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Research Article

ANTIOXIDANT ACTIVITIES, TOTAL FLAVONOID, PHENOLIC, CAROTENOID OF VARIOUS SHELLS EXTRACTS FROM FOUR SPECIES OF LEGUMES

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ABSTRACT

Objectives: The objectives of this research were to study antioxidant capacity from various extracts of legumes shells using two methods of antioxidant testing, which were 2,2-diphenyl-1 picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) and correlation of total flavonoid, phenolic, and carotenoid content in various extracts of legumes shells with DPPH and FRAP antioxidant capacities.

Materials and Methods: Extraction was performed by reflux using different polarity solvents. The extracts were vaporated using rotavapor. 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 DPPH and FRAP antioxidant capacities was analyzed by Pearson method.

Results: TNH2 (ethyl acetate shells extract of peanut) had the highest DPPH scavenging capacity with IC_{50} 0.595 mg/mL, while MRH2 (ethyl acetate shells extract of red kidney bean) had the highest FRAP capacity with EC_{50} 294.781 mg/mL. TNH2 contained the highest total flavonoid (13.37 g quercetin equivalents/100 g), TNH3 (ethanolic shells extract of peanut) had the highest phenolic content (6.91 g gallic acid equivalents/100 g), and KDL2 (ethyl acetate shells extract of soybean) had the highest carotenoid 0.33 g beta-carotene equivalent/100 g.

Conclusions: There were positively and high correlation between total phenolic and total flavonoid content in Bogor peanut and peanut shells extracts with their antioxidant activity using DPPH assays. FRAP capacities in all of the shells extracts had no linier result with DPPH scavenging capacities.

Keywords: Antioxidants, 2,2-diphenyl-1 picrylhydrazyl, Ferric reducing antioxidant power, Legumes Shells, Flavonoid, Phenolic, Carotenoid.

INTRODUCTION

Antioxidant were known to have beneficial effects to reduce the risk of many diseases that related to oxidative stress. 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 [5-9] exhibited that phenolic content and flavonoid content in plants could be correlated to their antioxidant activities. Plants including vegetables contained phenolic and polyphenol compounds can act as antioxidant [10,11].

Some of antioxidant methods such as 2,2-diphenyl-1 picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) were used to predict antioxidant capacity of vegetables, fruits, beverages, and food [1]. In the previous study, [1,12] revealed that DPPH and FRAP methods could be used to determine antioxidant activity in many plants extracts. The previous study [5-7,13] demonstrated that legumes had antioxidant capacities using DPPH, FRAP, and ABTS assays.

The objective of this research was to study antioxidant capacities of various extracts (n-hexane, ethyl acetate, and ethanol) of shells from four species of legumes (soybean *Glycine max,* red kidney bean *Phaleosus vulgaris,* Bogor peanut *Vigna subterranea*, 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 extract.

MATERIALS AND METHODS

Materials

2,4,6-tripyridyltriazine (TPTZ), DPPH, gallic acid, quercetin, beta-carotene were purchased from Sigma-Aldrich (MO, USA), ferric chloride, shells from four species of legumes, methanol, ethanol. All other reagents were analytical grades.

Preparation of sample

Shells from four species of legumes were: Soybean (*G. max*) namely as KDL collected from Bogor, red kidney bean (*P. vulgaris)* as sample MRH from Garut, Bogor peanut (*V. subterranea*) as sample BGR from Sukabumi, peanut (*A. hypogaea*) as sample TNH from Kendal-Semarang, were thoroughly washed with tap water, wet sortation, cut, dried, and grinded into powder.

Extraction

A total of 300 g 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 with ethyl acetate. Finally, the remaining residue was extracted three times with ethanol. Hence, there were four n-hexane extracts (namely KDL1, MRH1, BGR1, and TNH1), four ethyl acetate extracts (KDL2, MRH2, BGR2, and TNH2) and four ethanolic extracts (KDL3, MRH3, BGR3, and TNH3).

Determination of DPPH scavenging capacity

Preparation of DPPH solution was adopted from Molyneux [14] and Blois [15] with minor modification. Each extract 50 µg/mL was pipetted into DPPH solution with concentration 50 µg/mL (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 516 nm using spectrophotometer UV-Vis Hewlett Packard 8435. Methanol was used as a blank and DPPH solution 50 µg/mL 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 [14,16].

Determination of FRAP capacity

Preparation of FRAP solution was adopted from Benzi and Strain [17]. FRAP solution was prepared in acetate buffer pH 3.6. Each extract

50 µg/mL was pipetted into FRAP solution 50 µg/mL (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 593 nm using spectrophotometer UV-Vis Hewlett Packard 8435. Acetate buffer was used as a blank and FRAP solution 50 µg/mL was used as a standard. Analysis was performed in triplicate for standard and each extract. Antioxidant capacity of each extract was determined based on increasing in Fe (II) - TPTZ absorbance by calculating percentage of antioxidant capacity [17].

Determination of total flavonoid content

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 solutions of quercetin with concentration 40-160 µg/mL were used to obtain a standard curve. The total flavonoid content was reported as a percentage of total quercetin equivalents per 100 g extract (g QE/100 g).

Determination of total phenolic content

Total phenolic content was measured using the modified Folin-Ciolcalteu method adapted from Pourmorad *et al*. [19]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Standard solutions of gallic acid with concentration 60-150 µg/mL were used to obtain a standard curve. The total phenolic content was reported as a percentage of total gallic acid equivalents per 100 g extract (g GAE/100 g).

Determination of total carotenoid content

Total carotenoid content was measured using the modified carotene method adapted from Thaipong *et al.* [1]. Each extract was diluted in n-hexane. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extract. Standard solutions of beta-carotene with concentration 10-40 µg/mL were used to obtain a standard curve. The total carotenoid content was reported as a percentage of total beta-carotene equivalents per 100 g extract (g BET/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* least significant difference procedure was carried out with SPSS 16.0 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

Antioxidant capacities of various shells extracts from four species of legumes using DPPH and FRAP assays

The antioxidant capacities using DPPH and FRAP assays of various shells extracts from four species of legumes were shown in Tables 1-3. In the DPPH method, free radical scavenging capacities of various shells extracts from four species of legumes ranged from 49.74% to 75.97%. TNH3 (ethanolic shells extract of peanut) had the highest DPPH radical scavenging capacity (75.97 %), while KDL1 (n-hexane shells extract of soybean) had the lowest DPPH antioxidant capacity (49.74 %).

In FRAP method, antioxidant capacities in the range of 3.04-16.45 %. MRH1 (n-hexane shells extract of red kidney bean) had the highest FRAP capacity (16.45 %), while the lowest capacity (3.04 %) was given by MRH2 shells extract.

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 extract from four species of legumes shells using DPPH and FRAP assays were shown in Figs. 1 and 2. IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP capacities of each extract were compared with IC_{50} and EC_{50} ascorbic acid as standard. The lowest EC_{50} or IC_{50} means had the highest antioxidant capacity.

Table 1: DPPH scavenging capacities and FRAP capacities of n-hexane shells extracts

Sample	DPPH scavenging capacity (%)	FRAP capacity (%)
KDL1	49.74±0.10[a]	5.06±0.06[a]
MRH1	51.94±0.22[b]	16.45±0.11[b]
BGR1	50.55±1.88[a]	14.06±2.28[b]
TNH1	50.86±0.76[a]	4.11±1.28[a]
Ascorbic acid	97.14±0.10	37.91±0.11
p value	<0.05	<0.05

[a, b]Within a column with the different letter were significantly different ($p<0.05$).
DPPH: 2,2-diphenyl-1 picrylhydrazyl, FRAP: Ferric reducing antioxidant power

Table 2: DPPH scavenging capacities and FRAP capacities of ethyl acetate shells extracts

Sample	DPPH scavenging capacity (%)	FRAP capacity (%)
KDL2	53.80±0.88[a]	8.49±0.68[a]
MRH2	58.72±0.53[b]	3.04±0.09[b]
BGR2	66.83±0.02[c]	4.89±0.08[c]
TNH2	68.90±1.05[d]	5.91±0.06[d]
Ascorbic acid	97.14±0.10	37.91±0.11
p value	<0.05	<0.05

[a-d]Within a column with the different letter were significantly different ($p<0.05$).
DPPH: 2,2-diphenyl-1 picrylhydrazyl, FRAP: Ferric reducing antioxidant power

Table 3: DPPH scavenging capacities and FRAP capacities of ethanolic shells extracts

Sample	DPPH scavenging capacitiy (%)	FRAP capacity (%)
KDL3	55.47±0.91[a]	4.76±0.64[a]
MRH3	69.02±2.58[b]	8.08±1.89[a]
BGR3	59.55±0.68[c]	4.75±1.24[a]
TNH3	75.97±0.66[d]	5.38±1.19[a]
Ascorbic acid	97.14±0.10	37.91±0.11
p value	<0.05	<0.05

[a-d]Within a column with the different letter were significantly different ($p<0.05$).
DPPH: 2,2-diphenyl-1 picrylhydrazyl, FRAP: Ferric reducing antioxidant power

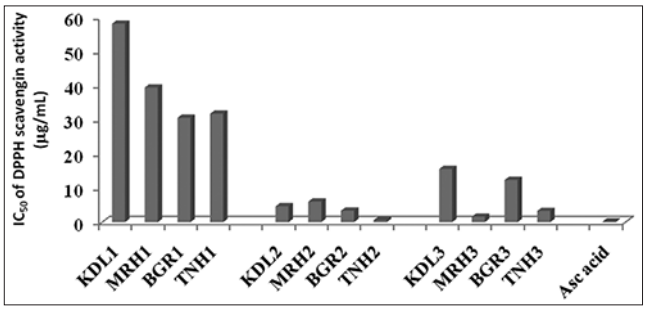

Fig. 1: IC_{50} of 2,2-diphenyl-1 picrylhydrazyl scavenging capacities in various shells extracts from four species of legumes

Total flavonoid in various shells extracts from four species of legumes

The total flavonoid content among the various extracts was expressed in terms of QE using the standard curve equation $y=0.034x-0.31$, $R^2=0.998$. The total flavonoid content in various shells extracts from four species of legumes showed a different result in the range of 1.64-13.37 g QE/100 g (Fig. 3). TNH2 (ethyl acetate shells extract of peanut) had the highest total flavonoid content (13.37 g QE/100 g) and the lowest (1.64 g QE/100 g) for KDL3 shells extract.

Total phenolic in various shells extracts from four species of legumes

The total phenolic content among the various extracts was expressed in terms of GAE using the standard curve equation y=0.0050 x+0.0475, R^2=0.9570. The total phenolic content in various shells extracts from four species of legumes showed a different result ranged from 0.54 to 6.91 g GAE/100 g. TNH3 (ethanolic shells extract of peanut) had the highest phenolic content (6.91 g GAE/100 g) (Fig. 4).

Total carotenoid in various shells extracts from four species of legumes

The total carotenoid content among the various extracts was expressed in term of BET using the standard curve equation y=0.017x+0.0008, R^2=0.998. The total carotenoid content in various shells extracts from four species of legumes showed a different result in the range of 0.026-0.33 g BET/100 g (Fig. 5). The highest carotenoid content (0.33 g BET/100 g) for KDL2 shells extract, while the lowest carotenoid (0.026 g BET/100 g) for TNH1 shells extract.

Correlations between total phenolic, flavonoid, carotenoid content, and DPPH scavenging capacities, FRAP capacities in various shells extracts from four species of legumes

Pearson's correlation coefficient was positively high if 0.68 ≤ r ≤ 0.97 [1]. The highest and positive correlation between total phenolic content and DPPH scavenging activity (r=0.985, p<0.01) for sample MRH, followed by sample TNH (r=0.984, p<0.01) (Table 4).

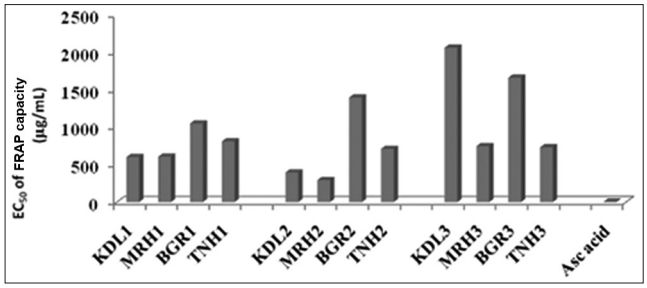

Fig. 2: EC_{50} of ferric reducing antioxidant power capacities in various shells extracts from four species of legumes

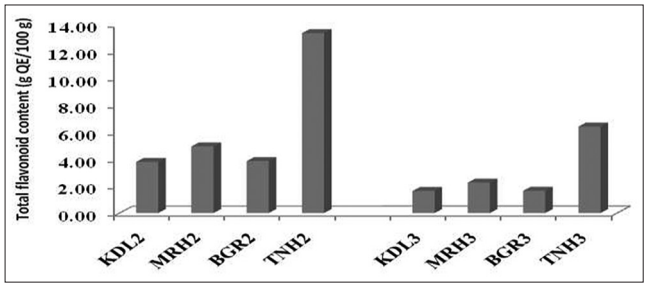

Fig. 3: Total flavonoid content in various legumes shells extracts

Pearson's correlation coefficient between total flavonoid form various extracts of four species of legumes and their antioxidant capacities demonstrated that only total flavonoid in sample BGR (Bogor peanut shells extract) and TNH (peanut shells extract) had positively high correlation with DPPH scavenging capacities (r=0.958, p<0.01, and r=0.676, p<0.05).

The correlation between total carotenoid and their antioxidant capacities exposed that only sample KDL (soybean shells extract) and MRH (red kidney bean shells extract) had high and positive correlation with FRAP capacities (r=0.924, p<0.01) and r=0.846, p<0.01).

DISCUSSION

A study by Sebei [3], Win [5], Lin [6], Halvorsen [20], Kahkonen [21], Petchiammal [22], Heimler [23] exposed that legumes had antioxidant capacity. There was no study regarding antioxidant capacity of three various extracts (which were n-hexane, ethyl acetate and ethanol) of shells from four species of legumes using DPPH and FRAP assays.

Colors of DPPH would be changed when the free radicals were scavenged by antioxidant [24,25]. The strength of DPPH method is fast and simple method. The weakness is DPPH can soluble in the alcohol solvent, so that lipophilic compound cannot react well with DPPH solution. 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 shows characteristic absorption at wavelength 593 nm. Sample will act as antioxidant in FRAP assays if sample had reduction potential was lower than reduction potential of Fe (III)/Fe (II) that was 0.77 V, so the sample

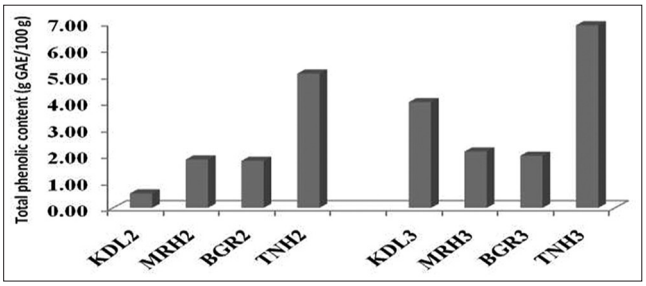

Fig. 4: Total phenolic content in various legumes shells extracts

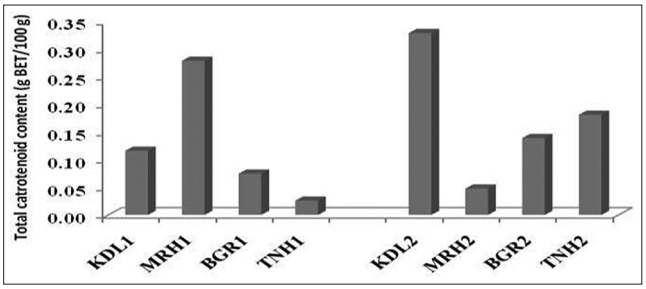

Fig. 5: Total carotenoid content in various legumes shells extracts

Table 4. Pearson's correlation coefficient of total flavonoid, phenolic, carotenoid of shells extract from four species of legumes and DPPH scavenging capacities, FRAP capacities

	Total flavonoid	Total phenolic	Total carotenoid	FRAP KDL	FRAP MRH	FRAP BGR	FRAP TNH
DPPH KDL	−0.541^{ns}	0.782*	−0.122^{ns}	0.143^{ns}			
DPPH MRH	−0.340^{ns}	0.985**	−0.872**		−0.484^{ns}		
DPPH BGR	0.958**	0.844**	0.392^{ns}			−0.840**	
DPPH TNH	0.676*	0.984**	0.114^{ns}				0.584^{ns}
FRAP KDL	0.805**	−0.436^{ns}	0.924**				
FRAP MRH	−0.946**	−0.859**	0.846**				
FRAP BGR	−0.767*	−0.966**	0.054^{ns}				
FRAP TNH	0.650^{ns}	0.592^{ns}	0.425^{ns}				

FRAP: FRAP capacity, DPPH: DPPH scavenging capacity, KD: Shells extract of KDL, MRH: Shells extract of MRH, BGR: Shells extract of BGR, TNH: Shells extract of TNH, ^{ns}: Not significant, *Significant at p<0.05, **Significant at p<0.01, DPPH: 2,2-diphenyl-1 picrylhydrazyl, FRAP: Ferric reducing antioxidant power

had reduced power to reduce Fe (III) to Fe (II) and this sample will be oxidized. Intensity of blue color depends on the amount of Fe (III) that is reduced to Fe (II).

The strength of FRAP method is simple, fast, and cheap. The weakness of this method is not all of the compound can act as an antioxidant agent to reduce Fe (III). The compounds that can donor electron and the compounds that have potential redox lower than Fe (III)/Fe (II) even though it has not antioxidant activity might contribute in this reaction, so that the result becomes higher.

In the present study, 50 µg/mL of ethanolic shells extract of four legumes (soybean, red kidney bean, Bogor peanut and peanut) which were reacted with 50 µg/mL DPPH solution gave DPPH scavenging capacity 55.47%, 69.02%, 59.55%, 75.97%, respectively. The previous research [22] revealed that antioxidant capacity of 1mg/mL water extract peanut which was reacted with 2 mL 0.1 mM DPPH had antioxidant activity 35.0% dan 1 mg/mL extract was reacted with 2 mL reagent FRAP exposed antioxidant capacity 19,00 µg AAE/mg sample. A study by Xu [7] regarding phenolic profiles and antioxidant activities of legumes, which used different solvents demonstrated that acidic 70 % acetone (+0.5% acetic acid) extract had the highest FRAP capacities for black bean, lentil, black soybean, and red kidney bean, while the 80 % acetone extract exposed that the highest DPPH scavenging activities were given by yellow pea, green pea, chickpea, and yellow soybean. In the research by Xu [8], regarding processing effect in total phenolic and antioxidant properties of eclipse black bean revealed that DPPH scavenging activity of beans with soaking, boiling, and steaming process were lower than raw beans. The previous study by Chon [25] exhibited that the cowpea and mung bean sprouts had higher DPPH scavenging capacity than soybean sprouts.

EC_{50} of FRAP capacity is concentration of sample or standard that can exhibit 50 % of FRAP capacity, while IC_{50} of DPPH scavenging capacity is concentration of sample or standard that can inhibit 50 % of DPPH scavenging capacity. The lowest IC_{50} or EC_{50} means had the highest antioxidant capacity. IC_{50} or EC_{50} was used to determine antioxidant capacity of the sample was compared to standard. Classification by Blois [15] stated that sample which had IC_{50} or EC_{50} <50 µg/mL, it was very strong antioxidant, 50-100 µg/mL strong antioxidant, 101-150 µg/mL medium antioxidant, while weak antioxidant with IC_{50} or EC_{50} >150 µg/mL.

In the DPPH method, antioxidant capacities of various extracts from four species of legumes ranged from 0.595 to 58.189 µg/mL. TNH2 (ethyl acetate shells extract of peanut) had the lowest IC_{50} of DPPH radical scavenging capacity 0.595 µg/mL, while ascorbic acid standard gave IC_{50} of DPPH scavenging capacity 0.181 µg/mL. Based on the value of IC_{50} of DPPH scavenging capacity, it can be concluded that all of sample extracts of legumes in this study (except n-hexane shells extract of soybean) can be categorized as very strong antioxidant. It is showed that the potency of TNH2 was one-third of potency of ascorbic acid using DPPH method. MRH2 (ethyl acetate shells extract red kidney bean) had the lowest EC_{50} of FRAP capacity (294.781 µg/mL) while ascorbic acid standard gave EC_{50} of FRAP capacity 7.004 µg/mL. It exhibited that the potency of ascorbic acid was around forty times of potency of MRH2 using FRAP assays. Study by Sebei [3] revealed that seed of peanut (*Arachis hypogea* L) from Trabilsia cultivar had the lowest IC_{50} of DPPH scavenging capacity (1550 µg/mL) compared to Massriya cultivar (720 mg/mL) and Sinya cultivar (820 mg/mL).

The presence of total phenolic might contribute to antioxidant capacity [10]. Phenolic acid might contribute in antioxidant capacity and cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid [26]. The present study exposed that total phenolic 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. In contrast with research by Mbagwu [27] revealed that ethanolic seeds extract of Bogor peanut *V. subterranea* (0.36 %) was higher than peanut *Arachis hypogea* and soybean *G. max*. Previous study [28] showed that total phenolic content in methanolic seeds extract of varieties soybean that grown in upland (693.8 µg/g) higher than lowland (630.2 µg/g). Chon [29] exhibited that total phenolic in soybean sprouts extract was higher than cowpea and mung bean sprouts extract. The research by Sebei [3]exposed that seed of Chounfakhi, Massriya, Sinya, and Trabilsia varieties had total phenolic 2.1 mg GAE/g, 1.35 mg GAE/g, 1.35 mg GAE/g and 1 mg GAE/g, respectively. Previous study [7] demonstrated that 50% acetone extract had the highest total phenolic for yellow pea, green pea, chickpea, and yellow soybean. Win [5] exposed that total phenolic of peanuts skin was higher than its hull, the raw kernel, and roasted kernel flour. Xu [8] stated that eclipse black beans (*Phaseolus vulgaris*) with soaking, boiling, and steaming process had lower total phenolic than raw beans, while study by Yao [17] showed that five black mung bean (*Vigna radiata* L.) had free phenolic acid and bound phenolic acid content ranged from 16.68 to 255.51 µg/g and ranged from 2284.53 to 5363.75 µg/g. Heimler [23] exhibited that total phenolic in 12 samples of common beans in the range of 1.17-4.40 mg GAE/g.

The present study revealed that the total flavonoid 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. Previous study [27] stated that the total flavonoid of ethanolic seeds extract of *Vigna uniguiculata* was the highest (0.33%), while the lowest was given by *Arachis hypogea* (0.18%). Total flavonoid content in soybean sprouts extract was higher than cowpea and mung bean [29] and ranged from 0.24 to 1.43 (+) catechin per g of dry seeds [23]. Yoshida [30] exposed that the flavonoid especially anthocyanin content was very high (0.2%) in acetonitrile peels extract of black colored seed coats of Vigna, Phaseolus, Glycine species.

The data in Table 4 exposed that there were positively high correlation between total phenolic content in all of shells extract sample (soybean, red kidney bean, Bogor peanut, peanut) with DPPH scavenging activities ($r=0.782$, $p<0.05$, $r=0.985$, $p<0.01$, $r=0.844$, $p<0.01$, $r=0.984$, $p<0.01$, respectively). Only total flavonoid in Bogor peanut shells extract and peanut shells extract had high, positive, and significant correlation with their DPPH scavenging capacities ($r=0.958$, $p<0.01$, $r=0.676$, $p<0.05$). The previous study by Lin [6] revealed that total flavonoid content in methanolic extract of legumes had high and positive correlation with their FRAP capacity ($r=0.9414$, $p<0.01$) and their total phenolic content positively and high correlation with DPPH scavenging activities ($r=0.6885$, $p<0.05$). Win [5]stated that total phenolic in methanolic extract of roasted kernel flour had good correlation with their DPPH scavenging activities ($r=0.8436$, $p<0.01$).

Phenolic compound included tannins, flavonoid, phenolic acid, qoumarine, quinone, and other compounds. Flavonoid will be included in phenolic groups if have OH in A ring and or B ring. Phenolic acid had lower antioxidant capacity than flavonoid [26]. Flavonoid would give higher antioxidant capacity which had OH in ortho C3', 4', OH in C3, oxo function in C4, a double bond at C2 and C3. 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 [26]. It could be seen in Fig. 3 that total flavonoid in TNH2 (ethyl acetate shells extracts of peanut) was higher than total flavonoid in TNH3 (ethanolic shells extracts of peanut), but DPPH scavenging activity of TNH2 (68.90%) was lower than TNH3 (75.97%). Based on the data above, it can be predicted that many flavonoids in TNH2 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 flavonoids in TNH3 were flavonoid that had OH in position which can influence antioxidant capacities.

The present study exposed that the total carotenoid in shells extract of soybean and red kidney bean had positive and significant correlation with its FRAP capacity ($r=0.924$, $p<0.01$, and $r=0.846$, $p<0.01$, respectively) and no significant and negative correlation with their 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 maximum 7 double bonds gave lower scavenging radical free capacity than more double bonds [32]. In the previous study by Kobayashi and Sakamoto [33] stated that increasing in lipophilicity of carotenoid would increase scavenging radical capacity. Lycopene was effective to reduce Fe (III), due to it had 11 conjugated double bonds. Carotenoid such as phytoene, phytofluene, and neurosporene that consisted of 3, 5, and 9 conjugated double bonds, respectively, did not show significant capacity to reduce Fe (III) [34]. Beta carotene was used as standard due to it had conjugation double bonds doe to its ability to scavenge free radicals [35]. KDL2 shells extracts had the highest carotenoid (0.33 BET g/100 g) and the lowest was given by TNH1 (0.026 g/100 g), but DPPH scavenging activity of KDL2 (53.80%) almost similar with TNH1 (50.86%). Based on this data, it could be seen that many carotenoids in KDL2 had double bonds lower than 7, that had no or low antioxidant capacity. In contrast, all of carotenoids in TNH1 had higher than 7 double bonds.

FRAP and DPPH methods had different mechanism reaction. Mechanism of DPPH that was electron transfer assays [36] and FRAP was the redox assays. Hence, the results of this study showed that DPPH scavenging activities in all of extracts sample were not linear with their FRAP capacities.

CONCLUSION

To assess the antioxidant capacity of the sample, variety of methods must be used in parallel, because different methods could give different results. All of shells extracts of legumes (except n-hexane shells extract of soybean) had IC_{50} of DPPH scavenging capacities <50 µg/mL that were very strong antioxidant. The positively and high correlation between total phenolic with DPPH scavenging capacities was given by all of shells extracts sample, but only Bogor peanut and peanut shells extract that had high, positive significant correlation between total flavonoid and DPPH scavenging capacities. Phenolic compounds were the major contributor in DPPH scavenging capacity of soybean and red kidney bean shells extract. Phenolic and flavonoid compounds were the major contributor in DPPH scavenging capacity of Bogor peanut and peanut. There was no liner correlation between DPPH and FRAP result in all of shells extract sample. Soybean, red kidney bean, Bogor peanut, and peanut's shells extracts may be exploited as a source of beneficial compounds for human health to alleviate oxidative stress.

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