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Research Article

Antioxidant Activities of Various Extracts from Ardisia SP Leaves Using DPPH and CUPRAC Assays and Correlation with Total Flavonoid, Phenolic, Carotenoid Content

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ABSTRACT

The objectives of this research were to study antioxidant activity from various leaves extracts of four Ardisia sp using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and CUPRAC (cupric ion reducing antioxidant capacity) and correlation of total flavonoid, phenolic and carotenoid content in various leaves extracts of four Ardisia sp with IC_{50} of DPPH and EC_{50} of CUPRAC antioxidant capacities. Extraction was performed by reflux using different polarity 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 sample extract was categorized as very strong antioxidant. Ethanolic leaves extract of *A.crenata* (CR3) had the lowest IC_{50} of DPPH scavenging activity with IC_{50} 0.49 µg/ml and ethyl acetate leaves extract of *A. elliptica* gave the lowest EC_{50} of CUPRAC capacity with EC_{50} 30.34 µg/ml. Ethyl acetate leaves extract of *A. cymosa* (CY2) contained the highest total flavonoid (8.24 g QE/100 g), ethanolic leaves extract of *A.elliptica* (EL3) showed the highest phenolic content (29.54 g GAE/100 g) and n-hexane leaves extract of *A.fuliginosa* (FU1) had the highest total carotenoid 13.37 g BE/100 g. There were negatively high correlation between total phenolic content in *A.crenata*, *A.cymosa* and *A.fuliginosa* leaves extracts with their IC_{50} of DPPH scavenging activities. There were negative and high correlation between total flavonoid in all of sample extract and EC_{50} of CUPRAC capacities. Total carotenoid content in *A.elliptica*, *A.cymosa* and *A.fuliginosa* leaves extracts had negative and high correlation with their CUPRAC capacities. *A.crenata* leaves extracts had linear result in DPPH and CUPRAC assays.

Keywords: Antioxidant, DPPH, CUPRAC, leaves, Ardisia sp, flavonoid, phenolic, carotenoid

INTRODUCTION

Antioxidant can reduce oxidative stress which related with the risk of many diseases. Phenolic compounds are commonly found in plants, and they have multiple biological effects, including antibacterial, anti-inflammatory and antioxidant activity[1-3]. Plants including Ardisia sp contained phenolic and flavonoid compounds[4-6]. Previous researches [7-10] reported that phenolic content and flavonoid content in plants could be correlated to their antioxidant activities.

Some of antioxidant methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl), CUPRAC (cupric ion reducing antioxidant capacity) and FRAP (ferric reducing antioxidant power) were used to test antioxidant activity of food, vegetables and fruits[7,11]. In previous study[12-13] exhibited that DPPH and CUPRAC methods could be used to determine antioxidant activity in many plants extracts. The previous study[4,14] exposed that *Ardisia elliptica* (*Ardisia humilis*) and *Ardisia crispa* had antioxidant capacities by using DPPH, FRAP and ABTS assays.

The objective of this research were to study antioxidant activities of different polarities extracts (n-hexane, ethyl acetate and ethanol) of leaves from four Ardisia sp (*Ardisia elliptica*, *Ardisia crenata*, *Ardisia cymosa* and *Ardisia fuliginosa*) using DPPH and CUPRAC assays and correlation of total flavonoid, phenolic, and carotenoid content in each extract with their antioxidant activities.

MATERIALS AND METHODS

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl), neocuproine, gallic acid, quercetin, beta carotene was purchased from Sigma-Aldrich (MO, USA), cupric chloride, leaves of four Ardisia sp, ethanol. All other reagents were analytical grades.

Preparation of sample

Leaves of four Ardisia sp were collected from West Java that were: *Ardisia elliptica* namely as EL collected from

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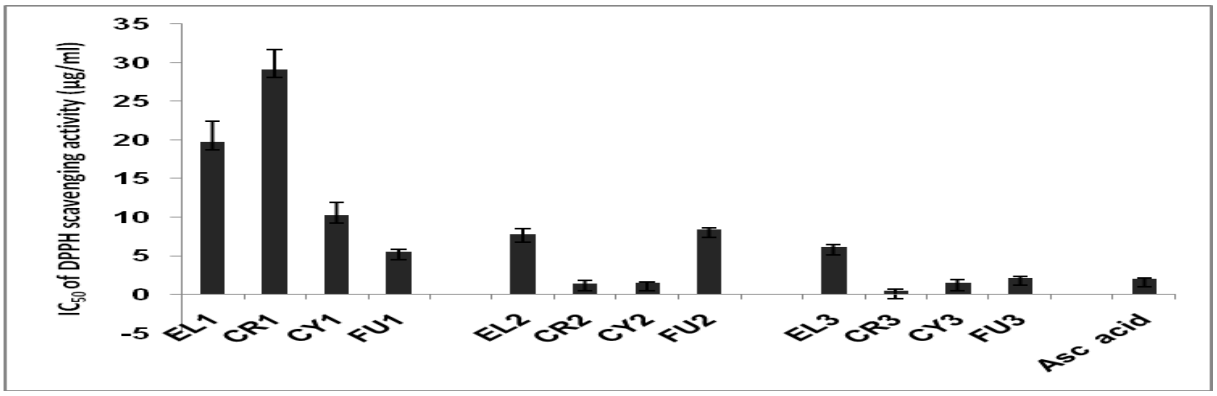

Figure 1: IC_{50} of DPPH scavenging activities in various leaves extracts of four Ardisia sp

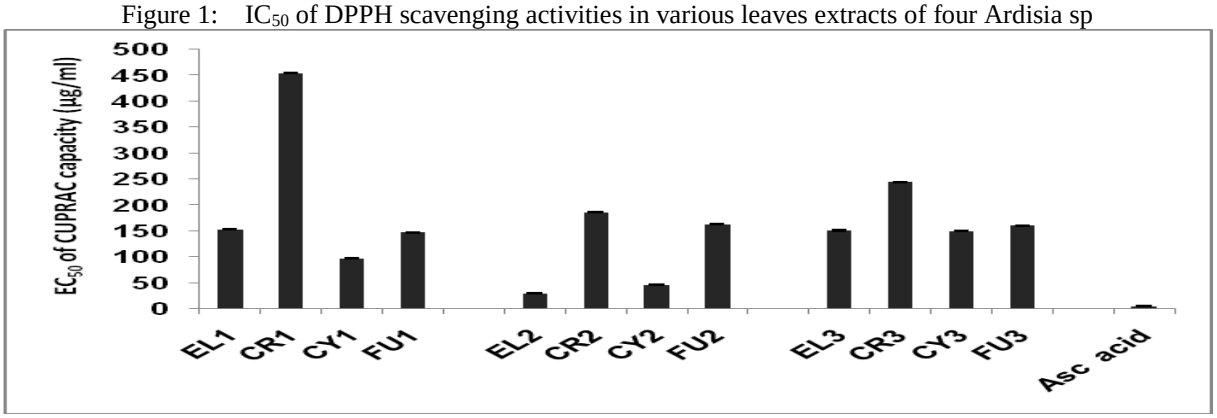

Figure 2: EC_{50} of CUPRAC capacities in various leaves extracts of four Ardisia sp

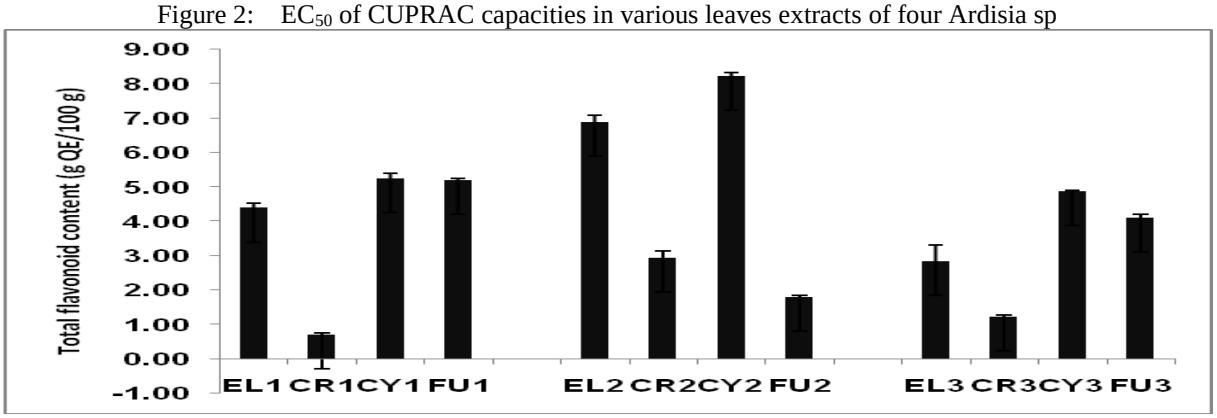

Figure 3: Total flavonoid content in various leaves extracts of four Ardisia sp

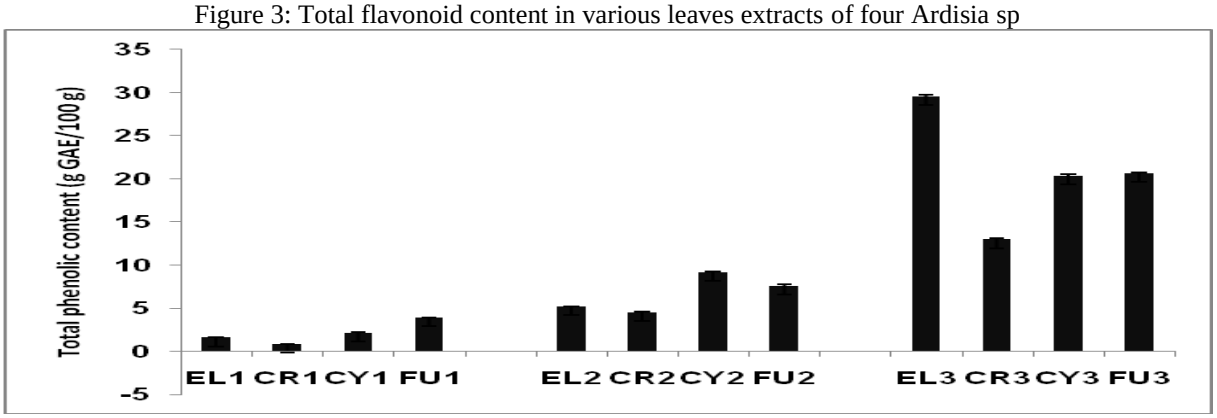

Figure 4: Total phenolic content in various leaves extracts of four Ardisia sp

Pangandaran, *Ardisia crenata* as sample CR from Bogor, *Ardisia cymosa* as sample CY and *Ardisia fuliginosa* as sample FU from Bandung, were thoroughly washed with tap water, wet sortation, cut, dried and grinded into powder.

Extraction

Three hundred grams of powdered sample was extracted by reflux using increasing gradient polarity solvents. The n-hexane extract was repeated three times. The remaining residue was then extracted three times with ethyl acetate. Finally the remaining residue was extracted three times with ethanol. So there were four n-hexane extracts (namely EL1, CR1, CY and FU1), four ethyl acetate extracts (EL2, CR2, CY2 and FU2) and four ethanolic extracts (EL3, CR3, CY3 and FU3).

IC_{50} of DPPH scavenging activity

Preparation of DPPH solution was adopted from Blois[15] with minor modification. Various concentration of each extract was pipetted into DPPH solution 50 µg/ml (1:1) to initiate the reaction for obtaining a calibration curve. After 30 minutes incubation, the absorbance was read at wavelength 515 nm by using spectrophotometer UV-Vis 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 was determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity[16]. IC_{50} of DPPH scavenging activity of each extract can be calculated using its calibration curve.

EC_{50} of CUPRAC capacity

Preparation of CUPRAC solution was adopted from Apak *et al*.[13]. The CUPRAC solution was prepared in ammonium acetate buffer pH 7. Various concentration of each extract was 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 spectrophotometer UV-Vis Hewlett Packard 8435. Ammonium acetate buffer was used as a blank. CUPRAC 50 µg/ml was as control. Ascorbic acid was used as standard. Analysis was done in triplicate for standard and each extract. Antioxidant capacity of each extract was determined based on increasing in Cu (I)-neocuproine absorbance by calculating percentage of antioxidant capacity[13]. EC_{50} of CUPRAC capacity of each extract can be calculated using its calibration curve.

Total flavonoid content (TFC)

Total flavonoid content was measured using adapted method from Chang *et al*[17]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Standard solution of quercetin with concentration 20-120 µg/ml were used to obtain a standard curve. The total flavonoid content was reported as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Total phenolic content (TPC)

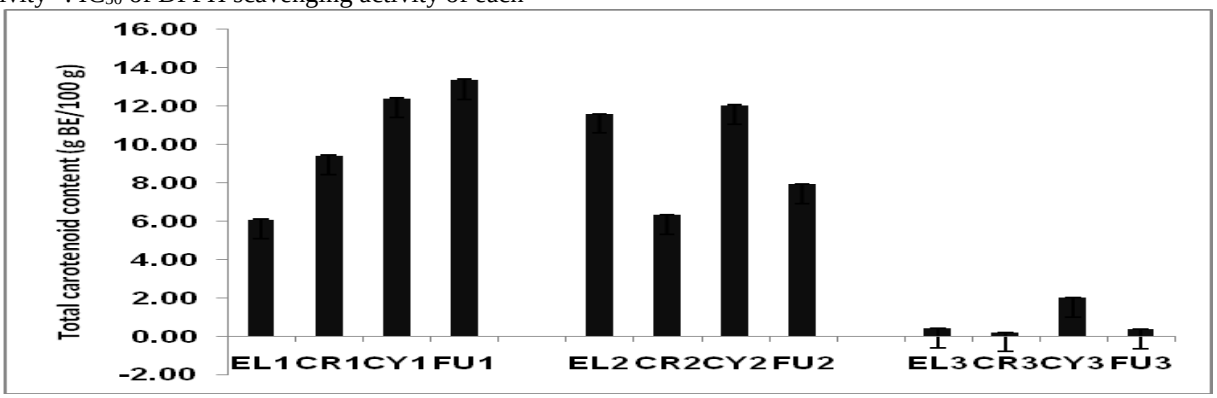

Figure 5: Total carotenoid content in various leaves extracts of four Ardisia sp

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

	Pearson's correlation coefficient (r)						
	TFC	TPC	TCC	IC_{50} DPPH EL	IC_{50} DPPH CR	IC_{50} DPPH CY	IC_{50} DPPH FU
IC_{50} DPPH EL	- 0,026 ^{ns}	- 0,0646 ^{ns}	0,118 ^{ns}				
IC_{50} DPPH CR	- 0,651 ^{ns}	- 0,748 *	0,773 *				
IC_{50} DPPH CY	- 0,294 ^{ns}	- 0,772 *	0,512 ^{ns}				
IC_{50} DPPH FU	- 0,640 ^{ns}	- 0,762 *	0,606 ^{ns}				
EC_{50} CUPRAC EL	- 0,927 **	0,398 ^{ns}	- 0,876 **	0,367 ^{ns}			
EC_{50} CUPRAC CR	- 0,813 **	- 0,574 ^{ns}	0,606 ^{ns}		0,967 **		
EC_{50} CUPRAC CY	- 0,947 **	0,618 ^{ns}	- 0,855 **			- 0,004 ^{ns}	
EC_{50} CUPRAC FU	- 0,853 **	0,516 ^{ns}	- 0,688 *				0,150 ^{ns}

Total phenolic content were measured using the modified Folin-Ciolcalteu method which was adapted from Pourmorad [10]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Standard solution of gallic acid with concentration 30-180 μg/ml were used to obtain a standard curve. The total phenolic content was reported as percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

Total carotenoid content (TCC)

Total carotenoid content was measured using the modified carotene method which was adapted from Thaipong *et al* [7]. Each extract was diluted in n-hexane. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extract. Standard solution of beta carotene with concentration 5-70 μg/ml were used to obtain a standard curve. The total carotenoid content was reported as percentage of total beta carotene equivalent per 100 g extract (g BE/100 g).

Statistical Analysis

Each sample analysis was performed in triplicate. All results presented are means (± standard deviation) of at least three independent experiments. Statistical analysis (ANOVA with a statistical significance level set at $p < 0.05$ with post-hoc Tukey procedure was carried out with SPSS 16 for Windows. Correlations between the total phenolic, flavonoid and total carotenoid content and antioxidant activities were analyzed using the Pearson's procedure ($p < 0.01$).

RESULTS

IC_{50} of DPPH scavenging activity and EC_{50} of CUPRAC capacity

The IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities in various leaves extracts of four Ardisia sp using DPPH and CUPRAC assays were shown in Fig 1 and Fig 2. IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities of each extract were compared to IC_{50} and EC_{50} ascorbic acid as standard. The lowest EC_{50} or IC_{50} means had the highest antioxidant activity.

TFC in various extracts of four Ardisia sp leaves

TFC among the various extracts were expressed in term of quercetin equivalent using the standard curve equation $y = 0.006 x - 0.0191$, $R^2 = 0.998$. The TFC in various leaves extracts of four Ardisia sp showed different result in the range of 0.71 – 8.24 g QE/100 g (Fig 3). Ethyl acetate leaves extract of *A. cymosa* (CY2) had the highest total flavonoid content (8.24 g QE/100 g) and the lowest (0.71 g QE/100 g) was given by n-hexane leaves extract of *A. crenata* (CR1).

TPC in various extracts of four Ardisia sp leaves

TPC among the various extracts were reported in term of gallic acid equivalent using the standard curve equation $y = 0.006x - 0.055$, $R^2 = 0.998$. The TPC in various leaves extracts of four Ardisia sp showed different result ranged from 0.87 to 29.54 g GAE/100 g. Ethanolic leaves extract of *A. elliptica* (EL3) had the highest phenolic content (29.54 g GAE/100 g) (Fig 4).

TCC in various extracts of four Ardisia sp leaves

TCC among the various extracts were demonstrated in term of beta carotene equivalent using the standard curve equation $y = 0.007x - 0.027$, $R^2 = 0.995$. The TCC in various leaves extracts of four Ardisia sp showed different result in the range of 0.20 – 13.37 g BE/100 g (Fig 5). The highest carotenoid content (13.37 g BE/100 g) was given by n-hexane leaves extract of *A. fuliginosa* (FU1), while the lowest carotenoid (0.20 g BE/100 g) for ethanolic leaves extract of *A. crenata* (CR3).

Correlations between total phenolic, flavonoid and carotenoid content in various leaves extracts of four Ardisia sp with their IC_{50} of DPPH scavenging activities, EC_{50} of CUPRAC capacities

Pearson's correlation coefficient between TFC in various leaves extracts of four Ardisia sp and their antioxidant activities demonstrated that TFC in all of sample had negatively high correlation with EC_{50} of CUPRAC capacities. TPC in sample CR, CY and FU had negative and high correlation with their IC_{50} of DPPH scavenging activities ($r = -0.748$; $r = -0.772$, $r = -0.762$, $p<0.01$, respectively), while TCC in sample EL, CY and FU had negatively high correlation with EC_{50} of CUPRAC capacities ($r = -0.876$, $r = -0.855$, $p <0.01$, and $r = -0.688$, $p<0.05$, respectively) (Table 1).

Note: IC_{50} DPPH = IC_{50} of DPPH scavenging activity, EC_{50} CUPRAC = EC_{50} of CUPRAC capacity, EL = leaves extract of EL, CR = leaves extract of CR, CY = leaves extract of CY, FU = leaves extract of FU, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

DISCUSSION

The previous study[4,8-9,11,13] reported that plants including Ardisia sp contained phenolic and flavonoid compounds which can act as antioxidant. There were no study regarding antioxidant activity of different polarities extracts (which were n-hexane, ethyl acetate and ethanol) of leaves from four Ardisia sp using DPPH and CUPRAC assays.

The DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 515-520 nm. Colors of DPPH would be changed when the free radicals were scavenged by antioxidant[7,16,18-19]. Reagent of CUPRAC is cupric (II) chloride that 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. 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 0.46 V, so the sample can reduce Cu (II) to Cu (I) and this sample will be oxidized.

IC_{50} of DPPH scavenging capacity is concentration of sample or standard that can inhibit 50 % of DPPH scavenging capacity, while EC_{50} of CUPRAC capacity is

concentration of sample or standard that can exhibit 50 % of CUPRAC capacity. The lowest IC_{50} or EC_{50} means had the highest antioxidant capacity. IC_{50} or EC_{50} were used to determine antioxidant capacity of sample was compared to standard. Classification by Blois [15] revealed that sample which had IC_{50} or EC_{50} < 50 µg/ml it 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} or EC_{50} >150 µg/ml.

In the present study demonstrated that IC_{50} of DPPH scavenging activities in various leaves extracts of four Ardisia sp ranged from 0.49 to 29.13 µg/ml. Ethanolic leaves extract of *A. crenata* (CR3) had the lowest IC_{50} of DPPH scavenging activity 0.49 µg/ml, while ascorbic acid standard gave IC_{50} of DPPH scavenging activity 2.03 µg/ml. Based on the value of IC_{50} of DPPH scavenging activity it can be concluded that all of leaves extracts of four Ardisia sp (*A. elliptica*, *A. crenata*, *A. cymosa* and *A. fuliginosa*) can be categorized as very strong antioxidant. It exposed that potency of ethanolic extract of *A. crenata* was around four times potency of ascorbic acid using DPPH method. Ethyl acetate leaves extract of *A. elliptica* (EL2) had the lowest EC_{50} of CUPRAC capacity (30 µg/ml) while ascorbic acid standard gave EC_{50} of CUPRAC capacity 0.099 µg/ml. It revealed that potency of ascorbic acid was around three hundred times of potency of EL2 using CUPRAC assay. In the previous study [14] reported that methanol leaves extract of *A. humilis* (synonym of *A. elliptica*) had IC_{50} of DPPH scavenging activity was 4.30 µg/ml. It was similar with the present study which expressed that IC_{50} of DPPH scavenging activity of ethanolic extract of *A. elliptica*, *A. crenata*, *A. cymosa* and *A. fuliginosa* were 6.13, 0.49, 1.5, 2.15 µg/ml, respectively.

Study by Azima[4] stated that antioxidant activity of citrate buffer (pH 3) fruit extract of *Ardisia elliptica* by FRAP, ABTS and ORAC methods were 19.60 mM TEAC, 0.24 mM TEAC and 4.63 µM TEAC, respectively. Research by Jindal[20] reported that fruit and leaves of *Ardisia crispa* which was extracted by using n-hexane, chloroform, methanol and water demonstrated that methanol fruit and leaves extracts had the highest percentage of DPPH scavenging activity (90.16 % and 82.24 %, respectively) and IC_{50} of DPPH scavenging activity were 900 µg/ml and 1500 µg/ml, respectively, which were categorized as weak antioxidant. It was different with the present result which exposed that IC_{50} of DPPH scavenging activity of ethanolic leaves extract of four Ardisia sp in the range from 0.49 to 6.13 µg/ml and can be classified as very strong antioxidant.

Antioxidant capacity might be related with the presence of total phenolic content, included phenolic acid[5,19]. Antioxidant activity of cinnamic acid was higher than phenyl acetic acid and benzoic acid[18]. The previous study[20] found that TPC in n-hexane, chloroform, methanol and water fruit extracts of *Ardisia crispa* were 0.095, 0.233, 0.814, 1.86 g GAE/100 g extract, respectively, while n-hexane, chloroform, methanol and water leaves extracts of *Ardisia crispa* were 0.067, 0.178, 0.557, 0.134 g GAE/100 g, respectively. The citrate buffer leaves extract of *Ardisia elliptica* had TPC 4.175 g GAE/100 g[4]. It was different with the present study which demonstrated that TPC in ethanolic leaves extract of *A. elliptica*, *A. crenata*, *A. cymosa* and *A. fuliginosa* were 29.54, 12.98, 20.32, 20.66 g GAE/100 g, respectively.

The previous study[4] stated that TFC in citrate buffer leaves extract of *Ardisia elliptica* was 3.691 g QE/100 g. It was similar with the current study which found that TFC in ethanolic extract of *A. elliptica*, *A. crenata*, *A. cymosa* and *A. fuliginosa* leaves extracts were 2.85, 1.23, 4.88, 4.12 g QE/100 g, respectively. The TFC in n-hexane, chloroform, methanol, water leaves extracts of *Ardisia crispa* were 0.161, 0.2, 0.312, 0.047 g QE/100 g, respectively, while in their fruit extracts were 0.187, 0.25, 0.654, 0.052 g QE/100 g extract, respectively[20].

Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$ [7] and negatively high if $-0.61 \leq r \leq -0.97$. Sample which had the lowest IC_{50} of DPPH scavenging activity or EC_{50} of CUPRAC capacity gave the highest antioxidant activity. So the good correlation between IC_{50} of DPPH or EC_{50} of CUPRAC with TPC, TFC and TCC will be given in negative and high correlation. It means increasing in TFC, TPC and TCC caused increasing in antioxidant activities, which was exposed by lower IC_{50} of DPPH scavenging activity and or EC_{50} of CUPRAC capacity.

The data in Table 1 exposed that TPC in *A. crenata*, *A. cymosa* and *A. fuliginosa* leaves extract had negatively high correlation with their IC_{50} of DPPH scavenging activities ($r = -0.748$, $p<0.05$, $r = -0.784$, $r = -0.762$, $p<0.01$). It demonstrated that increasing in TPC caused decreasing in IC_{50} of DPPH scavenging activities which showed increasing in antioxidant activities. Based on the data it can be concluded that antioxidant activities of *A. crenata*, *A. cymosa* and *A. fuliginosa* leaves extract by DPPH method can be predicted indirectly by determining their TPC.

The TFC in all of sample extract (*A. elliptica*, *A. crenata*, *A. cymosa* and *A. fuliginosa*) had negatively high correlation with their EC_{50} of CUPRAC capacity ($r = -0.91$, $r = -0.813$, $r = -0.948$, $r = -0.813$, $p<0.01$, respectively) and only TCC in *A. elliptica*, *A. cymosa* and *A. fuliginosa* leaves extract had negative and high correlation with their EC_{50} of CUPRAC capacity ($r = -0.853$, $r = -0.855$, $p<0.01$, $r = -0.718$, $p<0.05$, respectively). Based on the data it could be seen flavonoid and or carotenoid compounds in *A. elliptica*, *A. cymosa* and *A. fuliginosa* leaves extracts were the contributor in their antioxidant activities by CUPRAC method. It can be concluded also that antioxidant activities in *A. crenata* leaves extracts by CUPRAC method can predicted indirectly by determining their TFC, so flavonoid compounds were the major contributor in antioxidant activities of *A. crenata* leaves extract by CUPRAC method.

In the previous research by Azima[4] exposed that TFC in *Syzygium cumini* and *Ardisia elliptica* had positively high

correlation with their antioxidant activity by using FRAP and ABTS methods. It was similar with the present study which demonstrated that TFC in *A. elliptica*, *A. crenata*, *A. cymosa*, *A. fuliginosa* had negatively and high correlation with EC_{50} of CUPRAC capacity. It means increasing in TFC would decrease EC_{50} of CUPRAC capacity and increasing in antioxidant activity by CUPRAC method.

TPC in ethyl acetate leaves extract of *A.crenata* (CR2) 4.51 g GAE/100 g was lower than TPC in ethanolic leaves extract of *A. cymosa* (CY3) 9.13 g GAE/100 g, but IC_{50} of DPPH scavenging activity of CR2 (1.43 µg/ml) was similar with IC_{50} of DPPH scavenging activity of CY3 (1.45 µg/ml). Based on the data it can be supposed that many phenolic compounds in CR2 which had high antioxidant activities while many phenolic compounds in CY3 had low antioxidant activities. TPC in ethyl acetate leaves extract of *A. elliptica* (EL2) 5.19 g GAE/100 g was similar with TPC in ethyl acetate leaves extract of *A. crenata* (CR2) 4.51 g GAE/100 g, but EL2 had lower EC_{50} of CUPRAC capacity (30.34 µg/ml) which was categorized as very strong antioxidant than CR2 (186.37 µg/ml) which was classified as weak antioxidant. Based on this data it can be predicted that many phenolic compounds in EL2 had reduction potential (E^o) below 0.46 V, so it can reduce Cu(II) to Cu(I) and formed complex with neocuproine then given yellow color. In contrast with CR2 which was supposed that it contained many phenolic compounds which had reduction potential above 0.46 V.

Phenolic acid, tannins, flavonoid, qoumarine and quinone were included in phenolic compound. Flavonoid which have OH in A ring and or B ring will be included in phenolic groups. Flavonoid had higher antioxidant activity than phenolic acid[18]. Flavonoid which had -OH in ortho C-3', C-4', -OH in C3, oxo function in C-4, double bond at C-2 and C-3 would give higher antioxidant capacity. The -OH with ortho position in C-3'-C-4' had the highest influence to antioxidant activity of flavonoid. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides[18]. It could be seen in Fig 3 that TFC in n-hexane leaves extract of *A. fuliginosa* (FU1) 5.20 g QE/100 g was similar with TFC in ethanolic leaves extract of *A. cymosa* (CY3) 4.88 g QE/100 g, but IC_{50} of DPPH scavenging activity of CY3 (1.5 µg/ml) was lower than IC_{50} of DPPH scavenging activity of FU1 (5.47 µg/ml). Based on the data above it can predicted that many flavonoids in CY3 had -OH in position C3'-C4', -OH in C-3, double bond in C-2 - C-3, oxo in C-4, which had high antioxidant capacities. In contrast, FU1 contained many flavonoids that had -OH in other position which no or low influence in antioxidant activities. TFC in n-hexane leaves extract of *A. cymosa* (CY1) 5.63 g QE/100 g was similar with TFC in n-hexane leaves extract of *A. fuliginosa* (FU1) 5.20 g GAE/100 g), but EC_{50} of CUPRAC capacity of CY1 97.61 µg/ml which was categorized as medium antioxidant, was lower than EC_{50} of CUPRAC capacity of FU1 147.44 µg/ml which was classified as weak antioxidant. Based on this data it can be supposed that many flavonoid in CY1 that had reduction potential below 0.46 V so it could reduce Cu (II) to CU (I) then formed complex with neocuproine and gave yellow color.

Carotenoid had antioxidant capacity by scavenging free radical. More double bonds in carotenoid would give higher scavenging free radical capacity[21]. Carotenoid that consisted of more than 7 double bonds gave higher scavenging radical capacity[22]. Beta carotene was used as standard because of it had conjugation double bonds which had ability to scavenge free radicals[23]. In previous study[24-25] exposed that increasing in lipophilicity of carotenoid would increase scavenging radical capacity, it means give the lower IC_{50} of DPPH scavenging activity. TCC in n-hexane leaves extract of *A. cymosa* (CY1) 12.40 g BE/100 g was similar with TCC in ethyl acetate extract of *A. cymosa* (CY2) 12.04 g BE/100 g, but IC_{50} of DPPH scavenging activity of CY2 (1.45 µg/ml) was lower than IC_{50} of DPPH scavenging activity of CY1 (10.22 µg/ml). It can be supposed that many carotenoid in CY2 contained more than 7 double bonds and only a little carotenoid with more than 7 double bonds in CY1. TCC in ethyl acetate leaves extracts of *A. elliptica* (EL2) 11.60 g BE/100 g was similar with TCC in n-hexane leaves extract of *A. cymosa* (CY1) 12.40 g BE/100 g, but EC_{50} of CUPRAC capacity of EL2 (30.34 µg/ml) which was classified as very strong antioxidant, was lower than EC_{50} of CUPRAC capacity of CY1 (97.61 µg/ml) which was categorized as strong antioxidant. Based on the data it can be supposed that many carotenoid in EL2 had reduction potential below than reduction potential of Cu(II)/Cu(I) 0.46 V, so it can act as antioxidant and Cu(II) will be reduced to Cu(I).

DPPH and CUPRAC methods had different mechanism reaction. Mechanism of DPPH that was electron transfer assay[26] and CUPRAC was redox assay[13]. Only *A. crenata* leaves extracts expressed positively high correlation between IC_{50} of DPPH scavenging activities and EC_{50} of CUPRAC capacities. So the results of this study showed that IC_{50} of DPPH scavenging activities in *A. crenata* leaves extracts were linear with their EC_{50} of CUPRAC capacities.

CONCLUSION

Variety of methods should be used in parallel to assess the antioxidant capacity of sample, because different methods could give different results. All of leaves extracts of *A.elliptica*, *A. crenata*, *A. cymosa*, *A. fuliginosa* were very strong antioxidant. Phenolic compounds in *A. crenata*, *A. cymosa* and *A. fuliginosa* leaves extracts were the major contributor in antioxidant activities by DPPH method. Flavonoid and or carotenoid compounds were the contributor in antioxidant capacities in *A. elliptica*, *A. cymosa* and *A. fuliginosa* leaves extracts by CUPRAC method. Flavonoid compounds were the major contributor in antioxidant activities of *A. crenata* leaves extracts by CUPRAC method. There were linear correlation between IC_{50} of DPPH scavenging activities and EC_{50} CUPRAC capacities result in *A. crenata* leaves

extracts. *A. elliptica*, *A. crenata*, *A. cymosa* and *A. fuliginosa* leaves extracts may be exploited as natural antioxidant sources to alleviate oxidative stress.

REFERENCES

1. 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.
2. Souri E, Amin G, Farsan H, Barazandeh TM. Screening antioxidant activity and phenolic content of 24 medicinal plats extracts. DARU J Pharm Sci 2008; 16: 83 -87.
3. Halvorsen BL, Holte K, Myhrstad MC, Barikmo I, Hvattum E, Remberg SV. A systematic screening of total antioxidat in dietary plants. J Nutr 2002; 132: 461-471.
4. Azima AMS, Noriham A, Nurhuda M. Antioxidant activities of *Syzygium Cumini* and *Ardisia elliptica* in relation to their estimated phenolic compositions and chromatic properties. Int J Biosci, Biochem Bioinforma 2013; 3(4): 314-317.
5. Ling LT and Palanisamy UD. Review: Potential antioxidants from tropical plants, In: Valdez, B., editor, Food industrial processes-methods, Kuala Lumpur: In Tech; 1999. p.64-72.
6. Fidrianny I, Sari PI, Ruslan K. Antioxidant activities in various peel extracts of four varieties rambutan (*Nephelium lappaceum*) using DPPH, FRAP assays. Int J Pharmacog Phytochem Res 2015; 7(2): 280-285.
7. 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.
8. 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.
9. Kahkonen MH. Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 1999; 47: 3954-3962.
10. 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.
11. Boudjou S, Oomah BD, Zaidi F, Hosseinian F. Phenolics content and antioxidant and anti-inflammatory activities of legume fractions. Food Chem 2013; 138(2-3): 1543-1550.
12. Fidrianny I, Nurfitri H, Sukrasno. Invitro antioxidant activities, phenolic, flavonoid and carotenoid content from different polarity extracts of five citrus peels using DPPH and CUPRAC method 2015. Int J Chem Pharm Res; 7(4): 1525-1531.
13. Apak R, Guclu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu 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.
14. Khatun A, Rahman M, Kabir S, Akter MN, Chowdhury SA. Phytochemical and pharmacological properties of methanolic extract of *Ardisia humilis* Vahl. (Myrsinaceae). Int J Res Ayurveda Pharm 2013; 4(1): 38-41.
15. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958; 181: 1199-2000.
16. 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.
17. Bedawey AA. Characteristics of antioxidant isolated from some plants sources, Cairo: Shibin El-Kom; 2010. p. 1-11.
18. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002; 13: 572 -584.
19. 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.
20. Jindal HMK, Mohamad J. Antioxidant Activity of *Ardisia crispa* (Mata pelanduk). Sains Malaysiana 2012; 41(5): 539–545.
21. Foote CS. Free radicals in biology. 3rd ed. New York: Academic Press; 1976
22. Beutner S, Bloedorn B, Hoffmann T, Martin HD. Synthetic singlet oxygen quenchers. Methods Enzymol 2000; 319: 226-241.
23. Charles DJ. Antioxidant properties of spices shells and other. London: John Willey; 2013.
24. Kobayashi M, Sakamoto Y. Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. Biotech Lett 1999; 21: 265-269.
25. Müller L, Fröhlich K, Böhm V. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (aTEAC), DPPH assay and peroxyl radical scavenging assay. Food Chem 2011; 129: 139-148.
26. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005; 53: 1841 -1856.