1:1) to initiate the reaction for obtaining a calibration curve. The absorbance was measured after 30 minutes incubation at wavelength 515 nm by using UV-Vis spectrophotometer Hewlett Packard 8435. Methanol was used as a blank. DPPH solution 50 µg/ml was used as control. Ascorbic acid was used as standard. Analysis was done in triplicate for standard and each extract. Antioxidant activity of each extract by DPPH method was determined by calculating percentage of antioxidant activity using reduction of DPPH absorbance[20]. IC_{50} of DPPH scavenging activity of each extract can be determined using its calibration curve.

Statistical Analysis

Each sample analysis was conducted in triplicate. All results presented are means (± standard deviation) of at least three independent experiments. Statistical analysis using ANOVA with a statistical significance level set at $p < 0.05$ and post-hoc Tukey procedure was performed using SPSS 16 for Windows. Correlation between the total phenolic, flavonoid, carotenoid content and antioxidant activities, and correlation between two antioxidant activity methods were conducted using the Pearson's method.

RESULTS

Total phenolic content (TPC)

TPC among the various extracts were revealed in term of gallic acid equivalent using the standard curve equation $y = 0.006x - 0.055$, $R^2 = 0.998$. The TPC in eighteen extracts from three different processing of two cultivar papaya exposed different results ranged from 0.38 to 18.79 g QE/100 g for *C. papaya* cv. calina and 0 to 21.69 g QE/100 g for *C. papaya* cv. 1x2. N-hexane extract of pickled calina papaya (CLB1) gave the highest total flavonoid content (18.79 g QE/100 g), while n-hexane of pickled 1x2 papaya (SDA1) showed the highest TPC (21.69 g GAE/100 g).

Total flavonoid content (TFC)

TFC among the various extracts were reported in term of quercetin equivalent using the standard curve equation $y = 0.006 x - 0.0191$, $R^2 = 0.998$. The TFC in eighteen extracts from three different processing of two cultivar of *C. papaya* showed different results in the range of 0.01 – 10.03 g QE/100 g for *C. papaya* cv. calina and 0.03-6.57 g QE/100 g for *C. papaya* cv. 1x2. N-hexane extract of raw 1x2 papaya (SDA1) had the highest total flavonoid content (6.57 g QE/100 g) and the highest TFC in *C. papaya* cv. calina (10.03 g QE/100 g) was given by n-hexane extract of pickled calina papaya (CLB1).

Total carotenoid content (TCC)

TCC among the various extracts were exposed in term of beta carotene equivalent using the standard curve equation $y = 0.007x - 0.027$, $R^2 = 0.995$. The TCC in eighteen extracts from three different processing of two cultivar of *C. papaya* gave different results ranged from 0.13 to 4.10 g BE/100 g for *C. papaya* cv. calina and 0.04-1.35 g BE/100 g for *C. papaya* cv. 1x2. The highest TCC in *C. papaya* cv. calina was given by CLB1 (4.10 g BE/100 g) and SDA3 (1.35 g BE/100 g) for *C. papaya* cv. 1x2.

Antioxidant activity by DPPH method

Antioxidant activity was determined by measuring IC_{50} of DPPH scavenging activities in three different processing of two cultivar of papaya using DPPH assays were shown in Fig 1 and Fig 2. IC_{50} of DPPH scavenging activities of each extract were compared to IC_{50} ascorbic acid as standard. The lowest value of IC_{50} means had the highest antioxidant activity.

Correlations between total phenolic, flavonoid, carotenoid content in different processing extracts of two cultivar of papaya and IC_{50} of DPPH scavenging activities

Pearson's correlation coefficient between TPC in different processing extracts of two cultivar of papaya and their antioxidant activities exposed that TPC in raw calina papaya (CLA), candied calina papaya (CLC) and pickled 1x2 papaya (SDB) had negative and high significant correlation with their IC_{50} of DPPH scavenging activities (r = -0.873; r = -0.878; r = -0.981, p<0.01, respectively). TFC in candied 1x2 papaya extracts (SDC) had negatively high correlation with their IC_{50} of DPPH scavenging activities (r = -0.990, p<0.01) and only TCC in CLA extracts had negative and high correlation with their IC_{50} of DPPH scavenging activities (r = -0.943, p<0.01).

Table 1: Total phenol, flavonoid, carotenoid content in different processing of *Carica papaya* cv. calina

Sample	TPC (g GAE/100 g)	TFC (g QE/100 g)	TCC (g BE/100 g)
CLA1	3.52±0.07	1.73±0.01	0.18±0.01
CLB1	18.79±0.54	10.03±0.09	4.10±0.28
CLC1	9.46±0.08	1.36±0.00	0.14±0.01
CLA2	3.43±0.01	3.69±0.16	0.29±0.01
CLB2	2.37±0.01	3.97±0.16	0.38±0.01
CLC2	1.72±0.01	3.23±0.10	0.28±0.01
CLA3	0.69±0.01	0.04±0.01	0.13±0.01
CLB3	0.45±0.01	0.18±0.01	0.26±0.01
CLC3	0.38±0.01	0.01±0.01	0.40±0.01

CLA = raw papaya *C. papaya* cv. calina, CLB = pickled papaya *C. papaya* cv. calina, CLC = candied papaya *C. papaya* cv. calina, 1 = n-hexane extract, 2 = ethyl acetate extract, 3 = ethanolic extract, TPC = total phenolic content, TFC = total flavonoid content, TCC = total carotenoid content, GAE = gallic acid equivalent, QE = quercetin equivalent, BE = betacaroten equivalent

Table 2: Total phenol, flavonoid, carotenoid content in different processing of *Carica papaya* cv. 1x2

Sample	TPC (g GAE/100 g)	TFC (g QE/100 g)	TCC (g BE/100 g)
SDA1	6.53±0.15	6.57±0.05	0.44±0.01
SDB1	21.69±0.01	1.17±0.02	0.63±0.01
SDC1	0.00±0.00	4.34±0.01	0.58±0.01
SDA2	2.15±0.01	4.00±0.11	0.09±0.01
SDB2	2.23±0.01	3.51±0.09	0.13±0.01
SDC2	2.12±0.01	3.43±0.11	0.06±0.01
SDA3	1.09±0.03	1.52±0.02	1.35±0.02
SDB3	1.24±0.03	1.81±0.08	0.21±0.01
SDC3	0.61±0.01	0.03±0.01	0.04±0.01

SDA = raw papaya *C. papaya* cv. 1x2, SDB = pickled papaya *C. papaya* cv. 1x2, SDC = candied papaya *C. papaya* cv. 1x2, 1 = n-hexane extract, 2 = ethyl acetate extract, 3 = ethanolic extract, TPC = total phenolic content, TFC = total flavonoid content, TCC = total carotenoid content, GAE = gallic acid equivalent, QE = quercetin equivalent, BE = betacaroten equivalent

DISCUSSION

The previous research[10,16,21-23] reported that papaya had antioxidant capacity. There was no research regarding antioxidant activity in different processing of two cultivar of papaya from West Java- Indonesia using various polarity solvent which were n-hexane, ethyl acetate and ethanol by DPPH assay.

DPPH free radicals give characteristic absorption at wavelength 516 nm in methanol. Colors of DPPH would be changed from purple to yellow color when the free radicals were scavenged by antioxidant[24].

The IC_{50} of DPPH scavenging activities from various polariy extracts with three different processing of two cultivar of papaya using DPPH assay was reported in Fig 1 and Fig 2. The IC_{50} of DPPH scavenging activities in various extracts compared to IC_{50} of ascorbic acid standard. The lowest value of IC_{50} means showed the highest antioxidant activity. IC_{50} was used to determine antioxidant activity and compared to standard. Sample which had IC_{50} lower than 50 μg/ml was a very strong antioxidant, 50-100 μg/ml was a strong antioxidant, 101-150 μg/ml was a medium antioxidant, while a weak antioxidant with IC_{50} greater than 150 μg/ml[19].

In the present study exposed that IC_{50} of DPPH scavenging activities from various flesh extracts with different processing of two cultivar of papaya ranged from 13.20– 263.82 μg/ml for calina papaya and 46.27 – 273.54 μg/ml for 1x2 papaya. The lowest IC_{50} of DPPH was given by ethyl acetate flesh extract for pickled calina papaya (CLB2) 13.20 μg/ml and ethyl acetate flesh extract for raw 1x2 papaya (SDA2) 46.27 μg/ml, while IC_{50} of DPPH of ascorbic acid was 5.30 μg/ml. The result revealed that potency of ascorbic acid was around three times potency of CLB2 and nine times potency of SDA2 using DPPH method. The ethyl acetate flesh extract of

calina papaya and 1x2 papaya with three different processing gave IC_{50} of DPPH in the range of 13.20-20.16 µg/ml and 46.27-49.19 µg/ml, respectively. Based on the value of IC_{50} of DPPH, so it can be categorized as very strong antioxidant.

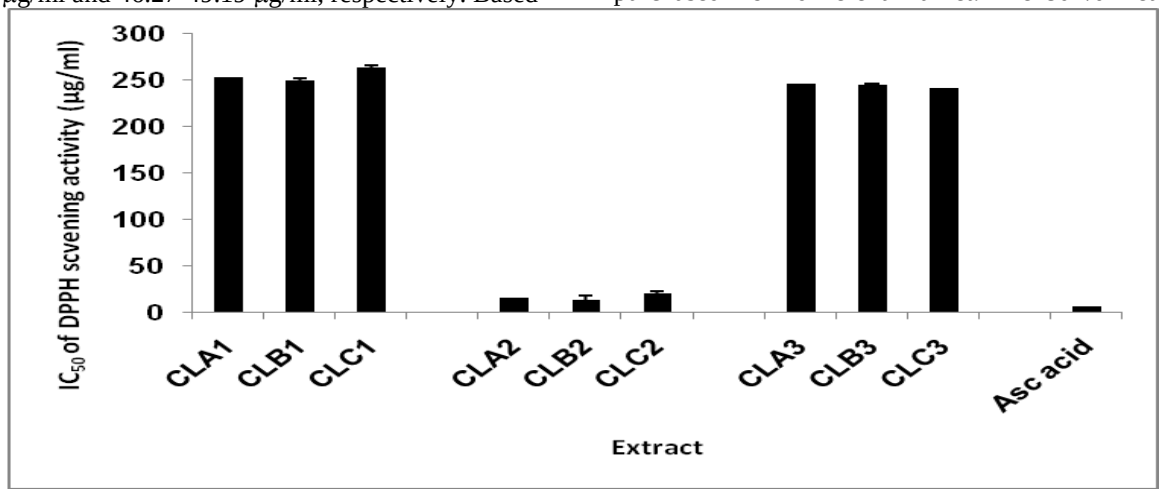

Figure 1: IC_{50} of DPPH scavenging activities in *Carica papaya* cv. calina extracts

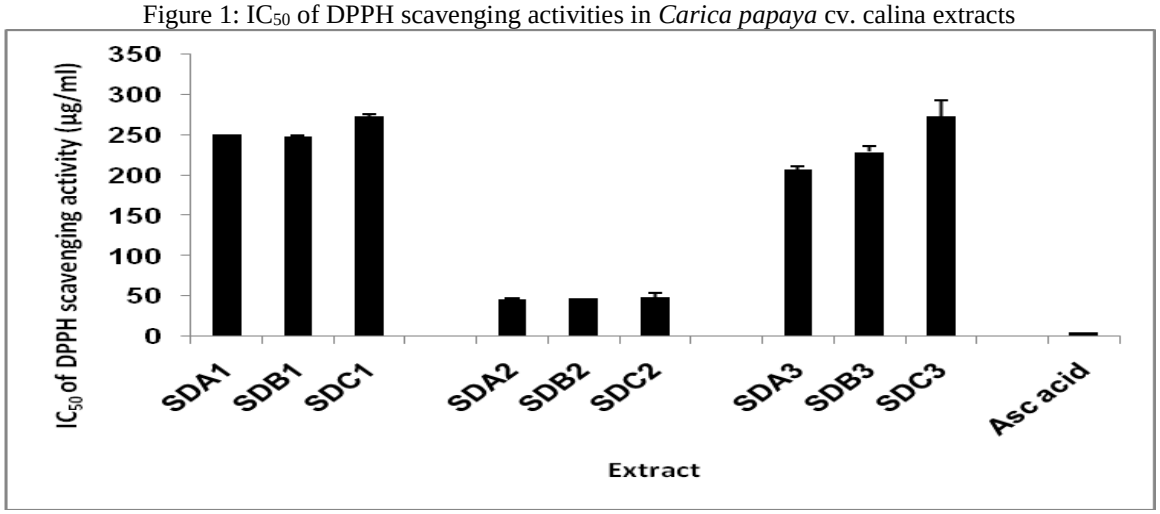

Figure 2: IC_{50} of DPPH scavenging activities in *Carica papaya* cv. 1x2 extracts

Study by Addai[16] stated that 50% methanol extract of different ripening stages of papaya fruit gave different results. Increasing in maturity stages correlated with increasing in antioxidant activity and antioxidant capacity. Papaya with maturity stages 20 weeks had the highest FRAP capacity 180 mg TE/100 g FW, percentage of DPPH and ABTS scavenging activities 72.19 % and 68.10 %, respectively. The previous research[22] which studied regarding antioxidant activities of 80 % aqueous methanol of young leaves, seed, ripe and unripe of *C. papaya* reported that their percentage of β-carotene linoleate bleaching activity were 90.01%, 58.97%, 88.12%, 90.67%, respectively, while their IC_{50} of DPPH of scavenging activities were 7800, 1000, 6500 and 4300 µg/ml respectively. It was contrary with the present study which demonstrated that ethanolic flesh extract from different processing (raw papaya, pickled papaya and candied papaya) had IC_{50} of DPPH of scavenging activities 246, 243, 241 µg/ml, respectively for calina papaya and 207, 229, 273 µg/ml, respectively for 1x2 papaya. Nurul[10] studied regarding antioxidant activity of fresh and pickled papaya. No statement regarding cultivar of *C. papaya*. The fresh papaya and pickled papaya was purchased from different market. The 80 % methanolic extract of fresh papaya had higher antioxidant activity by β-carotene linoleate bleaching assay than the pickled papaya. IC_{50} of DPPH scavenging activity of fresh papaya was 4280 µg/ml, while increasing in concentration of 1 to 8 mg/ml of pickled papaya gave scavenging activity below than 50 %, so the IC_{50} of DPPH of pickled papaya can't be detected. Reseacrh by Ozkan[21] stated that antioxidant activity of flesh juice of two different cultivars (Sunrise Solo and Red Lady) by DPPH assays were 52.1 and 63.4 ml PCJ/g DPPH, while IC_{50} of DPPH of ascorbic acid and BHT were 3.9 and 17.9 µg/ml. Ethyl acetate fraction of ethanol extract of dried and crushed papaya seeds had the highest antioxidant activity which showed the lowest IC_{50} of DPPH scavenging activity[23]. The dried and crushed papaya seeds was extracted using ethanol 95% and the fractionated with petroleum ether, ethyl acetate and n-butanol. The result reported that IC_{50} of DPPH scavenging activity of ethanolic extract, petroleum ether fraction, ethyl acetate fraction, n-butanol fraction and water fraction were 248, 1009, 64, 109, 1628 µg/ml, respectively, while their IC_{50} of ABTS scavenging activity were 2.08, 1.06, 2.48, 4.75, 0.29 m mol Trolox/g DW, respectively.

Table 3: Pearson's correlation coefficient of total phenolic, flavonoid, carotenoid content in different processing of two cultivar of papaya with their IC_{50} of DPPH scavenging activities

Antioxidant activities	Coefficient correlation Pearson (r)		
	TPC	TFC	TCC
IC_{50} DPPH CLA	-0.873**	-0.454^{ns}	-0.943**
IC_{50} DPPH CLB	0.150^{ns}	0.430^{ns}	0.489 ^{ns}
IC_{50} DPPH CLC	-0.878**	0.453^{ns}	0.135 ^{ns}
IC_{50} DPPH SDA	0.216^{ns}	0.518^{ns}	0.554 ^{ns}
IC_{50} DPPH SDB	-0.981**	0.538^{ns}	0.680*
IC_{50} DPPH SDC	0.313^{ns}	-0.990**	0.470^{ns}

CLA = raw papaya *C. papaya* cv. calina, CLB = pickled papaya *C. papaya* cv. calina, CLC = candied papaya *C. papaya* cv. calina, SDA = raw papaya *C. papaya* cv. 1x2, SDB = pickled papaya *C. papaya* cv. 1x2, SDC = candied papaya *C. papaya* cv. 1x2, TPC = total phenolic content, TFC = total flavonoid content, TCC = total carotenoid content, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

Ling[25] revealed that antioxidant activity might be related with the presence of total phenolic content which was included phenolic acid. Cinnamic acid had higher antioxidant activity than benzoic acid[26]. Flesh juice of *C. papaya* cv. Sunrise Solo and Red Lady showed TPC 65 and 53 mg GAE/100 g, respectively[21], while TPC in 80% methanol extract of fresh papaya and pickled papaya were 142 and 45 mg GAE/100 g, respectively[10]. Zhou[23] demonstrated that petroleum ether fraction, ethyl acetate fraction, n-butanol fraction, water fraction and ethanol extract of papaya seeds gave TPC 522, 1945, 832, 276 and 1132 mg GAE/100 g. It was similar to the present study which exposed that TPC in ethanolic extract from different processing (raw papaya, pickled papaya and candied papaya) were 690, 45, 380 mg GAE/100 g, respectively for *C. papaya* cv. calina and 1090, 1240, 610 mg GAE/100 g, respectively for *C. papaya* cv. 1x2. TPC in 50% methanol extract of different ripening stages of papaya fruit 12, 14, 16, 18, 20 weeks were 11.2, 17.43, 35, 39 and 60.4 mg GAE/100 g FW[16]. Maisarah[22] stated that 80 % aqueous methanol of ripe, unripe, seed and leaves of *C. papaya* gave TPC 273, 340, 30, 425 mg GAE/100 g, respectively.

Previous research[23] expressed that TFC in ethanol extract, ethyl acetate fraction, n-butanol fraction and water fraction of papaya seeds were 22.47, 117.48, 32.04 and 4.22 mg RE/g DW, respectively, while TPC in 80% aqueous methanol extract of ripe, unripe, seed and leaves were 93, 53, 60, 333 mg RE/100 g[22]. Study by Addai[16] exhibited that papaya fruit with ripening stages 20 weeks had the highest TFC 38 mg QE/100 g FW compared to ripening stages 12, 14, 16, 18 weeks (22.5, 24.1, 31, 33.2 mg QE/100 g FW, respectively). It was similar to the present study which reported that TFC in ethanolic extract from different processing of *C. papaya* cv. calina (raw papaya, pickled papaya and candied papaya) were 40, 180 and 10 mg QE/100 g, respectively, but contrast with TFC in ethanolic extract from different processing of *C. papaya* cv. 1x2 were 1520, 1810 and 30 mg QE/100 g, respectively.

Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$[12] and negatively high if $-0.61 \leq r \leq -0.97$. Sample which gave the lowest IC_{50} of DPPH scavenging activity showed the highest antioxidant activity. Therefore, the negatively and high correlation will be given in good correlation between TPC, TFC and TCC with IC_{50} DPPH. It means increasing in TFC, TPC and TCC caused increasing in antioxidant activities, which was reported by lower IC_{50} of DPPH scavenging activity.

The previous study[21] demonstrated that TPC in flesh juice of *C. papaya* cv. Sunrise Solo and *C. papaya* cv. Red Lady had negative and high correlation with their IC_{50} of DPPH scavenging activity. TPC and TFC in 80% aqueous methanol extract of ripe, unripe, seed and leaves of papaya had no correlation with their IC_{50} of DPPH scavenging activities, but its TPC had negatively high correlation with their β-carotene bleaching activity[22]. Research by Nurul[10] revealed that TPC and TFC in 80% methanol extract in fresh and pickled papaya had negative and high correlation with their IC_{50} of DPPH scavenging activities. It was similar to the present study (Table 3) it can be seen there were negative and high correlation between TPC and TCC in raw calina papaya extract (CLA) with their IC_{50} of DPPH ($r = -0.873$; $r = -0.943$, $p<0.01$, respectively) and for *C. papaya* cv. 1x2 only TPC in pickled papaya extract (SDB) had negatively and high correlation with its IC_{50} of DPPH ($r = -0.981$, $p<0.01$). Based on this results it can be concluded that phenolic and carotenoid compounds in CLA extracts were the major contributor in its antioxidant activities using DPPH methods. TPC in CLC and SDB extracts were the major contributor in its antioxidant activities using DPPH methods.

TPC in n-hexane flesh extracts of raw calina papaya (CLA1) 3.52 g GAE/100 g was lower than TPC in n-hexane flesh extracts pickled calina papaya (CLB1) 18.79 g GAE/100 g, but IC_{50} of DPPH of CLA1 (253.12 µg/ml) was similar to IC_{50} of DPPH of CLA1 250.01 µg/ml. In processing of pickled papaya used red chilies, which contain capsaicin and capsanthin. Capsaicin is a phenolic compound which soluble in n-hexane. Therefore, it can be seen that capsaicin could increase TPC in pickled papaya, so the TPC in CLB1 (18.79 g GAE/100 g) higher than CLA1 (3.52 g GAE/100 g). Based on the research it can be predicted that capsaicin could increase TPC in pickled papaya (CLB1), but couldn't increase its antioxidant activity. It revealed that capsaicin had low antioxidant, so antioxidant activity of CLB2 by DPPH method was similar to CLB1. TPC in ethyl acetate flesh extract of calina papaya (CLA2) 3.43 g GAE/100 g was

similar with CLA1 3.52 g GAE/100 g, but IC_{50} of DPPH CLA2 was 15.10 µg/ml, which classified as very strong antioxidant and IC_{50} of DPPH CLA1 was 253.12 µg/ml as weak antioxidant. It can be supposed that many phenolic compounds in CLA2 had high antioxidant activity, while many phenolic compounds in CLA1 had low antioxidant activity. Based on the same reason it can be concluded that many phenolic compounds in n-hexane flesh extract of pickled 1x2 papaya (SDB1) had low antioxidant activity, while many phenolic compounds in n-hexane flesh extract of raw 1x2 pepaya (SDA1) had high antioxidant activity.

Phenolic acid had lower antioxidant capacity than flavonoid[26]. Flavonoid which had ortho di OH at C-3'-C-4', OH in C-3, oxo function in C-4, double bond at C-2 and C-3 would give higher antioxidant capacity. The ortho position of di-OH in C-3'-C-4' had the highest influence to antioxidant capacity of flavonoid. The flavonoid aglycones would give higher antioxidant capacity than flavonoid glycosides[26]. It could be seen in Table 1 that TFC in n-hexane flesh extract of pickled calina papaya (CLB1) 10.03 g QE/100 g was higher than TFC in n-hexane flesh extract of raw calina papaya (CLA1) 1.73 g QE/100 g, but IC_{50} of DPPH CLB1 250 µg/ml was similar to IC_{50} of DPPH CLA1 253 µg/ml. Basic reaction in TFC determination is complex reaction between aluminium (III) chloride and ortho di-OH at C-3'-C-4' in ring B of flavonoid, or with oxo function at C-4 and OH at C-3 in ring C, or with oxo function at C-4 and OH at C-5 in ring A. Capsaicin which was used in pickled processing is phenolic compound which has ortho di OH - OCH_3. The fact in TFC determination, aluminium (III) chloride not only react with ortho di OH in flavonoid, but also react with ortho di OH or ortho di OH - OCH_3 in any phenol structure. In this reaction, capsaicin will react with aluminium (III) chloride and was calculated in TFC, so the TFC in CLB1 higher than CLA1. Based on this reaction and the explanation in TPC above, it can be seen capsaicin had low antioxidant. TFC in n-hexane flesh extract of candied papaya (SDC1) 4.34 g QE/100 g was higher than TFC in ethyl acetate flesh extract of candied papaya (SDC2) 3.43 g QE/100 g, but IC_{50} of DPPH of SDC1 was 274 µg/ml which was a weak antioxidant and IC_{50} of DPPH SDC2 49 µg/ml as very strong antioxidant. Based on the result it can be concluded that many flavonoid compounds in SDC2 had high antioxidant activity and many flavonoid compounds in SDC1 had low antioxidant activity.

Carotenoid had antioxidant capacity by scavenging free radical and more double bonds in carotenoid would give higher scavenging free radical capacity[27]. Carotenoid which had more than 7 double bonds gave higher scavenging radical capacity[28]. Beta carotene was used as standard because it had conjugation double bonds which had ability to scavenge free radicals[29]. Previous research[30] reported that increasing in lipophilicity of carotenoid would give higher scavenging radical activity, it means give the lower IC_{50} of DPPH scavenging activity. TCC in n-hexane flesh extract of pickled calina papaya (CLB1) 4.10 g BE/100 g was higher than TCC in n-hexane flesh extracts of raw calina papaya (CLA1) 0.18 g BE/100 g, but IC_{50} of DPPH scavenging activity of CLB1 (250 µg/ml) was almost similar to IC_{50} of DPPH scavenging activity of CLA1 (253 µg/ml). The usage of red chilies for processing pickled papaya can increase TCC in CLB1, because red chilies contain zeaxanthin, capsanthin, capsorubicin and lutein[31], which was included carotenoid compounds and soluble in n-hexane, so totally TCC in CLB1 was higher than TCC in CLA1, but zeaxanthin capsanthin, capsorubicin and lutein from red chilies only give little influence in IC_{50} of DPPH CLB1.

CONCLUSION

Antioxidant activity of sample should be measured by different methods in parallel, because various methods could give different results. Ethyl acetate flesh extracts of two cultivar of papaya (calina papaya and 1x2 papaya) were very strong antioxidant, using DPPH assays. Phenolic and carotenoid compounds in raw calina papaya extracts were the major contributor in its antioxidant activities using DPPH methods. Ethyl acetate flesh extracts of two cultivar of papaya with different processing (raw papaya, pickled papaya and candied papaya) may be exploited as natural antioxidant sources.

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