ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 4, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

ANTIOXIDANT ACTIVITIES FROM VARIOUS LEAVES EXTRACTS OF THREE CULTIVARS OF PAPAYA FROM WEST JAVA-INDONESIA

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Received: 12 April 2016, Revised and Accepted: 26 April 2016

ABSTRACT

Objectives: The goals of this research were to determine antioxidant activity from various leaves extracts of three cultivars of papaya using two methods of antioxidant testing which were 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), and correlation of total phenolic content (TPC), total flavonoid content (TFC), and total carotenoid content (TCC) in various extracts of papaya leaves with their IC_{50} of DPPH and IC_{50} of ABTS.

Methods: Extraction was carried out by reflux method using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH and ABTS assays, determination of TPC, TFC, and TCC were performed by ultraviolet-visible spectrophotometry and correlation with their IC_{50} of DPPH and IC_{50} of ABTS scavenging activities were analyzed by Pearson's method.

Results: The lowest IC_{50} of DPPH scavenging activity 0.84 µg/ml was given by ethanolic leaves extract of calina papaya, whereas ethanolic leaves extract of Bangkok papaya showed the lowest IC_{50} of ABTS scavenging activity 1.79 µg/ml. Ethanolic leaves extract of burung papaya had the highest phenolic content and its ethyl acetate extract had the highest total flavonoid. There were significantly negative correlation between TPC in Bangkok papaya and calina papaya extract with their IC_{50} of DPPH.

Conclusions: All of the leaves extracts from three cultivars of papaya were categorized as very strong antioxidant by DPPH and ABTS methods. Phenolic compounds in Bangkok papaya and calina papaya were the major contributor in IC_{50} of DPPH scavenging activities. DPPH and ABTS gave no linear result in antioxidant activities of leaves extract of three cultivars of papaya.

Keywords: 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, Leaves, Papaya, Three cultivars.

INTRODUCTION

Previous research [1-3] demonstrated that phenolic and flavonoid content could be correlated to their antioxidant activities. Phenolic compound can be found in plants, and they have been reported to have multiple biological effects, including antioxidant and antibacterial activity [4,5]. The excessive of oxidative stress which can cause many diseases can be prevented by consumption of antioxidant. Plants included legumes, banana, sweet potatoes, papaya, tea, cocoa, and coffee contained phenolic and flavonoid compounds [2,4,6-8].

The previous studies [6,9,10] reported that 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), cupric ion reducing antioxidant capacity, and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), methods could be used to determine antioxidant activity in many plants extracts. DPPH ABTS and FRAP can be performed to evaluate antioxidant activity of fruits, vegetables, and food [2,7,10,11]. Leaves of papaya contained many compounds such as flavonoid, tannin, anthraquinone, Vitamin A, and Vitamin C, which can act as antioxidant [12]. Papaya had antioxidant activities by using DPPH and β-carotene linoleate bleaching assays [13].

The goal of this research were to evaluate antioxidant activities in different polarities extracts (n-hexane, ethyl acetate, and ethanol) of leaves from three cultivar of papaya grown in West Java-Indonesia using DPPH and ABTS assays, and correlations of total phenolic content (TPC), total flavonoid content (TFC), and total carotenoid content (TCC) with their antioxidant activities.

MATERIALS AND METHODS

Materials

DPPH, ABTS, gallic acid, quercetin, β-carotene were purchased from Sigma-Aldrich (MO, USA), leaves of three cultivar of papaya. All of the other reagents were analytical grades.

Preparation of sample

Leaves of the three cultivars of papaya (*Carica papaya*) were collected from Bogor, West Java-Indonesia, which were as follows: Bangkok papaya namely as BAN, burung papaya as sample BUR and calina papaya as sample CAL, were thoroughly washed with tap water, sortation while wet, cut, dried, and grinded into powder.

Extraction

About 300 g of powdered samples were extracted by reflux using different polarities solvents. Extraction using n-hexane was repeated 3 times. The remaining residue was then extracted 3 times by using ethyl acetate. Finally, the remaining residue was extracted 3 times using ethanol. Hence, totally there were nine extracts: Three n-hexane extracts (namely BAN1, BUR1, and CAL1), three ethyl acetate extracts (BAN2, BUR2, and CAL2), and three ethanolic extracts (BAN3, BUR3, and CAL3).

IC_{50} DPPH scavenging activity

Preparation of DPPH solution was performed by Blois's method [14] with minor modification. 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 ultraviolet (UV)-Vis spectrophotometer Beckman Coulter DU 720. Methanol was used as a blank. DPPH solution 50 µg/ml was used as control. Ascorbic acid was used as standard. Analysis was carried out in triplicate for standard and each extract. Antioxidant activity of each extract by DPPH method was done by calculating the percentage of antioxidant activity using reduction of DPPH absorbance [15]. IC_{50} of DPPH scavenging activity of each extract can be determined using its calibration curve.

IC_{50} of ABTS scavenging activity

Preparation of ABTSsolution was conducted using Li *et al.* [16] method with minor modification. ABTS diammonium salt solution 7.6 mM in aquadest and potassium persulfate solution 2.5 mM in aquadest were prepared. Each solution was left in dark room for 12 hrs. The two solutions were mixed with 30 minutes incubation, left the mixture in refrigerator for 24 hrs, then diluted in ethanol [17]. Various concentration of each extract were pipetted into ABTS solution 50 µg/ml (volume 1:1) to initiate the reaction for obtaining a calibration curve. The absorbance was read at wavelength 734 nm using UV-Vis spectrophotometer Beckman Coulter DU 720. Ethanol (95%) was used as a blank, ABTS solution 50 µg/ml as control and ascorbic acid as standard. Analysis was done in triplicate for standard and each extract. Antioxidant capacity of each extract by ABTS method was evaluated by calculating the percentage of antioxidant activity using reduction of ABTS absorbance [15]. IC_{50} of ABTS scavenging activity of each extract can be calculated using its calibration curve.

TPC determination

TPC were determined using the modified Folin-Ciolcalteu method which reported by Pourmorad *et al.* [18]. The absorbance was measured at wavelength 765 nm. Analysis was performed in triplicate for each extract. Standard solution of gallic acid (105-200 mg/ml) was used to obtain a calibration curve. TPC was exposed as percentage of total gallic acid equivalent per 100 g extract (g GAE/100 g).

TFC determination

TFC was conducted by Chang *et al.* [19] method with minor modification. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Standard solution of quercetin (36-100 mg/ml) was used to obtain a calibration curve. The TFC was reported as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

TCC determination

TCC was performed using modified method from Thaipong *et al.* [10]. Each extract was diluted in n-hexane [6]. The absorbance was evaluated at wavelength 470 nm. Analysis was carried out in triplicate for each extract. Standard solution of β-carotene (30-100 mg/ml) was used to obtain a calibration curve. The TCC was revealed as the percentage of total β-carotene equivalent per 100 g extract (g BE/100 g).

Statistical analysis

Each sample analysis was performed in triplicate. All of the presented results 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 carried out with SPSS 16 for Windows. Correlation between the TPC, TFC, TCC, and antioxidant activities, and correlation between two antioxidant activity methods were performed using the Pearson's method.

RESULTS

IC_{50} of DPPH and ABTS scavenging activity

The IC_{50} of DPPH and IC_{50} of ABTS scavenging activities in various leaves extracts from three cultivars of papaya using DPPH and ABTS assays were shown in Figs. 1 and 2. IC_{50} of DPPH and IC_{50} of ABTS 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.

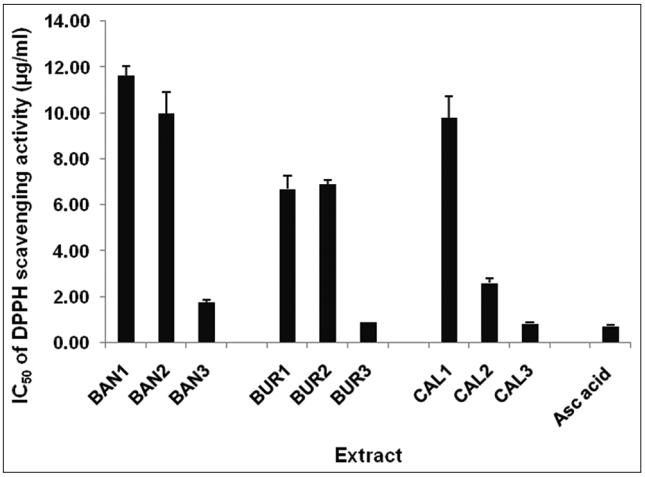

Fig. 1: IC_{50} of 2,2-diphenyl-1-picrylhydrazyl scavenging activities in various leaves extracts of papaya (n=3)

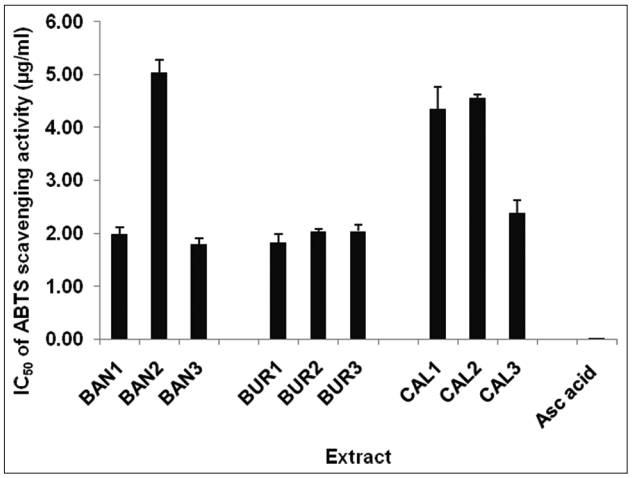

Fig. 2: IC_{50} of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) scavenging activities in various leaves extracts of papaya (n=3)

TPC in various leaves extracts of papaya

TPC among the various leaves extracts were reported in term of GAE using the standard curve equation $y=0.005x-0.198$, $R^2=0.9971$. The TPC in various leaves extracts from three cultivars of papaya had different result in the range of 1.88-5.70 g GAE/100 g. The highest phenolic content (5.70 g GAE/100 g) was given by ethanolic leaves extract of burung papaya (BUR3) and the lowest for its n-hexane leaves extract of calina papaya (CAL1) 1.88 g GAE/100 g (Fig. 3).

TFC in various leaves extracts of papaya

TFC among the various extracts were exhibited in term of QE using the standard curve equation $y=0.007x+0.001$, $R^2=0.9991$. The TFC in various leaves extracts from three cultivars of papaya expressed ranged from 1.82 to 12.63 g QE/100 g (Fig. 4). Ethyl acetate leaves extract of burung papaya (BUR2) had the highest TFC (12.63 g QE/100 g) and the lowest was given by its ethanolic extract (BUR3).

TCC in various leaves extracts of papaya

TCC among the various extracts were reported in terms of b-carotene equivalent using the standard curve equation $y=0.007x-0.002$, $R^2=0.9979$. The TCC in various leaves extracts from three cultivars of papaya gave different results in the range of 5.03-31.68 g BE/100 g (Fig. 5). Ethyl acetate leaves extract of burung papaya (BUR2) showed the highest carotenoid content (31.68 g BE/100 g), whereas the lowest carotenoid (5.03 g BE/100 g) for ethanolic leaves extract of calina papaya (CAL3).

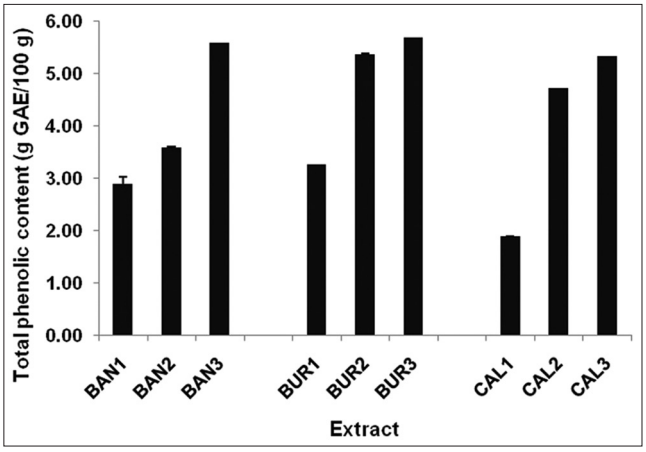

Fig. 3: Total phenolic content in various leaves extracts of papaya (n=3)

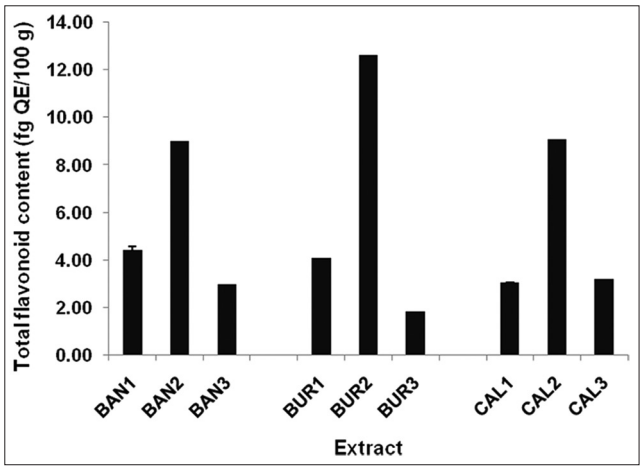

Fig. 4: Total flavonoid content in various leaves extracts of papaya (n=3)

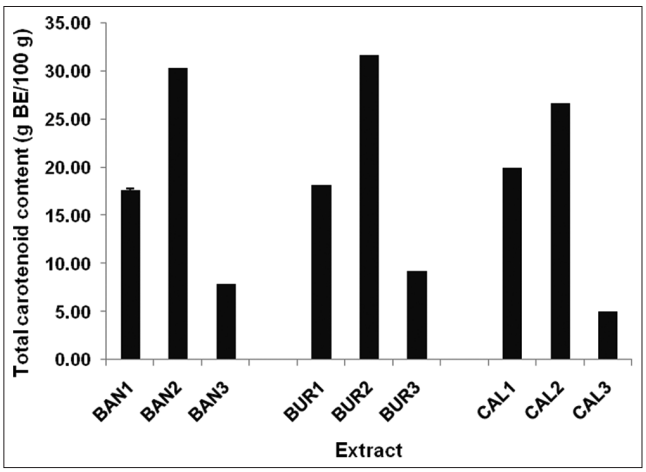

Fig. 5: Total carotenoid content in various leaves extracts of papaya (n=3)

Correlations between TPC, TFC, TCC in various leaves extracts of papaya and IC_{50} of DPPH, IC_{50} of ABTS scavenging activities

Pearson's correlation coefficient between TPC in various leaves extracts from three cultivars of papaya and their antioxidant activities revealed that TPC in Bangkok papaya (BAN) and calina papaya (CAL) had significantly negative correlation with their IC_{50} of DPPH scavenging activities (r=−0.982; r=−0.994, p<0.01, respectively) (Table 1).

DISCUSSION

The previous study [13,20] exposed that papaya had antioxidant capacity. Antioxidant activity of unripe, ripe, seeds, and leaves of papaya (*C. papaya*) had been studied using DPPH and β-carotene linoleate bleaching methods [13]. There was no research regarding antioxidant activity of various leaves extracts (which were n-hexane, ethyl acetate, and ethanol) from three cultivars of papaya using DPPH and ABTS assays.

DPPH free radicals dissolve in methanol give absorption at wavelength 516 nm, while ABTS free radicals dissolve in ethanol have characteristic absorption at l 734 nm. Colors of DPPH and ABTS would be changed when the free radicals were scavenged by antioxidant [16]. DPPH changed from purple to yellow, while ABTS would be changed from turquoise to white.

IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities in various leaves extracts of three cultivars of papaya can be seen in Figs. 1 and 2. The IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities in various leaves extracts of papaya were compared to IC_{50} of ascorbic acid standard. The lowest value of IC_{50} means had the highest antioxidant activity. 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, whereas a weak antioxidant with IC_{50} greater than 150 µg/ml [14].

The present research revealed that IC_{50} of DPPH scavenging activities of various leaves extract from three cultivars of papaya in the range of 0.84-11.62 µg/ml, whereas its IC_{50} of ABTS scavenging activities were 1.79-5.04 µg/ml. It can be seen that antioxidant activities in various leaves extract from three cultivars of papaya can be classified as very strong antioxidant by DPPH and ABTS methods. The highest antioxidant activity which was stated by the lowest IC_{50} of DPPH was given by ethanolic leaves extract of calina papaya (CAL3) 0.84 µg/ml, while ascorbic acid had IC_{50} of DPPH 0.73 µg/ml. Based on their IC_{50} value, it can be concluded that antioxidant activity of CAL3 was similar with ascorbic acid using DPPH method. Study by Zhou [21] demonstrated that ethyl acetate fraction of ethanol extract of dried and crushed papaya seeds gave the lowest IC_{50} of DPPH scavenging activity which showed the highest antioxidant activity. The seeds of dried and crushed papaya was extracted using ethanol 95% and the fractionated with petroleum ether, ethyl acetate and n-butanol. The ethanolic extract, petroleum ether fraction, ethyl acetate fraction, n-butanol fraction, and water fraction had IC_{50} of DPPH scavenging activity 248, 1009, 64, 109, 1628 µg/ml, respectively, whereas their IC_{50} of ABTS scavenging activity were 2.08, 1.06, 2.48, 4.75, 0.29 m mol Trolox/g DW, respectively. Previous study [20] used fresh and pickled papaya for evaluating its antioxidant activity. The fresh papaya and pickled papaya was purchased from different market and no statement regarding cultivars of *C. papaya*. The 80% methanolic extract of fresh papaya had higher antioxidant activity than the pickled papaya, by ß-carotene linoleate bleaching assay. IC_{50} of DPPH scavenging activity of fresh papaya was 4280 µg/ml, whereas IC_{50} of DPPH of pickled papaya cannot be detected because increasing in concentration of 1 to 8 mg/ml of pickled papaya gave scavenging activity below than 50%. Previous research [22] expressed that 50% methanol extract from different ripening stages of papaya fruit had different result. Increasing in antioxidant activity related with increasing in stages of maturity. Papaya with maturity stages 20 weeks had the highest FRAP capacity 180 mg TE/100 g FW, percentage of ABTS and DPPH scavenging activities 68.10% and 72.19%, respectively.

Study by Maisarah *et al.* [13] which studied regarding antioxidant activities of 80% aqueous methanol of ripe and unripe, young leaves, and seed of *C. papaya* reported that their IC_{50} of DPPH of scavenging activities were 6500, 4300, 7800, and 1000 µg/ml, respectively, which

Table 1: Pearson's correlation coefficient of total phenolic, flavonoid, carotenoid content in various leaves extracts from three cultivars of papaya with their IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities

Antioxidant activity	Pearson's correlation coefficient (r)					
	TPC	TFC	TCC	IC_{50} ABTS BAN	IC_{50} ABTS BUR	IC_{50} ABTS CAL
IC_{50} DPPH BAN	−0.982**	0.558ns	0.723*	0.414ns		
IC_{50} DPPH BUR	−0.576ns	0.681*	0.816**		−0.372ns	
IC_{50} DPPH CAL	−0.994**	−0.352ns	0.388ns			0.553ns
IC_{50} ABTS BAN	−0.319ns	0.978**	0.919**			
IC_{50} ABTS BUR	0.712*	0.189ns	0.037ns			
IC_{50} ABTS CAL	−0.553ns	0.537	0.948**			

IC_{50} DPPH: IC_{50} DPPH scavenging activity, IC_{50} ABTS: IC_{50} ABTS scavenging activity, BAN: Bangkok papaya, BUR: Burung papaya, CAL: Calina papaya, ns: Not significant, *Significant at p<0.05, **Significant at p<0.01. DPPH: 2,2-diphenyl-1-picrylhydrazyl, ABTS: 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid), TPC: Total phenolic content, TFC: Total flavonoid content , TCC: Total carotenoid content

was categorized as weak antioxidant, whereas their percentage of β-carotene linoleate bleaching activity were 88.12%, 90.67%, 90.01%, and 58.97%, respectively. It was contrary with the present study which reported that the ethanolic leaves extract of three cultivars of papaya (Bangkok papaya, burung papaya, and calina papaya) had IC_{50} of DPPH of scavenging activities 1.75, 0.91, 0.84 µg/ml, respectively, and classified as very strong antioxidant. Previous research [23] stated that IC_{50} of DPPH of flesh juice of two different cultivars of papaya (Sunrise Solo and Red Lady) were 52.1 and 63.4 ml PCJ/g DPPH, whereas ascorbic acid and BHT had IC_{50} of DPPH 3.9 and 17.9 µg/ml.

Previous research [16] exposed that antioxidant activity can be related with the TPC which was included phenolic acid. Cinnamic acid had higher antioxidant activity than benzoic acid [24]. Study by Imaga [12] expressed that aqueous-methanol (1:3) leaves extract of papaya contained alkaloid, flavonoid, glycoside, tannin, saponin, anthraquinone, and Vitamin A and C. The presence of flavonoid, tannin, anthraquinone, and Vitamin A and C might be contributed in antioxidant activity of papaya leaves extract. In the present study, it was reported that TPC in ethanolic leaves extract of Bangkok papaya, burung papaya, and calina papaya were 5.58, 5.70, and 5.34 g GAE/100 g, respectively. It was different from with the previous research [20] which demonstrated that TPC in 80% methanol extract of fresh papaya and pickled papaya were 142 and 45 mg GAE/100 g, respectively, whereas TPC in flesh juice of *C. papaya* cv. Sunrise Solo and Red Lady were 65 and 53 mg GAE/100 g, respectively [23]. Study by Maisarah *et al*. [13] reported that TPC in 80% aqueous methanol of seed, leaves, ripe and unripe of *C. papaya* were 30, 425, 273, and 340 mg GAE/100 g, respectively. Previous studies expressed that ethanol extract, petroleum ether fraction, ethyl acetate fraction, n-butanol fraction, and water fraction of papaya seeds gave TPC 1132, 522, 1945, 832, and 276 mg GAE/100 g [21], whereas 50% methanol extract of different ripening stages of papaya fruit 12, 14, 16, 18, and 20 weeks showed TPC 11.2, 17.43, 35, 39, and 60.4 mg GAE/100 g FW, respectively [22].

Zhou *et al.* [21] stated that ethanol extract, ethyl acetate fraction, n-butanol fraction and water fraction of papaya seeds gave TFC 22.47, 117.48, 32.04, and 4.22 mg RE/g DW, respectively, whereas Maisarah *et al.* [13] figured that 80% aqueous methanol of seed, leaves, ripe, and unripe extracts had TFC 60, 333, 93, and 53 mg RE/100 g. It was different from with the present study which showed that TFC in ethanolic leaves extract of Bangkok papaya, burung papaya, and calina papaya were 2.95, 1.82, and 3.19 g QE/100 g, respectively. Previous research [22] revealed that papaya fruit with ripening stages 12, 14, 16, 18, and 20 weeks had TFC 22.5, 24.1, 31, 33.2, and 38 mg QE/100 g FW, respectively.

Thaipong *et al.* [10] exposed that Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$ and negatively high if $-0.61 \leq r \leq -0.97$. The lowest IC_{50} of DPPH and IC_{50} of ABTS scavenging activity showed the highest antioxidant activity. Hence, the good correlation between TPC, TFC, and TCC with IC_{50} of DPPH or IC_{50} of ABTS if significantly negative correlation [25]. It means increasing in TFC, TPC, and TCC caused increasing in antioxidant activities, which was expressed by lower IC_{50} of DPPH scavenging activity and or IC_{50} of ABTS scavenging activity.

Previous study by Ozkan *et al*. [23] reported that TPC in flesh juice of *C. papaya* cv. Sunrise Solo and *C. papaya* cv. Red Lady had significantly negative correlation with their IC_{50} of DPPH scavenging activity. TPC and TFC in 80% aqueous methanol extract of seed, leaves, ripe, and unripe of papaya gave no correlation with their IC_{50} of DPPH scavenging activities, but its TPC had negative and significant correlation with their ß-carotene bleaching activity [13]. It was different from with the present study which expressed that TPC in leaves extract of Bangkok papaya and calina papaya had negative and significant correlation with their IC_{50} of DPPH scavenging activities ($r=-0.982$; $r=-0.994$, $p<0.01$, respectively). It can be predicted that antioxidant activities of leaves extract of Bangkok papaya and calina papaya can be estimated by determining its TPC. Research by Nurul [20] revealed that TPC and TFC in 80% methanol extract in fresh and pickled papaya had significantly negative correlation with their IC_{50} of DPPH scavenging activities.

Tannins, phenolic acid, flavonoid, qoumarine, and quinone are included in phenolic groups. Flavonoid compound, which have OH in A ring and or B ring, will be included in phenolic groups. Flavonoid had higher antioxidant activity than phenolic acid. The flavonoid glycosides would give lower antioxidant activity than flavonoid aglycones [24]. Flavonoid which had ortho di OH at C-3'-C4', OH at C-3, oxo function at C-4, double bond at C-2 and C-3 have high antioxidant activity. The ortho with di OH position at C-3'-C-4' had the highest influence to antioxidant activity of flavonoid. In Fig. 3, it could be seen that TPC in ethyl acetate leaves extract of burung papaya (BUR2) 5.38 g GAE/100 g was similar with ethanolic leaves extract of calina papaya (CAL3) 5.34 g GAE/100 g, but IC_{50} of DPPH scavenging activity of CAL3 (0.84 µg/ml), which was similar with IC_{50} of DPPH of ascorbic acid (0.73 µg/ml) and smaller than IC_{50} of DPPH scavenging activity of BUR2 (6.89 µg/ml). It can be supposed that many phenolic compounds in CAL3 which had influence high antioxidant capacities, whereas only a little phenolic compounds in BUR2 with high antioxidant activities. TFC in n-hexane leaves extract of calina papaya (CAL1) 3.02 g QE/100 g was smaller than TFC in ethanolic leaves extract of calina papaya (CAL3) 3.19 g QE/100 g, but IC_{50} of DPPH scavenging activity of CAL3 (0.84 µg/ml) was smaller than IC_{50} of DPPH of CAL1 (9.81 µg/ml). In TFC determination, a flavonoid compound will form a complex with aluminum (III) chloride if a flavonoid has ortho di OH at C-3'-C4' or OH at C-3 and oxo function at C-4, or OH at C-5 and oxo fuction at C-4. The weakness of this method is a complex with aluminum (III) chloride also will be happened in any phenolic compound which has ortho di OH-OCH_3 at C3'-C-4' or ortho di OCH_3 at C-3'-C-4'. Based on this data, it can be predicted that CAL1 contained many compound included flavonoid that has ortho di OH-OCH3 at C3'-C-4' or ortho di OCH3 at C-3'-C-4' which soluble in n-hexane solvent, but had no or low influence in antioxidant activities. In contrast, almost all of the flavonoids in CAL3 was flavonoid that had ortho di OH at C-3'-C4', OH at C-3, oxo function at C-4, double bond at C-2 and C-3.

Carotenoid have antioxidant capacity by scavenging free radical [26] and carotenoid which contain more double bonds will give higher scavenging free radical activity. Research by Beutner *et al*. [27]

expressed that carotenoid would give higher scavenging radical activity if contain greater than 7 double bonds. ß-carotene was used as standard because it has conjugation double bonds which have the ability to scavenge free radicals [28]. Increasing in lipophilicity of carotenoid would increase antioxidant activity which was revealed by lower IC_{50} of DPPH scavenging activity [29]. TCC in n-hexane leaves extract of burung papaya (BUR1) 18.14 g BE/100 g was lower than TCC in ethyl acetate leaves extract of burung papaya (BUR2) 31.68 g BE/100 g, but IC_{50} of DPPH and IC_{50} of ABTS scavenging activities of BUR1 was similar to with BUR2. It can be predicted that BUR1 contained many carotenoid compound with more than 7 double bonds which soluble in n-hexane solvent and had high antioxidant activities, whereas BUR2 contained many carotenoid with maximum 7 double bonds.

DPPH and ABTS assays have the same principle which was electron transfer, but in the present study showed no correlation between IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities in all of leaves extracts of three cultivars of papaya. It could be seen that IC_{50} of DPPH scavenging activities of leaves extract of Bangkok papaya, burung papaya, and calina papaya demonstrated no linear result with their IC_{50} of ABTS.

CONCLUSION

Antioxidant activity of sample should be determined using different methods in parallel, because various methods could give different results. All of leaves extracts of three cultivars of papaya (Bangkok papaya, burung papaya, and calina papaya) were very strong antioxidant using DPPH and ABTS assays. TPC in leaves extracts of Bangkok papaya and calina papaya had significantly negative correlation with their IC_{50} of DPPH scavenging activities. Phenolic compounds in leaves extracts of Bangkok papaya and calina papaya were the major contributor in IC_{50} of DPPH scavenging activity. There were no correlation between IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging of all of extracts sample. Bangkok papaya, burung papaya, and calina papaya may be exploited as natural antioxidant sources to prevent oxidative stress.

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