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Research Article

In Vitro Antioxidant Activity of Different Organs Extracts of Corn Grown in Cimahi-West Java-Indonesia

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ABSTRACT

The aims of this research were to determine antioxidant activity from different polarities organs extract of corn using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and CUPRAC (Cupric Reducing Antioxidant Capacity) and correlation of total phenolic, flavonoid and carotenoid content in different polarities extracts of corn organs with their IC_{50} of DPPH and IC_{50} of CUPRAC antioxidant activities. Extraction was conducted by reflux using different polarities solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH and CUPRAC assays, determination of total phenolic, flavonoid and carotenoid content were performed by UV-visible spectrophotometry and its correlation with IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities were analyzed by Pearson's method. All of corn leaves extracts (n-hexane, ethyl acetate and ethanolic extracts) were strong to very strong antioxidant, using DPPH assay. Flavonoid and carotenoid compounds in corn leaves extracts were the major contributor in antioxidant activity by DPPH method. DPPH and CUPRAC methods gave no linear result for antioxidant activity of corn cob, corn leaves and corn husk extracts.

Keywords: Antioxidant, DPPH, CUPRAC, organs, corn

INTRODUCTION

Plants included papaya, tea, legumes, rice, citrus, guava and onion consisted of phenolic and flavonoid compounds[1-7]. Phenolic compounds have been reported to have multiple biological effects, included antibacterial and antioxidant activity[8,9] which commonly found in plants. Oxidative stress condition lead to the excessive of free radicals in body which is related with many degenerative diseases such as cancer and hypercholesterolemia. Free radical can be scavenged by antioxidant[2,10]. Previous researches[5,11,12] expressed that their antioxidant activities could be correlated to phenolic and flavonoid content. Antioxidant activity of vegetables, fruits and food can be determined using DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)[6,13]. Previous studies[6,14] exhibited that DPPH, CUPRAC, FRAP and ABTS methods could be used to evaluate antioxidant activity in many plants extracts. The other research[15] exposed that corn had antioxidant activities by using DPPH, FRAP and ABTS assays. The objectives of this research were to investigate antioxidant activities of different polarities organs extract (n-hexane, ethyl acetate and ethanol) of corn grown in Cimahi - West Java-Indonesia using DPPH and CUPRAC assays, and correlations of total phenolic, flavonoid and carotenoid content with their antioxidant activities.

MATERIALS AND METHODS

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl), CUPRAC (Cupric Reducing Antioxidant Capacity), neocuproine, cupric (II) chloride, gallic acid, quercetin, beta carotene were purchased from Sigma-Aldrich (MO, USA), organs of corn. All of other reagents were analytical grades.

Preparation of sample

Organs of corn (*Zea mays*) were: corn cob namely as COB, corn leaves as LEV and corn hull as HUL were collected from Cimahi- West Java, were thoroughly washed with tap water, sorted while wet, cut, dried and grinded into powder.

Extraction

Extraction of three hundred gram of powdered sample was performed by reflux using different polarities 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, there were nine extracts: three n-hexane extracts (namely COB1, LEV1 and HUS1), three ethyl acetate extracts (COB2, LEV2 and HUS2) and three ethanolic extracts (COB3, LEV3 and HUS3).

Total phenolic content (TPC)

Total phenolic content determination was conducted using Folin-Ciolcalteu reagent[16]. The absorbance was read at wavelength 765 nm. Analysis was conducted in triplicate for each extract. Gallic acid standard solution (105-200 μg/ml) was used to obtain a calibration curve.

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Total phenolic content was figured as percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

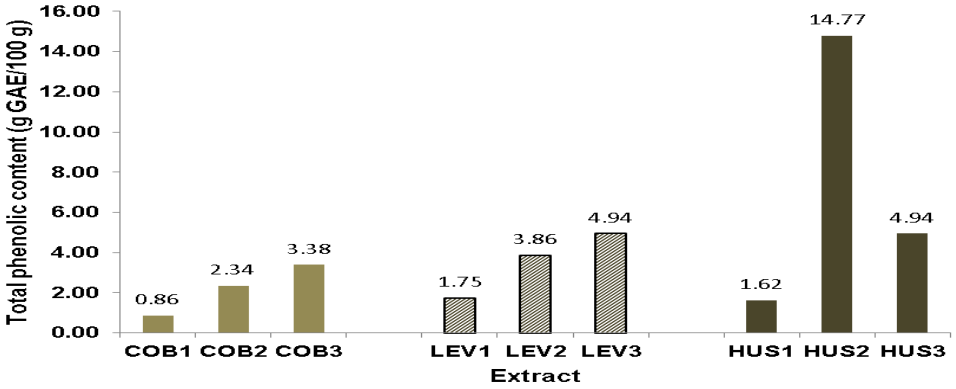

Figure 1: Total phenolic content in corn organs extracts

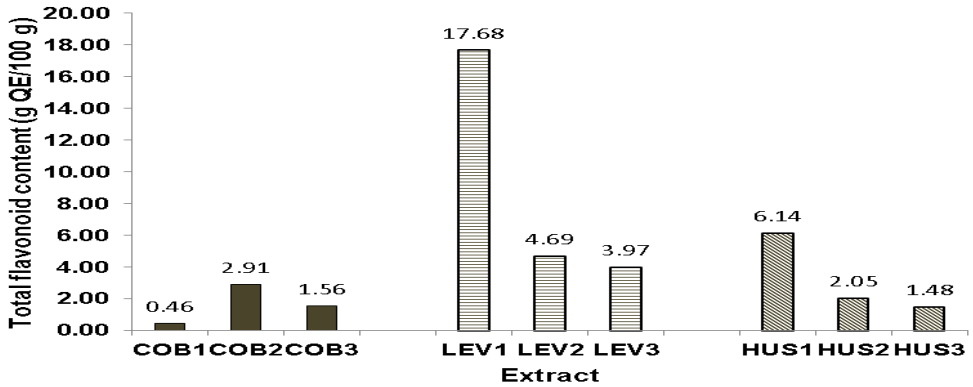

Figure 2: Total flavonoid content in corn organs extracts

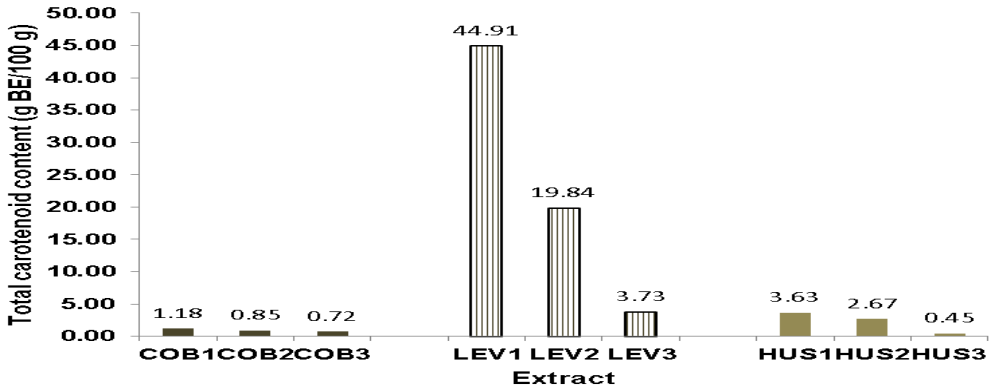

Figure 3: Total carotenoid content in corn organs extracts

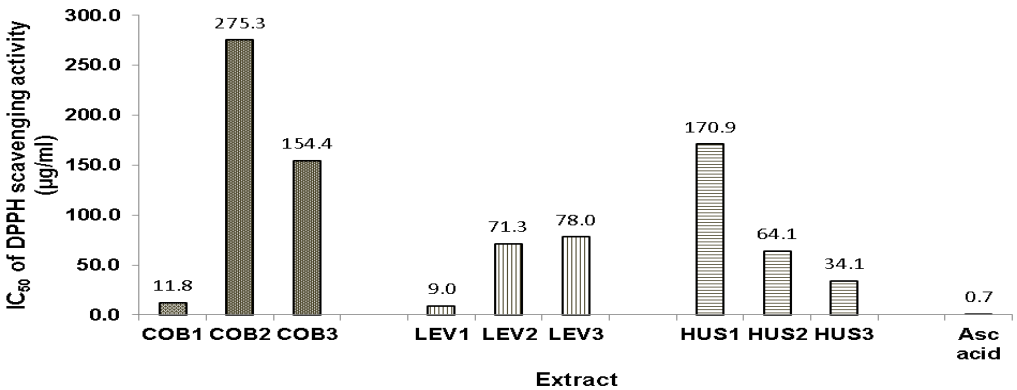

Figure 4: IC_{50} of DPPH scavenging activities in corn organs extract

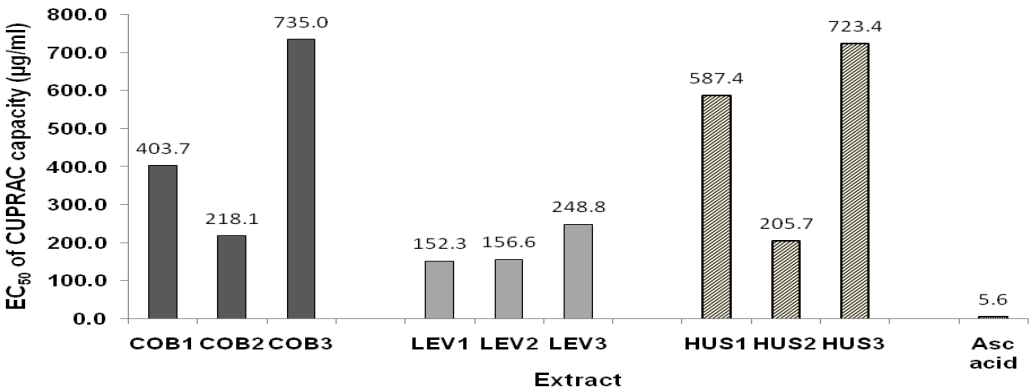

Figure 5: EC_{50} of CUPRAC capacities in corn organs extract

Total flavonoid content (TFC)

Modification of Chang *et al.*[17] was used for calculating total flavonoid content. The absorbance was measured at wavelength 415 nm. Analysis was done in triplicate for each extract. Quercetin standard solution (36-100 µg/ml) was used to obtain a calibration curve. The total flavonoid content was expressed as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Total carotenoid content (TCC)

Total carotenoid content was calculated using method from Thaipong *et al*[6] with minor modification. Each extract was diluted in n-hexane[18]. The absorbance was measured at wavelength 470 nm. Analysis was performed in triplicate for each extract. Beta carotene standard solution (30-100 µg/ml) was used to obtain a calibration curve. The total carotenoid content was reported as percentage of total beta carotene equivalent per 100 g extract (g BE/100 g).

DPPH scavenging activity

Blois's method[10] with minor modification was used in preparing DPPH solution. Various concentration of each extract were mixed with DPPH solution 50 µg/ml (volume 1:1) to obtain a calibration curve. The absorbance was seen after 30 minutes incubation at wavelength 515 nm by using UV-Vis spectrophotometer Beckman Coulter DU 720. Methanol was used as a blank, DPPH 50 µg/ml as control and ascorbic acid as standard. Analysis was performed 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[19]. IC_{50} of DPPH scavenging activity of each extract can be calculated using its calibration curve.

CUPRAC capacity

Preparation of CUPRAC solution was adopted from method of Apak *et al*.[20]. The CUPRAC solution was prepared in ammonium acetate buffer pH 7. Each extract were prepared in various concentrations and pipetted into CUPRAC 50 µg/ml (1:1) to initiate the reaction for obtaining a calibration curve. After 30 minutes incubation, the absorbance was read at wavelength 450 nm by using UV-Vis spectrophotometer Beckman Coulter DU 720. Ammonium acetate buffer was used as a blank, CUPRAC 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 was evaluated based on increasing in Cu (I)-neocuproine absorbance by calculating percentage of antioxidant capacity [20]. EC_{50} of CUPRAC capacity of each extract can be calculated using its calibration curve.

Statistical Analysis

Each sample analysis was conducted in triplicate. All of 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 total phenolic, flavonoid, carotenoid content and antioxidant activities, and correlation between two antioxidant activity methods were evaluated using the Pearson's method.

RESULTS

Determination of TPC in corn organs extract

TPC among the various extracts were exposed in term of gallic acid equivalent using the standard curve equation $y = 0.005 x - 0.1983$, $R^2 = 0.9971$. The TPC in various extracts of corn organs had different results, varied from 0.86 to 14.77 g GAE/100 g. The highest phenolic content (14.77 g GAE/100 g) was given by ethyl acetate extract of corn husk (HUS2) and the lowest given by n-hexane extract of corn cob (COB1).

Determination of TFC in corn organs extract

TFC among the various extracts were stated in term of quercetin equivalent using the standard curve equation $y = 0.0076 x + 0.0012$, $R^2 = 0.9991$. The TFC in various extracts of corn organs gave different result ranging from 0.46 to 17.68 g QE/100 g (Fig 2). N-hexane extract of corn leaves (LEV1) had the highest total flavonoid content (17.68 g QE/100 g), while n-hexane extract of corn cob (COB1) gave the lowest total flavonoid content (0.46 g QE/100 g).

Determination of TCC in corn organs extract

TCC among the various extracts were demonstrated in term of beta carotene equivalent using the standard curve equation $y = 0.0074x - 0.0025$, $R^2 = 0.9979$. The TCC in various extracts of corn organs had result in the range of 0.45 – 44.91 g BE/100 g (Fig 3). The highest carotenoid content (44.91 g BE/100 g) was figured by n-hexane extract of corn leaves (LEV1), while the lowest carotenoid (0.45 g BE/100 g) for ethanolic extract of corn husk (HUS3).

Table 1: Pearson's correlation coefficient of total phenolic, flavonoid, carotenoid content in various corn organs extracts with their IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities

Antioxidant activities	Coefficient correlation Pearson (r)					
	TPC	TFC	TCC	EC_{50} CUPRAC COB	EC_{50} CUPRAC LEV	EC_{50} CUPRAC HUS
IC_{50} DPPH COB	0.623 ns	0.994**	-0.732*	-0.309 ns		
IC_{50} DPPH LEV	0.964**	-0.998**	-0.951**		0.606 ns	
IC_{50} DPPH HUS	-0.53 ns	0.992**	0.856**			0.057 ns
EC_{50} CUPRAC COB	0.551 ns	-0.407	-0.416 ns			
EC_{50} CUPRAC LEV	0.783*	-0.572 ns	-0.820**			
EC_{50} CUPRAC HUS	-0.876**	0.153 ns	-0.463 ns			

IC_{50} DPPH = IC_{50} of DPPH scavenging activity, EC_{50} CUPRAC = EC_{50} of CUPRAC capacity, COB = corn cob, LEV = corn leaves, HUS = corn husk, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

DPPH scavenging activity and CUPRAC capacity

Antioxidant activities by DPPH and CUPRAC assays in various extracts of corn organs were given in Fig 4 and Fig 5. IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacity of each extract were compared to IC_{50} and EC_{50} of ascorbic acid as standard. The lowest value of IC_{50} means had the highest antioxidant activity.

Correlations between total phenolic, flavonoid, carotenoid content in various corn organs extracts and IC_{50} of DPPH scavenging activities, EC_{50} of CUPRAC capacities

TPC in extracts of corn husk had negative and significant correlation with their EC_{50} of CUPRAC capacities ($r = -0.876$, $p<0.01$. TFC and TCC in corn leaves extract gave significantly negative correlation with their IC_{50} of DPPH scavenging activities ($r = -0.998$; $r = -0.951$, $p<0.01$, respectively) (Table 1).

DISCUSSION

The previous research[21,22] reported that corn had antioxidant capacity. There was no study regarding antioxidant activity of various organs extracts (which were n-hexane, ethyl acetate and ethanol) of corn from Cimahi-West Java- Indonesia using DPPH and CUPRAC assays. Total phenolic content can be correlated with antioxidant activity[2,3,11,12]. Cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid[23]. Study by Sarepoua *et al*.[21] regarding TPC, TFC, TAC and antioxidant activity in corn silk of ten varieties of corn revealed that 95% ethanol corn silk extract of purple wax corn (PWC2 and PWC1 varieties) had the highest TPC (11.71 and 11.64 g GAE/100 g, respectively). It was similar to the present study which figured that TPC in ethanolic corn husk extract was 14.77 g GAE/100 g. Previous research[24] stated that TPC in 80% ethanolic extract of stigma maydis (corn silk) was 400 mg GAE/100 g which was higher than 80 % ethanolic extract of corn husk and corn cob. It was similar to TPC result of the other solvents (water, 50% ethanol, 50% methanol, 80% methanol and ethyl acetate extracts) which figured that TPC in corn silk had the highest TPC compared to corn cob and corn husk. Ku *et al*.[25] studied regarding TPC in forty corns with different kernel phenotype expressed that WX3 sample with light yellow kernel phenotype gave the highest TPC 467 μg GAE/ml. Research by Khampas *et al*.[15] expressed that TPC in corn kernel of 13 varieties of corn ranging from 2.1 to 4.5 mg GAE/g for dry kernel and 1.3 to 3.1 mg GAE/g for fresh kernel. The highest TPC was given by WP genotype of corn for dry kernel and SWWY genotype for fresh kernel. Balasubramanian[22] studied regarding TPC in different time after sowing. The result figured that sample 10 days after sowing had the highest TPC (5 mg/g leaves) compared to 5, 15, 20, 25 and 30 days after sowing. Dong *et al*.[24] stated that 80% ethanolic extract of corn cob showed the highest TFC (1.15 RE g/100 g) compared to TFC in 80 % ethanolic extract of corn silk (0.9 g RE/100 g) and corn husk (0.8 g RE/100 g). It was similar to the present study which exposed that TFC in ethanolic extract of corn cob, cob leaves and corn husk were 1.56, 3.97 and 1.48 g QE/100 g, respectively. The previous researches exposed that TFC in 95% ethanol of corn silk of PWC1 and PWC2 had the highest TFC (88.8 μg RE/g and 83.4 μg RE/g, respectively) compared to the other varieties[21], TFC in ten days after sowing was 4.5 mg/g leaves higher than 5, 15 and 20 days after sowing[22], 60 % methanol containing 1% HCl leaves extract of corn with purple kernel phenotype (C29 sample) gave the highest TFC (515 μg naringin equivalent/ml) among 40 kernel phenoptype[25]. Corn kernel and corn cob of purple corn contained anthocyanin were cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside[26]. Many studies determined total anthocyanin content (TAC) in corn. Previous researches stated that corn kernel of WP variety had the highest TAC 1.52 mg cyanidin-3-glucoside equivalent (C3GE)/g dry weight (DW) and 1.65 mg C3GE/g fresh weight (FW) among corn kernel from 13 corn varieties[15], 95% ethanol corn silk extracts of PWC2 and PWC1 varieties had the highest TAC 72.9 and 68.7 μg C3GE/g among corn silk extracts of ten varieties[21]. Khu *et al*.[25] demonstrated that the highest TAC was given by 60% methanol containing 1 % HCl of C29 sample with purple kernel phenotype (90 μg C3GE/g). In the previous study[22] reported that TCC in in ten days after sowing gave 32 mg/g leaves, which was the highest compared to 15, 20 and 25 days. Khampas *et al*.[15] expressed that corn kernel of FC genotype showed the highest TCC 35.6 μg/g DW and 23.3 μg/g FW among 13 corn varieties. C11 sample with yellow kernel phenotype exhibited the highest TCC (564 μg/100 g)[25]. It was

contrary with the present study which reported that TCC in ethanolic extract of corn cob (COB3), corn leaves (LEV3) and corn husk (HUS3) were 0.72, 3.73 and 0.45 g BE/100 g, respectively, while the n-hexane extract of corn cob, corn leaves and corn husk gave higher TCC (1.18, 44.91 and 3.63 g BE/100 g, respectively). The IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities in various organs extracts compared to IC_{50} or EC_{50} of ascorbic acid standard. The lowest IC_{50} means showed the highest antioxidant activity. Sample which had IC_{50} or EC_{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[10]. The IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities in various organs extract of corn using DPPH and CUPRAC assays were given in Fig 4 and Fig 5. Research by Dong *et al*.[24] water extract of corn silk had the highest DPPH scavenging activities (22 µmol TE/100 g), followed by water extract of corn husk (20 µmol TE/100 g). It was contrary with the present study which revealed antioxidant activity using IC_{50} of DPPH value. The highest antioxidant activity would give the lowest IC_{50} of DPPH value, which expressed by n-hexane extract of corn leaves LEV1 (9.0 µg/ml). N-hexane extract of corn cob (COB1) and corn leaves (LEV1), also ethanolic exract of corn husk (HUS3) can be categorized as very strong antioxidant because their IC_{50} of DPPH (11.8, 9.0 and 34.1 µg/ml, respectively) were lower than 50 µg/ml. Previous studies[15,21] exposed antioxidant activity by DPPH method using percentage of DPPH scavenging activity. The 95% ethanolic corn silk extract of PWC1 and PWC2 gave the highest percentage of DPPH scavenging activities (75.6 % and 74.8 %, respectively) [21] and corn kernel of WP1 variety had the highest percentage of DPPH scavenging activity (68.9%) for fresh kernel and WP variety (62.8 %) for dry kernel[15]. The other antioxidant activity method was performed in the present study using CUPRAC method. The lowest EC_{50} of CUPRAC was given by n-hexane extract of corn leaves LEV1 (152.3 µg/ml), which was similar to DPPH method it gave the lowest IC_{50} of DPPH. Study by Ku *et al*.[25] expressed that C24 sample with light yellow kernel phenotype showed the highest antioxidant capacity (12.7 mmol Trolox/g) by FRAP method and C29 sample with purple kernel phenotype gave the highest antioxidant activity (14.4 mmol Trolox/g) by ABTS assay among forty kernel phenotype of corns. Previous research[24] figured that 80% ethanol corn cob extract showed the highest antioxidant activity (260 µmol TE/100 g) by ABTS method and 80% ethanol extract of corn silk had the highest antioxidant capacity (980 µmol TE/100 g) by FRAP method, compared to water extract, 50% ethanol extract, 50% methanol extract, 80% methanol extract and ethyl acetate extract of three different parts of corn (corn husk, corn cob and corn silk). Pearson's correlation coefficient was negatively significant if $-0.61 \leq r \leq -0.97$ and positively high if $0.61 \leq r \leq 0.97$[6]. Antioxidant activity was expressed in IC_{50} of DPPH scavenging activity and EC_{50} of CUPRAC capacity. Sample which had the lowest IC_{50} of DPPH scavenging activity and EC_{50} of CUPRAC capacity had the highest antioxidant activity. Increasing in TPC, TCC and TCC caused increasing in antioxidant activities, which was stated by small value of IC_{50} of DPPH scavenging activity and or EC_{50} of CUPRAC capacity. So the good correlation between TPC, TFC and or TCC with their IC_{50} of DPPH or EC_{50} of CUPRAC if significantly negative correlation[27]. Data in Table 1 demonstrated that there were negatively significant correlation between TFC and TCC in corn leaves extracts with their IC_{50} of DPPH scavenging activities ($r = -0.998$; $r = -0.951$, $p<0.01$, respectively). TPC in corn husk extracts had significantly negative correlation with their EC_{50} of CUPRAC capacities ($r = -0.876$, $p<0.01$). Based on the result it can be concluded flavonoid and carotenoid compounds were the major contributor in antioxidant activities of corn leaves extracts using DPPH method, whereas phenolic compounds were the major contributor in corn husk extracts using CUPRAC method. It means antioxidant capacities of corn leaves extracts using DPPH method can be estimated indirectly by determining TFC and TCC. It was different to the previous studies which expressed antioxidant activity by DPPH, FRAP and ABTS as mmol trolox equivalent (TE)/g or µmol TE/g or percentage of scavenging activity[15,24]. In these cases the good correlation between TPC, TFC TAC or TCC with their antioxidant activities were the positively and significant correlation. Previous researches stated that TPC and TFC in 60% methanol containing 1% HCl from forty samples of corn with different kernel phenotype had positive correlation with their FRAP capacity which was reported as mmol TE/g ($r = 0.840$, $p<0.01$; $0 = 0.746$, $p<0.05$, respectively)[25], TPC in corn cob, corn leaves and corn husk extracts gave positive and significant correlation with their DPPH and ABTS which was expressed as µmol TE/100 g ($r = 0.709$, $p<0.05$; $r = 0.871$, $p<0.01$, respectively), while their TFC had positive correlation with their FRAP which exposed as µmol Fe (II)/100 g[24], TPC and TFC in 95% ethanol corn silk extracts of ten varieties of corn had positive and significant correlation with percentage of DPPH scavenging activity ($r = 0.71$; $r = 0.63$ $p<0.05$, respectively)[21] and also research by Khampas *et al*.[15] which figured that TPC in fresh and dry corn kernel extracts from 13 varieties of corn had significant and positive correlation with their FRAP (as µmol Fe (II)/g), ABTS (as µmol TE/g) and DPPH (percentage of DPPH scavenging activity). Colors of DPPH would be changed from purple to yellow when the free radicals were scavenged by antioxidant[10,28]. The DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors give absorption at wavelength 515-520 nm. Reagent of CUPRAC is $CuCl_2$ which was combined with neocuproine in ammonium acetate buffer pH 7. Cu (II) will be reduced to Cu (I). Complex Cu (I) – neocuproine gives yellow color and show characteristic absorption at wavelength 450 nm[20]. Intensity of yellow color depends on amount of Cu (II) that is reduced to Cu (I). If a sample reduces Cu (II) to Cu (I), at the same time

it will be oxidized, so that sample can act as antioxidant. Sample will act as antioxidant in CUPRAC assay if sample had reduction potential lower than reduction potential of Cu (II)/Cu (I) which was 0.159 V. Flavonoid, coumarin, quinone, tannin and phenolic acid were included in phenolic groups. 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 di OH at C-3'-C-4' had the highest influence to antioxidant activity of flavonoid. The flavonoid glycosides would give lower antioxidant activity than flavonoid aglycones. Flavonoid had greater antioxidant activity than phenolic acid[23]. In Figure 1 it could be seen that TPC in ethanol corn leaves extract (LEV3) 4.94 g GAE/100 g was higher than ethanol corn husk extract (HUS3) 4.94 g GAE/100 g, but IC_{50} of DPPH scavenging activity of LEV3 (78 µg/ml) which was classified as strong antioxidant higher than IC_{50} of DPPH of HUS3 (34 µg/ml) as very strong antioxidant. Cinnamic acid has higher antioxidant activity than benzoic acid[23]. Dong *et al*.[24] stated that corn contained phenolic compounds such as gallic acid, procatechuic acid, chlorogenic acid, caffeic acid, ferulic acid, rutin, resveratrol and kaempferol. It can predicted that many phenolic compounds in HUS3 had high antioxidant activity, might be cinnamic acid such as chlorogenic acid, ferulic acid and caffeic acid, while many phenolic compounds in LEV3 which had lower antioxidant activity compared to phenolic compounds in HUS3, such as gallic acid and protocatechuic acid which was belong to benzoic acid. TPC in n-hexane corn leaves extract (LEV1) 1.75 g GAE/100 g was similar to TPC in n-hexane corn husk extract (HUS1) 1.62 g GAE/100 g, but EC_{50} CUPRAC of LEV1 (152.3 µg/ml) was lower than EC_{50} CUPRAC of HUS1 (587.4 µg/ml). Based on the result it can be seen that many phenolic compounds in LEV1 has reduction potential lower than reduction potential of Cu (II)/Cu I 1.59 V, whereas many phenolic compounds in HUS1 with reduction potential higher than 1.59 V. TFC in ethyl acetate corn husk extract (HUS2) 2.05 g QE/100 g was higher than TFC in ethanolic corn husk extract (HUS3) 1.48 g QE/100 g, but IC_{50} of DPPH of HUS3 (34.1 µg/ml) which was classified as very strong antioxidant was lower than IC_{50} of DPPH of HUS2 (64.1 µg/ml) as strong antioxidant. Corn contained flavonoid compounds likes rutin, kaempferol, cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside[24,26]. Kaempferol is flavonoid aglycones which soluble in ethyl acetate, while rutin, cyanidin-3-glucoside, pelargonidin-3-glucoside and peonidin-3-glucoside soluble in ethanol. Kaempferol has no ortho di OH at C-3'-C-4', so the kaempferol will be have lower antioxidant activity than rutin and cyanidin-3-glucoside which have ortho di OH at C-3'-C-4'. Peonidin-3 glucoside which has ortho di OH-OCH_3 at C-3'-C-4' still react with aluminum (III) chloride reagent in determination flavonoid content, but have low antioxidant activity. Based on explanation above it can be supposed that HUS2 contained many flavonoid compounds such as kaempferol which had lower antioxidant activity, while HUS3 contained many flavonoid compounds such as rutin and cyanidin-3-glucoside which had higher antioxidant activity than kaempferol. TCC in n-hexane corn cob extract (COB1) 1.18 g BE/100 g was higher than TCC in ethyl acetate corn cob extract (COB2) 0.85 g BE/100 g, but IC_{50} of DPPH scavenging activity of COB1 (11.8 µg/ml) which was very strong antioxidant was lower than IC_{50} of DPPH scavenging activity of COB2 (275.3 µg/ml) as weak antioxidant. The higher scavenging radical activity will be given by carotenoid which contained more than seven double bonds[29]. Increasing in lipophilicity of carotenoid would increase scavenging radical activity and will give the lower IC_{50} of DPPH scavenging capacity[30]. Beta carotene was used as standard because it had conjugation double bonds which had ability to scavenge free radicals[31]. Corn consisted of carotenoid compounds, with dominant compound such as lutein and zeaxanthin [32]. It can be estimated that COB1 contained many carotenoid compounds which has more than seven conjugated double bonds, such as lutein (10 double bonds) and zeaxanthin (11 double bonds), while COB2 contained many carotenoid compound which has little conjugated double bonds such as neoxanthin (8 double bonds). DPPH and CUPRAC had different mechanism reaction. Mechanism of CUPRAC was redox assay [20] whereas DPPH that was electron transfer assay[33]. The previous study[25] which exposed that antioxidant activities of corn kernel by ABTS assay showed no linear result their antioxidant activity by FRAP method. The other research reported that antioxidant activity of corn kernel from 13 varieties by DPPH method had linear result with their antioxidant activities by FRAP and ABTS methods[15]. In the present study reported that IC_{50} of DPPH scavenging activities of corn cob, corn leaves and corn cob extracts gave no correlation with their EC_{50} of CUPRAC capacities.

CONCLUSION

Different methods antioxidant activity could give different results, so determination of antioxidant activity should be measured using different methods in parallel. All of corn leaves extracts (n-hexane extract, ethyl acetate extract and ethanol extract), ethyl acetate and ethanolic extracts of corn husk were strong to very strong antioxidant using DPPH assay. TFC and TCC in corn leaves extracts had significantly negative correlation with IC_{50} of DPPH scavenging activities. Flavonoid and carotenoid compounds in corn leaves extract were the major contributor in antioxidant activity by DPPH assay. There was no linear correlation between IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities of three different parts of corn. Corn cob, corn leaves and corn husk may be exploited as sources of natural antioxidant to degenerative diseases.

REFERENCES

1. Imaga NA, Gbenle GO, Okochi VI, Adenekan S, Emmanuel TD, Oyeniyi B, Dokai PN, Oyenuga M, Otumara A, Ekeh FC. Phytochemical and antioxidant nutrient constituents of *Carica papaya* and *Parquetina*

nigrescens extracts. Sci Res Essays 2010; 5(16), 2201-2205.
2. Zielinski AAF, Haminiuk CWI, Alberti A, Nogueira A, Demiate IM, Granato D. A comparative study of the phenolic compounds and the in vitro antioxidant activity of different Brazilian teas using multivariate statistical techniques. Food Res Int 2014; 60: 246–254.
3. Xu BJ, Chang SK. Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods. J Food Sci 2008; 73(2): H19-27.
4. Sompong R, Ehn SS, Martin GL, Berghofer E. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. Food Chem 2011; 124: 132–140.
5. Ghasemi K, Ghasemi Y, Ebrahimzadeh MA. Antioxidant activity, phenol and flavonoid contents of 13 citrus species peels and tissues. Pak J Pharm Sci 2009; 22(3): 277-281.
6. Thaipong K, Boonprakob U, Crosby K, Zevallos LC, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal 2006; 19: 669-675.
7. Fidrianny I, Permatasari L, Wirasutisna KR, Antioxidant activities from various bulbs extracts, of three kinds allium using DPPH, ABTS assays and correlation with total phenolic, flavonoid, carotenoid content. Int. J. Res. Pharm Sci 2013; 4(3): 438-444.
8. Mokbel MS, Hashinaga F. Antibacterial and antioxidant activities of banana (*Musa*, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol 2005; 1(3): 125-131.
9. Abirami MS, Muthuswamy. Antioxidant potential, total phenolic and total flavonoids content of various extracts from whole plant of *Polycarpaea corymbosa* Lam. Asian J Pharm Clin Res 2013; 6(4): 121-124.
10. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958; 181: 1199-2000.
11. Mashkor IMAAL. Phenolic content and antioxidant activity of fenugreek seeds extract. Int J Pharmacogn Phytochem Res 2014; 6(4): 841-844.
12. Win M, Hamid A, Baharin B, Anwar FS, Pak-Dek MS. Phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour. Pak J Bot 2011; 43(3): 1635-1642.
13. Pellegrini N, Serafini M, Colombi B, Rio DD, Salvatore S. Total antioxidant capacity of plant food, beverages and oils consumed in Italy assessed by three different in vitro assays. J Am Coll Nutr 2003; 133: 2812-2819.
14. Prasad MP, Rajkumar RA. In vitro antioxidant assay of citrus species using DPPH method. Indian J Adv Plant Res 2014; 1(4): 1-3.
15. Khampas S, Lertrat K, Lomthaisong K, Suriharn, B. Variability in phytochemicals and antioxidant activity in corn at immaturity and physiological maturity stages. Int Food Res J 2013; 20(6): 3149-3157.
16. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid content of some selected Iranian medicinal plants. Afr J Biotechnol 2006; 5(11): 1142-1145.
17. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002; 10: 178-182.
18. Fidrianny I, Windyaswari AS, Wirasutisna KR. Antioxidant capacities of various leaves extract from five colors varieties of sweet potatoes tubers using ABTS, DPPH assays and correlation with total flavonoid, phenolic, carotenoid content. Res J Med Plant 2013; 7(3): 130-40.
19. Bedawey AA. Characteristics of antioxidant isolated from some plants sources, Shibin El-Kom: Cairo; 2010. p. 1-11.
20. Apak R, Kubilay G, Birsen D, Mustava O, Saliha EC, Burcu B, Berker KI, Ozyurt D. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules 2007; 12: 1496-1547.
21. Sarepoua E, Tangwongchai R, Suriharn B, Lertrat K. Relationships between phytochemicals and antioxidant activity in corn silk. I Food Res J 2013; 20(5): 2073-2079.
22. Balasubramanian K, Padma PR. Screening of antioxidant properties of *Zea mays* leaves at different time periods of growth. J Pharm Res 2012; 5(8): 4034-4037.
23. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002; 13: 572 - 584.
24. Dong J, Cai L, Zhu X, Huang X, Yin T, Fang H, Ding Z. Antioxidant activities and phenolic compounds of cornhusk, corncob and stigma maydis. J Braz Chem Soc 2014; 25 (11): 1956-1964.
25. Ku KM, Kim HS, Kim SK, Kang YH. Correlation analysis between antioxidant activity and phytochemicals in Korean colored corns using principal component analysis. J Agric Sci 2014; 6(4): 1-8.
26. Yang Z, Zhai W. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (*Zea mays* L.). Innov Food Sci Emerg Technol 2010; 11(1): 169–176.
27. Fidrianny I, Johan Y, Sukrasno. Antioxidant activities of different polarity extracts from three organs of makrut lime (*Citrus hystrix* DC) and correlation with total flavonoid, phenolic, carotenoid content. Asian J Pharm Clin Res 2015; 8(4): 239-243.
28. Li XC, Wang XZ, Chen DF, Chen SZ. Antioxidant activity and mechanism of protochatechuic acid in vitro. J Funct Food Health Dis 2011; 1: 232-244.
29. Beutner S, Bloedorn B, Hoffmann T, Martin HD. Synthetic singlet oxygen quenchers. Methods Enzymol 2000; 319: 226-241.
30. Kobayashi M, Sakamoto Y. Singlet oxygen quenching ability of astaxanthin esters from the green alga

Haematococcus pluvialis. Biotech Lett 1999; 21: 265-269.
31. Charles DJ. Antioxidant properties of spices shells and other. London: John Willey; 2013.
32. Preedy VR, Watson RR, Patel VB (eds.). Flour and breads and their fortification in health and disease prevention. San Diego: Academic Press; 2011.
33. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005; 53: 1841 -1856.