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Research Article

Antioxidant Capacities of Various Leaves Extracts from Three Species of Legumes and Correlation with Total Flavonoid, Phenolic, Carotenoid Content

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ABSTRACT

The objectives of this research were to study antioxidant capacity from various leaves extracts of legumes using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) and correlation of total flavonoid, phenolic and carotenoid content in various leaves extracts of legumes with IC_{50} of DPPH and EC_{50} of FRAP antioxidant capacities. Extraction was performed by reflux using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant capacities using DPPH and FRAP assays, determination of total phenolic, flavonoid and carotenoid content were performed by spectrophotometry UV-visible and its correlation with IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP capacities were analyzed by Pearson's method. Ethanolic leaves extract of green bean (GB3) had the lowest IC_{50} of DPPH scavenging capacity with IC_{50} 1.9 μg/ml and the lowest EC_{50} of FRAP capacity with EC_{50} 46.9 μg/ml. Ethyl acetate leaves extract of green bean (GB2) contained the highest total flavonoid (6.7 g QE/100 g), ethanolic leaves extract of green bean (GB3) the highest phenolic content (26.2 g GAE/100 g) and n-hexane leaves extract of soybean (SB1) had the highest total carotenoid 18.42 g BE/100 g. There were negatively and high correlation between total phenolic content in soy bean and peanut leaves extracts with their IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities. All of leaves extracts from three species of legumes had linear result in DPPH and FRAP assays.

Keywords: Antioxidant, DPPH, FRAP, leaves, legumes, flavonoid, phenolic, carotenoid

INTRODUCTION

Oxidative stress related to the risk of many diseases which can be reduced by antioxidant. Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antibacterial, anti-inflammatory and antioxidant activity[1-4]. Many studies[5-8] demonstrated that phenolic content and flavonoid content in plants could be correlated to their antioxidant activities. Plants including legumes contained phenolic and polyphenol compounds can act as antioxidant[9-11].

Some of antioxidant methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) were used to predict antioxidant capacity of vegetables, fruits and food[4,12]. In previous study[4,13] exhibited that DPPH and FRAP methods could be used to determine antioxidant activity in many plants extracts. The previous study[6-8,14] exposed that legumes had antioxidant capacities by using DPPH, FRAP and ABTS assays.

The objective of this research were to study antioxidant capacities of different polarities extracts (n-hexane, ethyl acetate and ethanol) of leaves from three species of legumes (green bean *Phaseolus radiatus*, soybean *Glycine max* and peanut *Arachis hypogaea*) using antioxidant testing DPPH and FRAP assays and correlations of their antioxidant capacities with total flavonoid, phenolic, and carotenoid content in each extracts.

MATERIALS AND METHODS

Materials

TPTZ (2,4,6-tripyridyltriazine), DPPH (2,2-diphenyl-1-picrylhydrazyl), gallic acid, quercetin, beta carotene was purchased from Sigma-Aldrich (MO, USA), ferric chloride, leaves from three species of legumes, ethanol. All other reagents were analytical grades.

Preparation of sample

Leaves from three species of legumes were: green bean (*Phaseolus radiatus*) namely as GB collected from Muaro Jambi, soybean (*Glycine max*) namely as SB from Dhamasraya-West Sumatra, peanut (*Arachis hypogaea*) as sample PN from Purwakarta-West Java, were thoroughly washed with tap water, wet sortation, cut, dried and grinded into powder.

Extraction

Three hundred grams of powdered samples were extracted by reflux using increasing gradient polarity solvents. The n-hexane extract was repeated three times. The remaining residue was then extracted three times

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with ethyl acetate. Finally the remaining residue was extracted three times with ethanol. So there were three n-hexane extracts (namely GB1, SB1 and PN1), three ethyl acetate extracts (GB2, SB2 and PN2) and three ethanolic extracts (GB3, SB3 and PN3).

IC_{50} of DPPH scavenging activity

Preparation of DPPH solution was adopted from Blois[15] with minor modification. Various concentration of each extract were 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 FRAP capacity

Preparation of FRAP solution was adopted from Benzi[17]. The FRAP solution were prepared in acetate buffer pH 3.6. Various concentration of each extract were pipetted into FRAP 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 593 nm by using spectrophotometer UV-Vis Hewlett Packard 8435. Acetate buffer was used as a blank. FRAP 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 capacity of each extracts were determined based on increasing in Fe (II) - TPTZ absorbance by calculating percentage of antioxidant capacity[17]. EC_{50} of FRAP 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.*[18]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Standard solution of quercetin with concentration 36-120 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)

Total phenolic content were measured using the modified Folin-Ciolcalteu method adapted from Pourmorad [19]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Standard solution of gallic acid with concentration 40-165 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 adapted from Thaipong *et al.*[4]. Each extract were 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 15-55 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 (±SD) 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 capacities were made using the Pearson procedure ($p < 0.01$).

RESULTS

IC_{50} of DPPH scavenging capacity and EC_{50} of FRAP capacity

The IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP capacities in various leaves extracts from three species of legumes using DPPH and FRAP assays were shown in Fig 1 and Fig 2. IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP 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 capacity.

TFC in various leaves extracts from three species of legumes

TFC among the various extracts were revealed 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 from three species of legumes showed different result in the range of 1.42 – 6.7 g QE/100 g (Fig 3). Ethyl acetate leaves extract of green bean (GB2) had the highest total flavonoid content (6.7 g QE/100 g) and the lowest (1.42 g QE/100 g) was given by ethanolic leaves extract of soybean (SB3) leaves extract.

TPC in various leaves extracts from three species of legumes

TPC among the various extracts were exposed in term of gallic acid equivalent using the standard curve equation $y = 0.004 x + 0.0025$, $R^2 = 0.998$. The TPC in various leaves extracts from three species of legumes showed different result ranged from 1.12 to 26.2 g GAE/100 g. GB3 (ethanolic leaves extract of green bean) had the highest phenolic content (26.2 g GAE/100 g) (Fig 4).

TCC in various leaves extracts from three species of legumes

TCC among the various extracts were expressed in term of beta carotene equivalent using the standard curve equation $y = 0.015x + 0.002$, $R^2 = 0.9999$. The TCC in various leaves extracts from three species of legumes showed different result in the range of 0.14 – 18.42 g BET/100 g (Fig 5). The highest carotenoid content (18.42 g BE/100 g) was given by n-hexane leaves extract of soybean (SB1), while the lowest carotenoid (0.14 g BE/100 g) for ethanolic leaves extract of soybean (SB3).

Correlations between IC_{50} of DPPH scavenging ativities, EC_{50} of FRAP capacities, total phenolic, flavonoid and

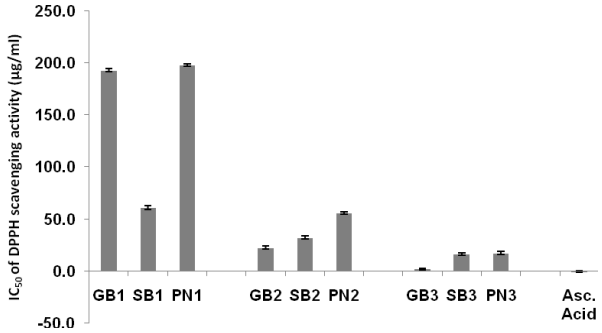

Fig 1: IC_{50} of DPPH scavenging capacities in various leaves extracts from three species of legumes

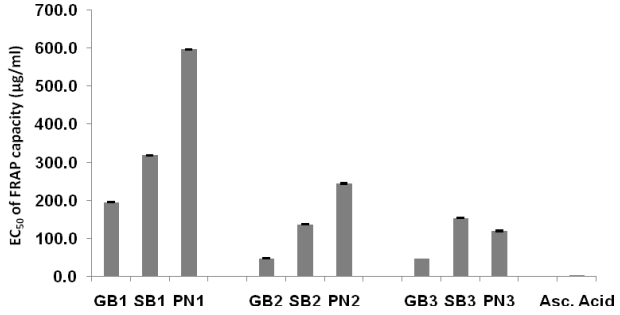

Fig 2: EC_{50} of FRAP capacities in various leaves extracts from three species of legumes

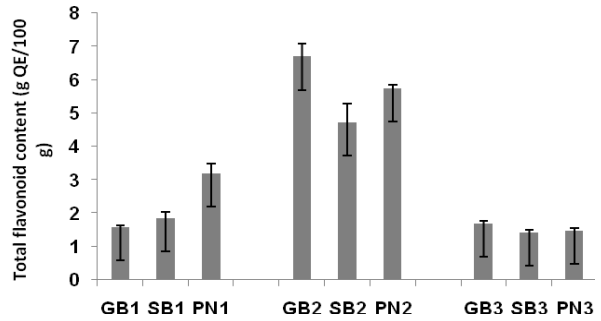

Fig 3: Total flavonoid content in various legumes leaves extracts

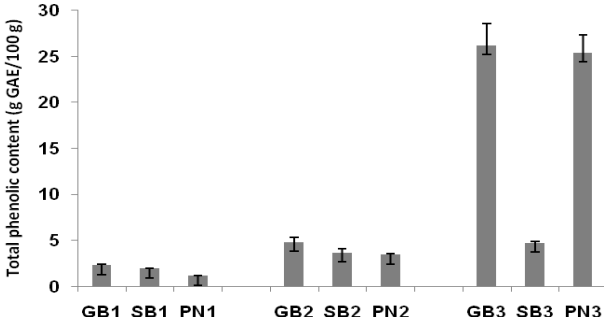

Fig 4: Total phenolic content in various legumes leaves extracts

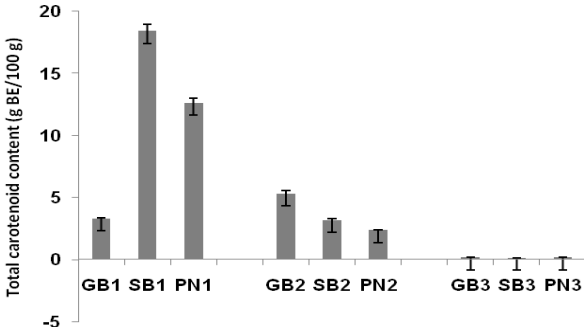

Fig 5: Total carotenoid content in various legumes leaves extracts

carotenoid content in various leaves extracts from three species of legumes

Pearson's correlation coefficient between TFC in various leaves extracts of three species of legumes and their antioxidant activities demonstrated that TFC had no significant correlation with IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities. TPC in sample GB, PN and SB had negative and high correlation with their IC_{50} of DPPH scavenging activities ($r = -0.65$; $r = -0.721$, $p<0.05$, $r = 0.975$, $p<0.01$) and only sample SB and PN had negatively high correlation with EC_{50} of FRAP capacities ($r = 0.871$, $p <0.01$, and $r = 0.757$, $p<0.05$) (Table 4).

DISCUSSION

The previous study[2, 6-7, 11, 20-23] revelaed that legumes had antioxidant capacity. There were no study regarding antioxidant capacity of different polarities extracts (which were n-hexane, ethyl acetate and ethanol) of leaves from three species of legumes using DPPH and FRAP 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[24-25]. Reagent of FRAP is $FeCl_3$ that combined with TPTZ in acetate buffer pH 3.6. Fe (III) will be reduced to Fe (II). Complex Fe (II) - TPTZ gives blue color and show characteristic absorption at wavelength 593 nm. Intensity of blue color depends on amount of Fe (III) that is reduced to Fe (II). If a sample reduces Fe (III) to Fe (II), at the same time it will be oxidized, so that sample can act as antioxidant. Sample will act as antioxidant in FRAP assays if sample had reduction potential lower than reduction potential of Fe (III)/Fe (II) which was 0.77 V, so the sample had the reducing power to reduce Fe (III) to Fe (II) 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 FRAP capacity is concentration of sample or standard

Table 1. Pearson's correlation coefficient of IC_{50} of DPPH scavenging activities, EC_{50} of FRAP capacities and total flavonoid, phenolic, carotenoid content in various leaves extracts of three species of legumes

	TFC	TPC	TCC	IC_{50} DPPH GB	IC_{50} DPPH SB	IC_{50} DPPH PN
EC_{50} FRAP GB	-0.43^{ns}	-0.65*	0.218^{ns}			
EC_{50} FRAP SB	-0.049^{ns}	-0.975**	0.972**			
EC_{50} FRAP PN	0.091^{ns}	-0.721*	0.999**			
EC_{50} DPPH GB	-0.508^{ns}	-0.581^{ns}	0.13^{ns}	0.996**		
EC_{50} DPPH SB	-0.454^{ns}	-0.871**	0.973**		0.903**	
EC_{50} DPPH PN	0.143^{ns}	-0.757*	0.995**			0.999**

Note: IC_{50} DPPH = IC_{50} DPPH scavenging capacity, EC_{50} FRAP = EC_{50} FRAP capacity, GB = leaves extract of GB, SB = leaves extract of SB, PN = leaves extract of PN, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

that can exhibit 50 % of FRAP 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] stated that sample which had IC_{50} or EC_{50} lower than 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} higher than 150 µg/ml. In the present study exposed that IC_{50} of DPPH scavenging capacities of various leaves extracts from three species of legumes ranged from 1.9 to 197.5 µg/ml. Ethanolic leaves extract of green bean (GB3) had the lowest IC_{50} of DPPH scavenging capacity 1.9 µg/ml, while ascorbic acid standard gave IC_{50} of DPPH scavenging capacity 0.11 µg/ml. Based on value of IC_{50} of DPPH scavenging capacity it can be concluded that all of ethyl acetate and ethanolic leaves extracts of legumes (green bean, soybean and peanut) can be categorized as very strong antioxidant. Its demonstrated that potency of ascorbic acid was around twenty times of ethanolic leaves extract of green bean (GB3) using DPPH method. Ethanolic leaves extract of green bean (GB3) had the lowest EC_{50} of FRAP capacity (46.9 µg/ml) while ascorbic acid standard gave EC_{50} of FRAP capacity 3.72 µg/ml. Its expressed that potency of ascorbic acid was around fifteen times of potency of GB3 using FRAP assay. In the previous study[11] exposed that n-hexane, ethyl acetate and ethanolic shells extract of peanut (*A. hypogea*) had IC_{50} of DPPH scavenging capacities were 6, 1.5 and 39 µg/ml, respectively. It was contrast with the current research showed that n-hexane, ethyl acetate and ethanolic leaves extract of peanut were 197, 55 and 17µg/ml. The previous research by Sebei[2] revealed that seed extract of peanut from Trabilsia cultivar had the lowest IC_{50} of DPPH scavenging capacity (1.55 mg/ml) compared to Massriya cultivar (720 mg/ml) and Sinya cultivar (820 mg/ml). The present study exposed that ethanolic leaves extract of green bean had the highest antioxidant capacity compared to ethanolic leaves extract of soy bean and peanut, which had the lowest IC_{50} of DPPH scavenging activity (1.9 µg/ml), while the previous study[11] revealed that ethanolic shells extract of soybean, peanut, bogor peanut (*Vigna subterranean)* and red kidney bean were 58, 31, 30, 39 µg/ml, respectively. In research by Xu[5] demonstrated that DPPH scavenging activity of eclipse black bean with soaking, boiling and steaming process were lower than raw bean, while Chon[26] exhibited that cowpea and mung bean sprouts had higher DPPH scavenging capacity than soybean sprouts. Previous research[8] revealed that 80 % acetone extract exposed that the highest DPPH scavenging activities were given by yellow pea, green pea, chickpea and yellow soybean, while in acidic 70 % acetone (+ 0.5% acetic acid) extract had the highest FRAP capacities for black bean, lentil, black soybean and red kidney bean. Antioxidant capacity might be related with the presence of total phenolic content, included phenolic acid[10]. Cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid[27]. The previous study[11] found that 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, while in the present study showed that TPC in ethanolic leaves extract of green bean, soybean and peanut were 26.2, 4.7, 25.4 g GAE/100 g, respectively. In contrast with the previous research[28] revealed that ethanolic seeds extract of bogor peanut *V. subterranea* (0.36 %) was higher than peanut *A. hypogea* and soybean *G. max*. Study by Chon[26] exhibited that TPC in soybean sprouts extract was higher than cowpea and mung bean sprouts extract. Previous research[2] exposed that seed of Chounfakhi, Massriya, Sinya and Trabilsia varieties had total phenolic 0.21, 13.5, 13.5 and 0.1 g GAE/100 g, respectively. Previous study by Cong[29] showed that TPC in methanolic seeds extract of varieties soybean that grown in upland (69.3 mg/100 g) was higher than lowland (63 mg/100 g). Research by Heimler[23] exposed that TPC in 12 samples of common beans in the range of 0.11-0.44 g GAE/100 g. Study by Xu[8] revealed that 50 % acetone extract had the highest TPC for yellow pea, green pea, chickpea and yellow soybean. TPC in peanut skin was higher than its hull, raw kernel and roasted kernel flour[7]. Xu[5] stated that TPC in raw bean of eclipse black beans (*P. vulgaris*) was higher than soaking, boiling and steaming process, while Yao[14] demonstrated that five black mung bean (*V. radiata*) had free phenolic acid and bound phenolic acid content ranged from 1.66 to 25.5 mg/100 g and ranged from 228.4 to 536.3 mg/100 g, respectively. The previous study[11] 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, while the present research exhibited that

ethanolic leaves extract of green bean, soybean and peanut were 1.69, 1.42 and 1.47 mg QE/100 g, respectively. Mbagwu[28] exposed that TFC in ethanolic seeds extract of *A. hypogea* (0.18 %) was the lowest, while *V. uniguiculata* was the highest (0.33 %). TFC in soybean sprouts extract was higher than cowpea and mung bean[26] and ranged from 0.24 to 1.43 (+) catechin per g of dry seeds[23]. Yoshida[30] revealed that flavonoid especially anthocyanin content was very high (0.2 %) in acetonitrile shells extract of black colored seed coats of Vigna, Phaseolus, Glycine species. Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$[4] 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 FRAP capacity gave the highest antioxidant activity. So the good correlation between IC_{50} DPPH or EC_{50} FRAP with TPC, TFC and TCC will be given in negatively and high correlation. It means increasing in TFC, TPC and TCC caused increasing in antioxidant activities, which was expressed by lower IC_{50} of DPPH scavenging activity and or EC_{50} of FRAP capacity. The data in Table 1 exposed that the highest and negatively correlation between TPC in leaves extract and IC_{50} of DPPH scavenging activities was given by sample SB ($r = -0.975$, $p<0.01$) and the highest and negatively correlation TPC in leaves extract and EC_{50} of FRAP capacity ($r = -0.871$, $p<0.01$) was given by sample SB also and only TPC in leaves extract of GB had no correlation with EC_{50} of FRAP capacities ($r = -0.581$). In the previous research by Fidrianny[11] Pearson's correlation coefficient which was investigated was different with the current study. Previous study[11] determined the correlation between TFC and percentage of DPPH scavenging activity, so the good correlation would 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 ($r = 0.958$, $p<0.01$, $r = 0.676$, $p<0.05$). Research by Win[7] expressed that TPC in methanolic extract of roasted kernel flour had good correlation with their percentage of DPPH scavenging activity ($r = 0.8436$, $p<0.01$). Lin[6] exhibited 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 their total phenolic content positively and high correlation with percentage of DPPH scavenging activity ($r = 0.6885$, $p<0.05$). TPC in ethyl acetate leaves extract of green bean (GB2) 4.79 g GAE/100 g was lower than TPC in ethanolic leaves extract of green bean (GB3) 26.2 g GAE/100 g, but EC_{50} of FRAP capacity of GB2 (48.1 µg/ml) was similar with EC_{50} of FRAP capacity of GB3 (46.9 µg/ml). Based on the data it can be supposed that many phenolic compounds in GB3 which had reduction potential (E^o) below 0.44 V, so it can not reduce Fe(III) to Fe(II) and all of phenolic compounds in GB2 had reduction potential above 0.44 V that can reduce Fe(III) to Fe(II) and form complex with TPTZ then given blue color. Tannins, flavonoid, phenolic acid, 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. Phenolic acid had lower antioxidant capacity than flavonoid[27]. 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 OH with ortho position in C3'-C4' had the highest influence to antioxidant capacity of flavonoid. The flavonoid aglycones would give higher antioxidant capacity than flavonoid glycosides[27]. It could be seen in Fig 3 that TFC in n-hexane leaves extract of green bean (GB1) 1.58 g QE/100 g was similar with TFC in ethanolic extract of green bean (GB3) 1.69 g QE/100 g, but IC_{50} of DPPH scavenging activity of GB3 (1.9 µg/ml) was lower than IC_{50} of DPPH scavenging activity of GB1 (192 µg/ml). Based on the data above it can predicted that many flavonoids in GB1 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 capacities. In contrast, almost all of flavonoid in GB3 were flavonoid that had OH in position which can influence high antioxidant capacities. In the present study exposed that TCC in leaves extract of soybean and peanut had positive and high correlation with IC_{50} of DPPH scavenging activities ($r = 0.972$, $r = 0.999$, $p<0.01$, respectively). It means increasing in TCC would give increasing in IC_{50} of DPPH scavenging activity and there were no good correlation between TCC and IC_{50} of DPPH scavenging capacities. In the current study there were no good correlation between TCC and EC_{50} of FRAP capacity, because of their positive and high correlation. Previous research[11] revealed that TCC in 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. More double bonds in carotenoid would give higher scavenging free radical capacity[31]. Carotenoid that consisted of more than 7 double bonds gave higher scavenging radical capacity[32]. Beta carotene was used as standard because of it had conjugation double bonds which had ability to scavenge free radicals[33]. In previous study[34] exposed that increasing in lipophilicity of carotenoid would increase scavenging radical capacity, it means give the lower IC_{50} of DPPH scavenging capacity. TCC in ethanolic leaves extract of green bean (GB3) 0.16 g BE/100 g was similar with TCC in ethanolic extract of peanut (PN3) 0.17 g BE/100 g, but IC_{50} of DPPH scavenging activity of GB3 (1.9 µg/ml) was lower than IC_{50} of DPPH scavenging activity of PN3 (17.2 µg/ml). It can be supposed that many of carotenoid in GB3 contained more than 7 double bonds and almost all of carotenoid in PN3 consisted maximum 7 double bonds.

Lycopene was effective to reduce Fe (III), because of it had 11 conjugated double bonds. Carotenoid such as phytoene, phytofluene, neurosporene that consisted of 3, 5 and 9 conjugated double bonds respectively, did not show significant capacity to reduce Fe (III)[35]. Ethyl

acetate leaves extract of soybean (SB2) 3.19 g BE/100 g was higher than ethanolic leaves extract of soybean (SB3) 0.14 g BE/100 g, but EC_{50} of FRAP capacity of SB2 (137 µg/ml) was similar with EC_{50} of FRAP capacity of SB3 (154 µg/ml). Based on the data it can be predicted that many carotenoid in SB3 had reduction potential lower than 0.44 V, so it could be reduced Fe(III) to Fe(II), and at the same time the carotenoid will be oxidized. Then it can act as antioxidant. FRAP and DPPH methods had different mechanism reaction. Mechanism of DPPH that was electron transfer assays[36] and FRAP was redox assays[17]. All of leaves extracts sample (green bean, soybean and peanut) expressed positively high correlation between IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities. So the results of this study showed that IC_{50} of DPPH scavenging activities in all of extracts sample were linear with their EC_{50} of FRAP capacities.

CONCLUSION

Variety of methods should be used in parallel to assess the antioxidant capacity of sample, because different methods could give different results. Ethyl acetate and ethanolic leaves extracts of green bean, soybean and peanut were very strong antioxidant. The negatively and high correlation between TPC with IC_{50} of DPPH scavenging capacities was given by all of leaves extracts sample. Only soybean and peanut leaves extracts had negatively high correlation with EC_{50} of FRAP capacity. Phenolic compounds in soybean and peanut leaves extract were the major contributor in IC_{50} of DPPH scavenging capacity and EC_{50} of FRAP capacity. There were linear correlation between IC_{50} of DPPH scavenging capacities and EC_{50} FRAP capacities result in all of leaves extracts sample. Green bean, soybean and peanut leaves extracts may be exploited as sources of natural antioxidant to alleviate oxidative stress.

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