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Research Article

In Vitro Antioxidant Activities in Various Beans Extracts of Five Legumes from West of Java-Indonesia Using DPPH and ABTS Methods

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ABSTRACT

The aim of this research were to determine antioxidant activity from different polarities beans extracts of five legumes beans using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and correlation of total phenolic, flavonoid and carotenoid content in various extracts of five legumes beans with their IC_{50} of DPPH and IC_{50} of ABTS antioxidant activities. Extraction was done by reflux using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH and ABTS assays, determination of total phenolic, flavonoid and carotenoid content were conducted by UV-visible spectrophotometry and its correlation with IC_{50} of DPPH and IC_{50} of ABTS scavenging activities were analyzed by Pearson's method. All of ethanol extracts from five legumes beans were categorized as very strong antioxidant by DPPH and ABTS methods. Phenolic compounds in red kidney bean extracts were the major contributor in IC_{50} of DPPH and IC_{50} of ABTS scavenging activities. DPPH and ABTS assays showed linear results in red kidney bean and bogor peanut sample.

keywords: Antioxidant, DPPH, ABTS, legumes beans, phenolic, flavonoid, carotenoid

INTRODUCTION

Negative effect of free radical can be inhibited by antioxidant. Antioxidant is compound that can inhibit oxidation reaction by scavenging free radical. The excessive of free radical related with oxidative stress condition which can cause many diseases. Many plants have active compounds which have antioxidant properties such as flavonoid and phenolic compounds, which have known to multiple biological effects, included antibacterial and antioxidant activity[1-3]. Previous study[4-8] expressed that phenolic and flavonoid content could be correlated to their antioxidant activities. Plants including legumes contained phenolic and flavonoid compounds[9-11]. DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) can be used to predict antioxidant activity of vegetables, fruits and food[10-11]. Previous researches[7,10,12] revealed that DPPH, FRAP, CUPRAC and ABTS methods could be used to determine antioxidant activity in many plants extracts. The previous studies[13-15] reported that legumes had antioxidant activities by using ABTS, DPPH, and FRAP assays. The aim of this research were to determine antioxidant activities of three different polarities extracts (n-hexane, ethyl acetate and ethanol) of five legumes beans using DPPH and ABTS assays, and correlations of total phenolic, flavonoid and carotenoid content with their antioxidant activities.

MATERIALS AND METHODS

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt), gallic acid, quercetin, beta carotene was purchased from Sigma-Aldrich (MO, USA), legumes beans, ethanol. All other reagents were analytical grades.

Preparation of sample

Legumes beans which were: soybean (*Glycine max*) namely as sample GM, green bean (*Phaseolus radiatus*) as PR, peanut (*Arachis hypogaea*) as AH, red kidney bean (*Phaseolus vulgaris*) as PV were collected from Cirebon-West of Java and bogor peanut (*Vigna subterranea*) as VS was collected from Bogor-West of Java. All of samples were thoroughly washed with tap water, wet sortation, cut, dried and grinded into powder.

Extraction

Three hundred gram of powdered sample was extracted 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. So totally there were fifteen extracts: five of n-hexane extracts (namely GM 1, PR 1, AH 1, PV 1 and VS 1), five of ethyl acetate extracts (GM 2, PR 2, AH 2, PV 2 and VS 2) and five of ethanolic extracts (GM 3, PR 3, AH 3, PV 3 and VS 3).

IC_{50} of DPPH scavenging activity

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Preparation of DPPH solution was performed using Blois's method with minor modification[16]. 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 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 by DPPH method was determined by calculating percentage of antioxidant activity using reduction of DPPH absorbance[17]. IC_{50} of DPPH scavenging activity of each extract can be calculated using its calibration curve.

IC_{50} of ABTS scavenging activity

Preparation of ABTS solution was conducted using Li method with minor modification[18]. 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 hours. Both solutions were mixed with 30 minutes incubation, left the mixture in refrigerator for 24 hours, then diluted in ethanol. 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 Hewlett Packard 8435. 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 determined by calculating percentage of antioxidant activity using reduction of ABTS absorbance[17]. IC_{50} of ABTS scavenging activity of each extract can be calculated using its calibration curve.

Total phenolic content (TPC)

Total phenolic content was conducted by using the modified Folin-Ciolcalteu[6]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Gallic acid standard solution (40-165 µg/ml) was used to obtain a calibration curve. Total phenolic content was expressed as percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

Total flavonoid content (TFC)

Total flavonoid content was adapted from Chang *et al*[19] with minor modification. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Quercetin standard solution (36-120 µg/ml) was used to obtain a calibration curve. The total flavonoid content was expressed as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Total carotenoid content (TCC)

Total carotenoid content was measured using modified method which was adapted from Thaipong *et al*[10]. Each extract was diluted in n-hexane. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extract. Beta carotene standard solution (15-55 µg/ml) was used to obtain a calibration curve. The total carotenoid content was expressed as percentage of total beta carotene equivalent per 100 g extract (g BE/100 g).

Statistical Analysis

Each sample analysis was conducted in triplicate. All results presented are means (± standard deviation) of at least three independent experiments. Statistical analysis using ANOVA with a statistical significance level set at $p < 0.05$ and post-hoc Tukey procedure was 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 performed using the Pearson's method.

RESULTS

IC_{50} of DPPH and IC_{50} of ABTS scavenging activity

The IC_{50} of DPPH and IC_{50} of ABTS scavenging activities in different polarities extracts from five legumes beans using DPPH and ABTS assays were shown in Fig 1 and Fig 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.

Total phenolic content (TPC)

TPC among the various extracts were revealed in term of gallic acid equivalent using the standard curve equation $y = 0.006x - 0.055$, $R^2 = 0.998$. The TPC in fifteen extracts from three different regions of legumes beans exposed different result in the range of 0.19 – 3.04 g GAE/100 g. Ethanolic extract of bogor peanut beans (VS3) revealed that the highest phenolic content (3.04 g GAE/100 g) and the lowest was given by n-hexane extract of soybean (GM1).

Total flavonoid content (TFC)

TFC among the various extracts were reported in term of quercetin equivalent using the standard curve equation $y = 0.006 x - 0.0191$, $R^2 = 0.998$. The TFC in fifteen extracts from three different regions of legumes beans showed different result ranged from 0.27 to 8.69 g QE/100 g. Ethyl acetate extract of red kidney beans (PV2) had the highest total flavonoid content (8.69 g QE/100 g).

Total carotenoid content (TCC)

TCC among the various extracts were expressed in term of beta carotene equivalent using the standard curve equation $y = 0.007x - 0.027$, $R^2 = 0.995$. The TCC in fifteen extracts from three different regions of legumes beans gave different result in the range of 0.03 – 2.96 g BE/100 g. The highest carotenoid content (2.96 g BE/100 g) was given by ethanol extract of peanut (AH3).

Correlations between total phenolic, flavonoid, carotenoid content in various legumes beans extracts and IC_{50} of DPPH, IC_{50} of ABTS scavenging activities

Pearson's correlation coefficient between TPC in various extracts of legumes beans and their antioxidant activities exposed that TPC in red kidney bean and bogor peanut extracts (PV and VS) had negative and high significant correlation with IC_{50} of DPPH scavenging activities ($r = -0.888$; $r = -0.865$; $p<0.01$, respectively) and TPC in green bean, peanut and red kidney bean extracts had negatively

Table 1: Total phenol, flavonoid, carotenoid content in different polarities extracts from five legumes beans

Sample	TPC (g GAE/100 g)	TFC (g QE/100 g)	TCC (g BE/100 g)
GM 1	0.19 ± 0.01	0.99 ± 0.07	0.03 ± 0.003
PR 1	1.50 ± 0.01	2.80 ± 0.36	0.03 ± 0.001
AH 1	0.63 ± 0.03	2.16 ± 0.19	0.03 ± 0.002
PV 1	0.33 ± 0.01	2.93 ± 0.20	0.05 ± 0.010
VS 1	0.36 ± 0.02	2.58 ± 0.07	0.06 ± 0.010
GM 2	1.51 ± 0.01	4.56 ± 0.16	0.75 ± 0.010
PR 2	0.76 ± 0.07	4.35 ± 0.25	0.17 ± 0.001
AH 2	1.38 ± 0.07	3.22 ± 0.20	0.09 ± 0.003
PV 2	1.50 ± 0.03	8.32 ± 0.23	0.15 ± 0.010
VS 2	1.08 ± 0.04	8.70 ± 0.27	2.03 ± 0.080
GM 3	0.34 ± 0.01	0.37 ± 0.03	0.53 ± 0.003
PR 3	1.01 ± 0.08	0.66 ± 0.09	0.03 ± 0.001
AH 3	0.80 ± 0.03	0.44 ± 0.03	2.96 ± 0.040
PV 3	0.63 ± 0.01	0.27 ± 0.02	1.19 ± 0.002
VS 3	3.04 ± 0.13	0.49 ± 0.03	0.55 ± 0.010

GM 1 = n-hexane soybean extract, PR 2 = ethyl acetate green bean extract, AH 3 = ethanolic peanut extract, PV = red kidney bean, VS = bogor peanut, TPC = total phenolic content, TFC = total flavonoid content, TCC = total carotenoid content, GAE = gallic acid equivalent, QE = quercetin equivalent, BE = betacaroten equivalent

Table 2: Pearson's correlation coefficient of total phenolic, flavonoid, carotenoid content in various legumes beans extracts with their IC_{50} of DPPH and IC_{50} of ABTS scavenging activities

Antioxidant activities	Coefficient correlation Pearson (r)							
	TPC	TFC	TCC	IC_{50} ABTS GM	IC_{50} ABTS PR	IC_{50} ABTS AH	IC_{50} ABTS PV	IC_{50} ABTS VS
IC_{50} DPPH GM	0.992**	0.977**	0.747*	0.357 ns				
IC_{50} DPPH PR	0.964**	-0.072	-0.634*		-0.922**			
IC_{50} DPPH AH	-0.273 ns	-0.907**	0.91**			-0.446 ns		
IC_{50} DPPH PV	-0.888**	-0.510 ns	-0.242 ns				0.985**	
IC_{50} DPPH VS	-0.865**	-0.853**	0.512 ns					0.793**
IC_{50} ABTS GM	0.414 ns	0.248 ns	0.756*					
IC_{50} ABTS PR	-0.980**	0.398 ns	0.849**					
IC_{50} ABTS AH	-0.721*	0.060 ns	-0.447 ns					
IC_{50} ABTS PV	-0.941**	-0.603 ns	-0.140 ns					
IC_{50} ABTS VS	-0.390 ns	0.994**	0.928**					

IC_{50} DPPH = IC_{50} DPPH scavenging activity, IC_{50} ABTS = IC_{50} ABTS scavenging activity, GM = soybean, PR = green bean, AH = peanut, PV = red kidney bean, VS = bogor peanut, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

high correlation with their IC_{50} of ABTS scavenging activities ($r = -0.980$, $p<0.01$; $r = -0.721$, $p<0.05$; $r = -0.941$, $p<0.01$, respectively). TFC in all of extracts had no significant correlation with their IC_{50} of DPPH scavenging activities (except peanut and bogor peanut extracts) and IC_{50} of ABTS scavenging activities, and only TCC in green bean extracts had negative and high correlation with their IC_{50} of DPPH scavenging activities ($r = -0.634$, $p<0.05$).

DISCUSSION

The previous research[12-15,20,21] reported that legumes had antioxidant capacity. There were no research regarding antioxidant activity of various beans extracts (which were n-hexane, ethyl acetate and ethanol) of five legumes from West Java- Indonesia using DPPH and ABTS assays. ABTS and DPPH free radicals give characteristic absorption at wavelength 734 nm and 516 nm, respectively in ethanol and methanol. Colors of DPPH and ABTS would be changed when the free radicals were scavenged by antioxidant[18]. DPPH would be changed from purple to yellow color, while ABTS changed from turquoise to white color. The IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities in various legumes beans extracts using DPPH and ABTS assays were shown in Fig 1 and Fig 2. The IC_{50} of DPPH and IC_{50} of ABTS scavenging activities in various extracts compared to IC_{50} of ascorbic acid standard. The lowest value of IC_{50} means had the highest antioxidant activity. IC_{50} were used to determine antioxidant activity was compared to standard. Sample which had IC_{50} lower than 50 μg/ml was a very strong antioxidant, 50-100

μg/ml was a strong antioxidant, 101-150 μg/ml was a medium antioxidant, while a weak antioxidant with IC_{50} greater than 150 μg/ml[16]. In the present study exposed that IC_{50} of DPPH and IC_{50} of ABTS scavenging activities of various beans extracts of five legumes in the range of 0.96 – 134.76 μg/ml and 0.54 – 199.84 μg/ml, respectively. The lowest IC_{50} of DPPH was given by ethyl acetate beans extract of red kidney bean (PV2) 0.96 μg/ml, while IC_{50} of DPPH of ascorbic acid was 2.36 μg/ml. It figured that potency of PV2 was around three times potency of ascorbic acid using DPPH method. N-hexane beans extract of soybean (GM1) had the lowest IC_{50} of ABTS scavenging activities (0.54 μg/ml) while ascorbic acid standard showed IC_{50} of ABTS scavenging activity 0.31 μg/ml. It revealed that antioxidant potency of GM1 was similar with potency of ascorbic acid using ABTS assay. IC_{50} of DPPH and IC_{50} of ABTS scavenging activities of ethanolic beans extracts ranged from 3.13 to 29.28 μg/ml and 1.26 to 32.42 μg/ml, respectively. Based on value of IC_{50} of DPPH and IC_{50} of ABTS scavenging activity it can be concluded that all of ethanolic beans extracts of five legumes (soybean, green bean, peanut, red kidney bean and bogor peanut) can be classified as very strong antioxidant. In the previous study[15] demonstrated that IC_{50} of DPPH scavenging activities of various leaves extracts from three species of legumes (green bean, soybean and peanut) in the range of 1.9 - 197.5 μg/ml and based on value of IC_{50} of DPPH scavenging activity it can be concluded that all of ethyl acetate and ethanolic leaves extracts of three species legumes (except ethyl acetate leaves extract of peanut) can be categorized as very strong antioxidant by DPPH method. It was similar with the present study which revealed that all of ethyl acetate and ethanolic beans extracts of five legumes: soybean, green bean, peanut, red kidney bean and bogor peanut (except ethyl acetate beans extract of bogor peanut) can be classified as very strong antioxidant using DPPH and ABTS methods. In other research[12] stated that IC_{50} of DPPH scavenging activity of n-hexane, ethyl acetate and ethanolic shells extracts of four legumes were classified as very strong antioxidant. Ethanolic beans extract of soybean, green bean, peanut, red kidney bean and bogor peanut (*Vigna subterranean*) had IC_{50} of DPPH scavenging activity 3.13, 22.64, 29.28, 17.56 and 19.16 μg/ml, respectively, while in previous study stated that IC_{50} of DPPH scavenging activity of ethanolic leaves extract of green bean, soybean and peanut were 1.9, 16.3 and 17.2 μg/ml, respectively [15] and ethanolic shells extract of soybean, peanut, bogor peanut (*Vigna subterranea)* and red kidney bean gave IC_{50} of DPPH 15.5, 3.2, 12.3 and 1.6 μg/ml, respectively[12]. Based on the research above it can be concluded that ethanolic beans extract of soybean had the lowest IC_{50} of DPPH compared to its ethanolic leaves and shells extracts, while ethanolic shells extract of peanut had the lowest IC_{50} of DPPH compared to its ethanolic beans and leaves extracts. The ethyl acetate shells extract of soybean showed the lowest IC_{50} of DPPH compared to its ethyl acetate leaves extract[15] and ethyl acetate beans extract. Ethyl acetate shells extract of peanut had the lowest IC_{50} of DPPH compared to it ethyl acetate leaves extract[15] and ethyl acetate beans extract. Previous study[22] reported that the highest DPPH scavenging activities were given by 80 % acetone extract of yellow pea, green pea, chickpea and yellow soybean, while the highest FRAP capacities were showed by acidic 70 % acetone (+ 0.5% acetic acid) extract of black bean, lentil, black soybean and red kidney bean. IC_{50} of DPPH scavenging capacity of peanut seed extract of from Trabilsia cultivar had the lowest (1.55 mg/ml) compared to Massriya cultivar (720 mg/ml) and Sinya cultivar (820 mg/ml)[14]. Chon[23] revealed that soybean sprouts had lower DPPH scavenging activity than cowpea and mung bean sprouts. DPPH scavenging activity of eclipse black bean by soaking, boiling and steaming process were lower than its raw bean[4]. The presence of total phenolic content, included phenolic acid can be related with its antioxidant activity [24]. Cinnamic acid had higher antioxidant activity than benzoic acid[25]. The present study showed that TPC in ethanolic beans extract of soybean, green bean, peanut, red kidney bean and bogor peanut were 0.34, 1.01, 0.80, 0.63 and 3.04 g GAE/100 g, respectively (Table 1). It was similar with TPC in ethanolic shells extract of soybean, red kidney bean, bogor peanut and peanut were 4.00, 2.13, 1.97, 6.91 g GAE/100 g, respectively[12] and contrast with the result of the previous study[15] which demonstrated that TPC in ethanolic leaves extract of green bean, soybean and peanut were 26.2, 4.7, 25.4 g GAE/100 g, respectively. Mbagwu [26] exposed that TPC in ethanolic seeds extract of bogor peanut *V. subterranea* (0.36 %) was higher than peanut (*A. hypogaea*) and soybean (*G. max*). The previous research revealed that TPC in soybean sprouts extract was higher than cowpea and mung bean sprouts extract[23]. Research by Heimler[27] expressed that TPC in 12 samples of common beans ranged from 0.11 to 0.44 g GAE/100 g. Sebei[14] reported that TPC in peanut seed extract of Chounfakhi, Massriya, Sinya and Trabilsia varieties were 0.21, 13.5, 13.5 and 0.1 g GAE/100 g, respectively. Previous research by Xu[22] found that the highest TPC were given by 50 % acetone extract of yellow pea, green pea, chickpea and yellow soybean. Cong[28] stated that TPC in methanolic seeds extract of varieties soybean that grown in lowland (63 mg/100 g) was lower than upland (69.3 mg/100 g). TPC in peanut shells was higher than its hull, raw kernel and roasted kernel flour[9]. Xu[4] demonstrated that TPC in raw bean of eclipse black beans (*P. vulgaris*) was higher than soaking, boiling and steaming process. The present study exposed that TFC in ethanolic beans extract of five legumes (soybean, green bean, peanut, red kidney bean and bogor peanut) were 0.37, 0.66, 0.44, 0.27 and 0.49 g QE/100 g (Table 1). It was contrast with the previous study which stated that TFC of ethanolic shells extract from four species legumes (soybean, red kidney bean, bogor peanut and peanut) were 1.64, 2.26, 1.65 and 6.42 mg QE/100 g, respectively[12], and ethanolic leaves extract of green bean, soybean and peanut were 1.69, 1.42 and 1.47 mg QE/100 g, respectively [15]. The previous research exposed that TFC in ethanolic seeds extract of *V. uniguiculata* was the

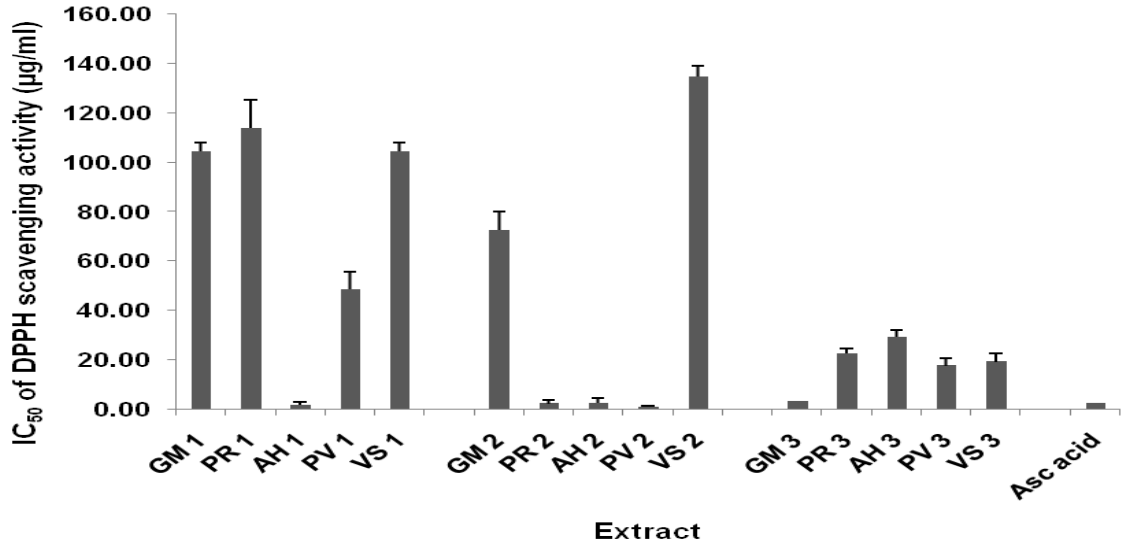

Figure 1: IC_{50} of DPPH scavenging activities in different polarities extracts from five legumes beans

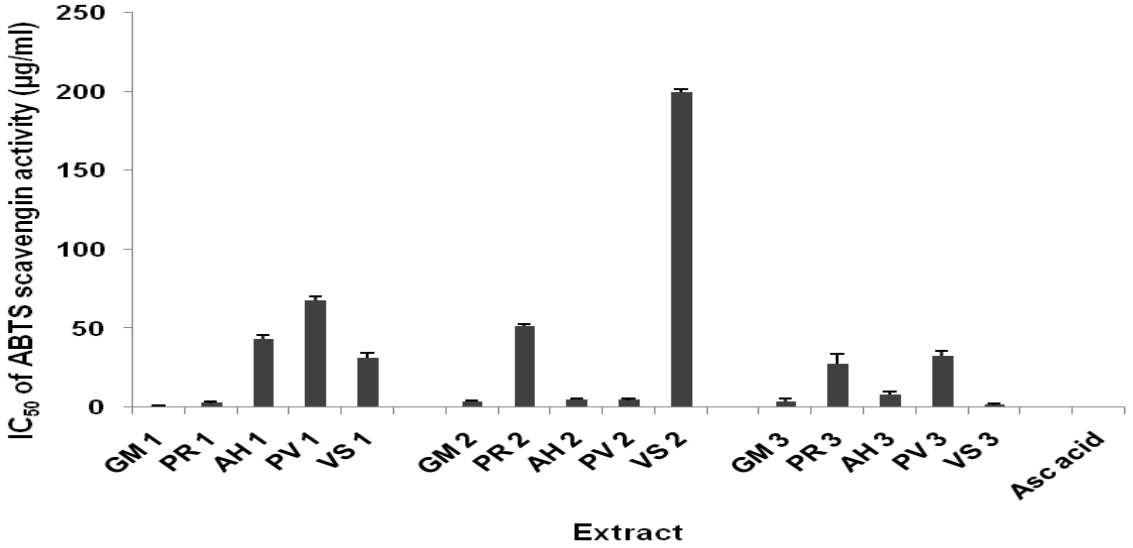

Figure 2: IC_{50} of ABTS scavenging activities in different polarities extracts from five legumes beans

highest (0.33 %), while *A. hypogea* (0.18 %) was the lowest[26]. TFC in soybean sprouts extract was higher than cowpea and mung bean[23] and ranged from 0.24 to 1.43 (+) catechin per g of dry seeds [27]. Anthocyanin content was very high (0.2 %) in acetonitrile shells extract of black colored seed coats of Vigna, Phaseolus, Glycine species[29]. Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$[10] and negatively high if $-0.61 \leq r \leq -0.97$. Sample which had the lowest IC_{50} of DPPH and ABTS scavenging activity showed the highest antioxidant activity. So the negatively and high correlation will be given in good correlation between TPC, TFC and TCC with IC_{50} DPPH or IC_{50} ABTS. It means increasing in TFC, TPC and TCC caused increasing in antioxidant activities, which was expressed by lower IC_{50} of DPPH and ABTS scavenging activity. In the present study (Table 2) it can be seen there were negative and high correlation between TPC in red kidney bean and bogor peanut extract sample with their IC_{50} of DPPH ($r = -0.888$; $r = -0.865$, $p<0.01$, respectively) and TPC in green bean, peanut and red kidney bean extracts with their IC_{50} of ABTS ($r = -0.980$, $p<0.01$; $r = 0.721$, $p<0.05$; $r = -0.941$, $p<0.01$, respectively). Based on this result it can be concluded that phenolic compounds in red kidney bean extracts were the major contributor in its antioxidant activities using DPPH and ABTS methods. TFC in bean extract and IC_{50} of DPPH for peanut and bogor peanut sample ($r = -0.907$; $r = -0.853$, $p<0.01$). In the previous research [12] Pearson's correlation coefficient which was investigated by different method with the present study. Previous study determined the correlation between TFC and percentage of DPPH scavenging activity, so the good correlation will be exposed in parallel position, increasing in TFC would give increasing in percentage of scavenging activity of DPPH. TFC in bogor peanut shells extract and peanut shells extract had high, positive and significant correlation with their percentage of DPPH scavenging activity ($r = 0.958$, $p<0.01$, $r = 0.676$, $p<0.05$). It was the same method with study by Lin [20] which demonstrated that TPC in methanolic extract of legumes had high and positive correlation with their percentage of FRAP capacity ($r = 0.9414$, $p<0.01$) and

percentage of DPPH scavenging activity ($r = 0.6885$, $p<0.05$). The previous research which expressed that TPC in methanolic extract of roasted kernel flour had good correlation with their percentage of DPPH scavenging activity ($r = 0.8436$, $p<0.01$) [9], while in previous study stated that TPC in leaves extracts of three legumes (green bean, soybean and peanut) had high and negative correlation with their IC_{50} of DPPH ($r = -0.65$, $p<0.05$; $r = - 0.975$, $p<0.01$; $r = -0.721$, $p<0.05$, respectively)[15]. TPC in ethanolic beans extracts of green bean (PR3) 1.01 g GAE/100 g was similar with TPC in ethyl acetate beans extracts of bogor peanut (VS2) 1.08 g GAE/100 g, but IC_{50} of DPPH and IC_{50} of ABTS of PR3 (22.6 µg/ml and 27.48 µg/ml, respectively) which can be categorized as a very strong antioxidant, was lower than IC_{50} of DPPH of VS2 (134.8 µg/ml) which was classified as a medium antioxidant and IC_{50} of ABTS of VS2 (199.84 µg/ml) as a weak antioxidant. Based on the data it can be predicted that many phenolic compounds in PR3 had high antioxidant activity, while many phenolic compounds in VS2 had low antioxidant activity. Flavonoid which has OH in A ring and or B ring will be included in phenolic groups. Phenolic acid had lower antioxidant capacity than flavonoid[25]. Flavonoid which had OH in ortho C 3',4', OH in C3, oxo function in C4, double bond at C2 and C3 would give higher antioxidant capacity. The ortho position of OH in C3'-C4' had the highest influence to antioxidant capacity of flavonoid. The flavonoid aglycones would give higher antioxidant capacity than flavonoid glycosides[25]. It could be seen in Table 1 that TFC in ethyl acetate beans extracts of soybean (GM2) 4.56 g QE/100 g was similar with TFC in ethyl acetate beans extracts of green bean (PR2) 4.35 g QE/100 g, but IC_{50} of DPPH scavenging activity of PR2 (2.30 µg/ml) was lower than IC_{50} of DPPH scavenging activity of GM2 (72.41 µg/ml). Based on the data above it can be predicted that many flavonoids in GM2 had OH in other position, example in C5, C7, or C3' only, or C4' only, or C3 only without oxo function in C4, that had no and low antioxidant activities. In contrast, almost all of flavonoids in PR2 were flavonoid that had OH in position which can influence high antioxidant activities. In the present study demonstrated that only TCC in green bean extracts had high and negative correlation with IC_{50} of DPPH scavenging activities ($r = -0.634$, $p<0.05$). It means increasing in TCC in green bean extracts would give increasing in antioxidant activity which was showed by lower IC_{50} of DPPH scavenging activity. In the previous research[15] there was no good correlation between TCC and IC_{50} of DPPH, EC_{50} of FRAP, because their positive and high correlation. Different method was done in previous research[12] which was revealed that shells extract of soybean and red kidney bean had positive and significant correlation with its percentage of FRAP capacity ($r =0.924$, $p<0.01$ and $r = 0.846$, $p<0.01$, respectively) and no significant and negative correlation with their percentage of DPPH scavenging activity. Carotenoid had antioxidant capacity by scavenging free radical and more double bonds in carotenoid would give higher scavenging free radical capacity[30]. Carotenoid that consisted of more than 7 double bonds gave higher scavenging radical capacity[31]. Beta carotene was used as standard because of it had conjugation double bonds which had ability to scavenge free radicals[32]. In previous study[33] stated that increasing in lipophilicity of carotenoid would increase scavenging radical activity, it means give the lower IC_{50} of DPPH scavenging activity. TCC in ethanolic beans extracts of bogor peanut (VS1) 0.06 g BE/100 g was lower than TCC in ethanolic beans extracts of red kidney bean (PV3) 1.19 g BE/100 g, but IC_{50} of DPPH scavenging activity of VS1 (31.24 µg/ml) was similar with IC_{50} of DPPH scavenging activity of PV3 (32.42 µg/ml). It can be supposed that almost all of carotenoid in PV3 consisted of maximum 7 double bonds which have low antioxidant activity and many of carotenoid in VS1 contained more than 7 double bonds which have high antioxidant activity. ABTS and DPPH methods had the same mechanism reaction, which were electron transfer assays[34]. Only beans extracts in red kidney bean and bogor peanut showed positively high correlation between IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities. So the results of the present study showed DPPH and ABTS methods gave linear result in beans extracts of red kidney bean and bogor peanut.

CONCLUSION

Antioxidant activity of sample should be measured by different methods in parallel, because various methods could give different results. Ethanolic beans extract of five legumes (soybean, green bean, peanut, red kidney bean and bogor peanut) were very strong antioxidant, using DPPH and ABTS assays. Phenolic compounds in red kidney bean extracts were the major contributor in its antioxidant activities using DPPH and ABTS methods. DPPH and ABTS methods gave linear result in red kidney bean and bogor peanut extracts. Beans extracts of five legumes (soybean, green bean, peanut, red kidney bean and bogor peanut) may be exploited as natural antioxidant sources.

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