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Research Article

In vitro Antioxidant Capacities of Three Organs of Bitter gourd (*Momordica charantia* L.) Form West Java-Indonesia Using DPPH and FRAP Assays

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ABSTRACT

The objectives of this research were to study antioxidant capacity from various organs extracts of bitter gourd 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 organs extracts of bitter gourd 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 UV-visible spectrophotometry and its correlation with IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP capacities were analyzed by Pearson's method. All of extracts sample (except n-hexane pedicel extract, n-hexane fruit extract and ethyl acetate fruit extract) were categorized as strong and very strong antioxidant by DPPH method. Phenolic compounds were the major contributor in antioxidant activities of various organs (leaves, pedicel and fruit) extracts of bitter gourd by DPPH and FRAP assays. All of organs extracts of bitter gourd had linear result in DPPH and FRAP assays.

Keywords: Antioxidant, DPPH, FRAP, organs, bitter gourd

INTRODUCTION

Antioxidant can reduce oxidative stress which was related with many diseases. Phenolic compounds are commonly found in plants, and they have been exposed to have multiple biological effects, including antibacterial and antioxidant activity[1-4]. Previous researches reported that phenolic content and flavonoid content in plants could have correlation with their antioxidant activities[5-7]. Plants including bitter gourd consisted of phenolic and polyphenol compounds which can act as antioxidant[8-10].

Some of antioxidant methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power), CUPRAC (Cupric ion Reducing Antioxidant Capacity) were used to predict antioxidant capacity of vegetables, fruits and food[1,11-12]. Previous study[2,11,13] demonstrated that DPPH and FRAP methods could be used to determine antioxidant activity in many plants extracts. The previous study[8-10,14] exposed that bitter gourd had antioxidant capacities by using DPPH, FRAP and CUPRAC assays.

The objective of this research were to study antioxidant capacities of different polarities extracts (n-hexane, ethyl acetate and ethanol) of three organs (leaves, pedicel and fruit) of bitter gourd (*Momordica charantia*) 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

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, three organs of bitter gourd, ethanol. All other reagents were analytical grades.

Preparation of sample

Three organs of bitter gourd (*Momordica ch*arantia L.) which were: leaves namely as LV, pedicel as PE and fruit as FR were collected from Cipatat - West Bandung, 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 with ethyl acetate. Finally the remaining residue was extracted three times with ethanol. So there were three n-hexane extracts (namely LV1, PE1 and FR1), three ethyl acetate extracts (LV2, PE2 and FR2) and three ethanolic extracts (LV3, PE3 and FR3).

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)

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to initiate the reaction for obtaining a calibration curve. After 30 minutes incubation, the absorbance was read at wavelength 515 nm by using UV-Vis spectrophotometer Beckman Coulter DU 720. 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 CUPRAC 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 UV-Vis spectrophotometer Beckman Coulter DU 720. 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 extract was 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 phenolic content (TPC)

Total phenolic content were measured using the modified Folin-Ciolcalteu method adapted from Pourmorad [6]. 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 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 carotenoid content (TCC)

Total carotenoid content was measured by using the modified of Thaipong *et al* [11] method. 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 (± 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 capacities were made using the Pearson's procedure ($p < 0.01$).

RESULTS

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

The IC_{50} of DPPH scavenging capacity and EC_{50} of FRAP capacity in various organs extracts of bitter gourd 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.

TPC in various organs extracts of bitter gourd

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 organs extracts of bitter gourd showed different result ranged from 0.46 to 3.05 g GAE/100 g. FR1 (n-hexane fruit extract of bitter gourd) had the lowest phenolic content (0.46 g GAE/100 g), while the highest (3.05 g GAE/100 g) was given by LV2 (ethyl acetate leaves extract of bitter gourd) (Fig 3).

TFC in various organs extracts of bitter gourd

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 organs extracts of bitter gourd showed different result in the range of 0.32 – 5.45 g QE/100 g (Fig 4). Ethanol extract of pedicel (PE3) had the lowest total flavonoid content (0.32 g QE/100 g) and the highest (5.45 g QE/100 g) was given by ethyl acetate extract of leaves (LV2).

TCC in various organs extracts of bitter gourd

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 organs extracts of bitter gourd showed different result in the range of 0.07 – 11.32 g BE/100 g (Fig 5). The lowest carotenoid content (0.07 g BE/100 g) was given by ethanolic extract of pedicel (PE3), while the highest carotenoid (11.32 g BE/100 g) for n-hexane extract of leaves (LV1).

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

Pearson's correlation coefficient between TPC in various organs extracts of bitter gourd and their antioxidant activities demonstrated that TPC in all of sample had negatively high correlation with IC_{50} of DPPH

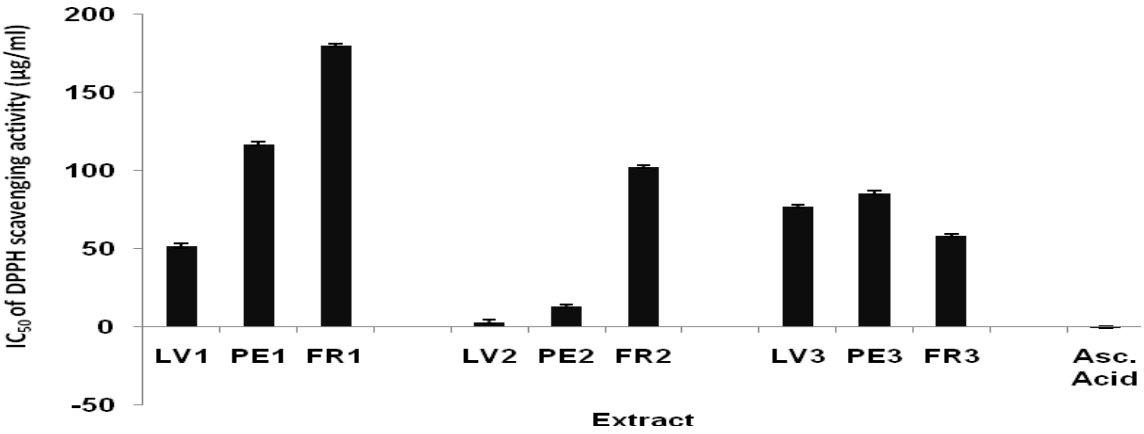

Figure 1: IC_{50} of DPPH scavenging capacities in various organs extracts of bitter gourd

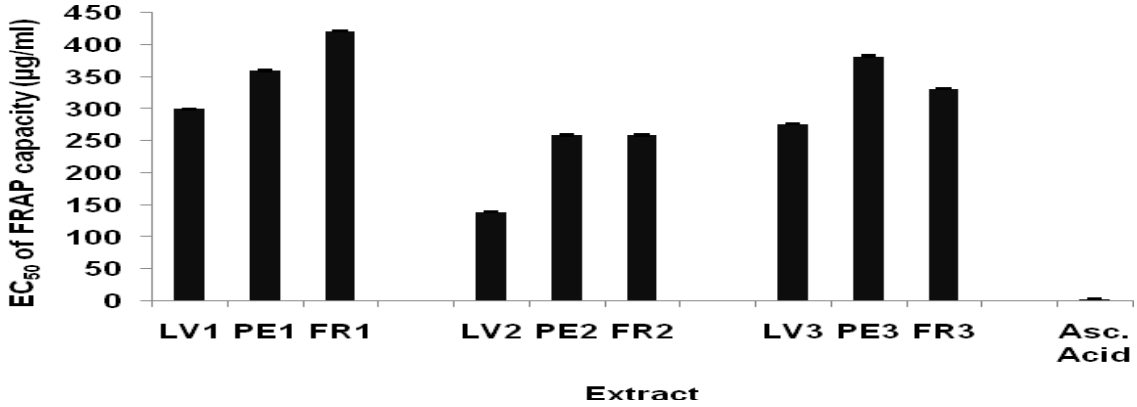

Figure 2: EC_{50} of FRAP capacities in various organs extracts of bitter gourd

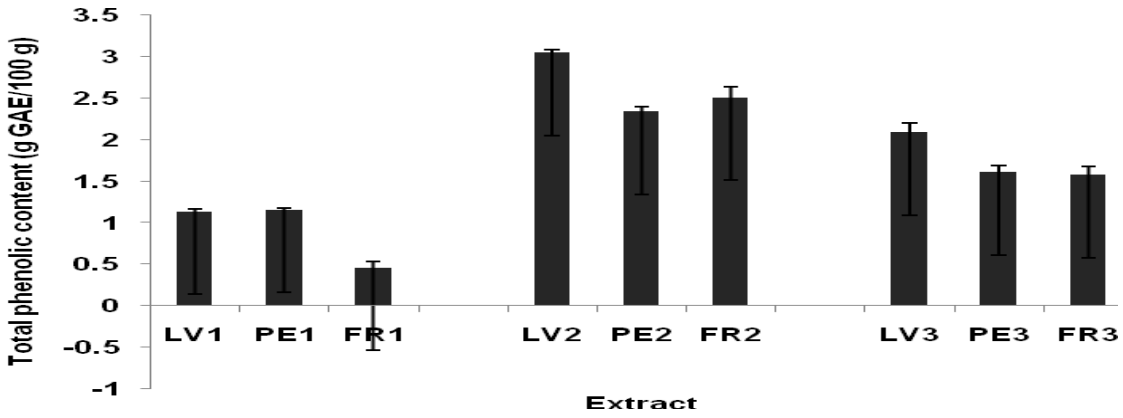

Figure 3: Total phenolic content in various organs extracts of bitter gourd

scavenging activities and EC_{50} of FRAP capacities. TFC in sample LV had negative and high correlation with their IC_{50} of DPPH scavenging activities ($r = -0.961$, $p<0.01$) and EC_{50} of FRAP capacities ($r = -0.723$, $p<0.05$). TCC in all of sample had no correlation with their IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities (Table 1).

DISCUSSION

The previous researches[5,8-10,1419-21] reported that bitter gourd had antioxidant capacity. There were no study regarding antioxidant capacity of different polarities extracts (which were n-hexane, ethyl acetate and ethanol) from various organs of bitter gourd using DPPH and FRAP assays.

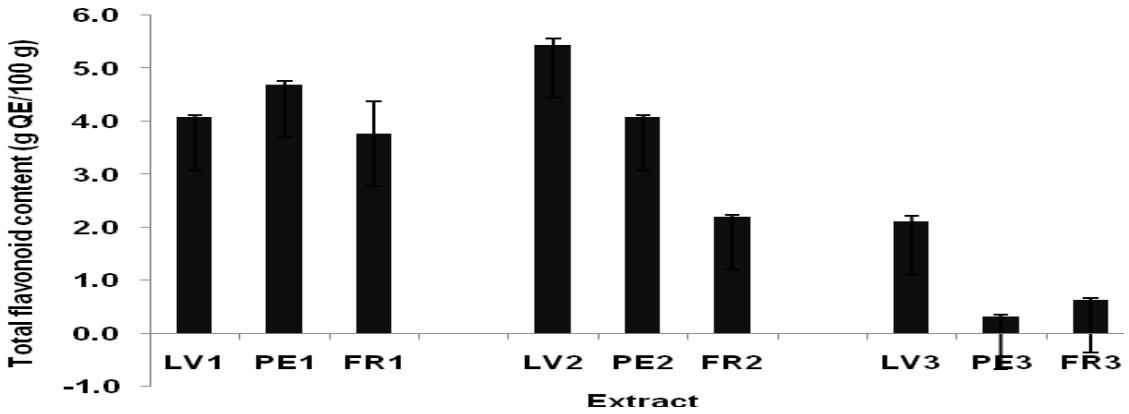

Figure 4: Total flavonoid content in various organs extracts of bitter gourd

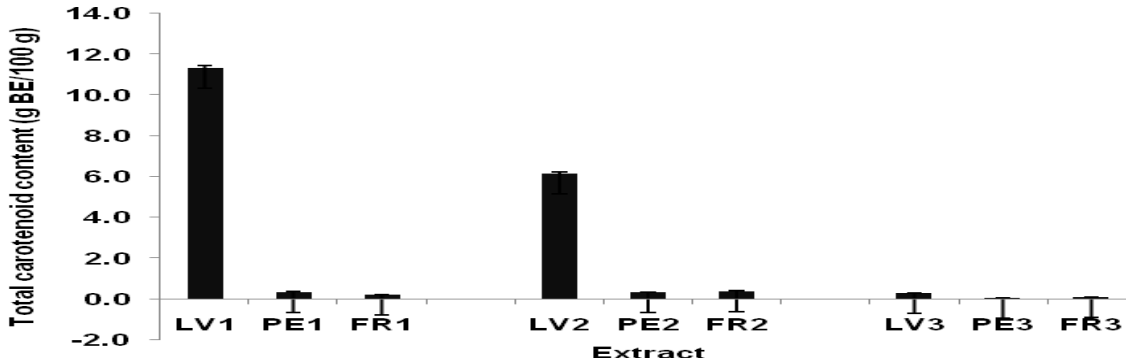

Figure 5: Total carotenoid content in various organs extracts of bitter gourd

The DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 515-520 nm. Antioxidant could scavenge the free radicals and would change the colors of DPPH[22-23]. FRAP reagent is $FeCl_3$ which was combined with TPTZ in acetate buffer pH 3.6. Intensity of blue color depends on amount of Fe (III) that is reduced to Fe (II). Complex Fe (II) - TPTZ gives blue color and show characteristic absorption at wavelength 593 nm. Sample can act as antioxidant if it can reduce Fe (III) to Fe (II), at the same time it will be oxidized. Reduction potential of Fe (III)/Fe (II) is 0.77 V. Sample will act as antioxidant in FRAP assays if sample had reduction potential lower than 0.77 V.

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 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] exposed 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 previous study[20] reported that IC_{50} of DPPH scavenging capacities of ethanolic fruit extract of bitter gourd was 111.87 µg/ml which was categorized as medium antioxidant and ethyl acetate fruit extracts and 157.03 µg/ml classified as weak antioxidant. It was contrast with the present study which exhibited that IC_{50} of DPPH scavenging activities of ethanolic extract of leaves, pedicel and fruit extracts of bitter gourd were 77.10, 85.61, 58.62 µg/ml, respectively, which were categorized as strong antioxidant. The ethyl acetate leaves and pedicel extracts of bitter gourd were 2.87 and 13.43 µg/ml, respectively, which were classified as very strong antioxidant, while ethyl acetate fruit extract of bitter gourd had IC_{50} of DPPH was 102.46 µg/ml categorized as medium antioxidant. Leelaprakash[24] revealed that IC_{50} of DPPH of water and methanol leaves extract of bitter gourd were 66.25 µg/ml and 73.30 µg/ml, respectively, which categorized as strong antioxidant. It was similar with the present study which showed that ethanolic extract of leaves, pedicel and fruit of bitter gourd were 77.10, 85.61, 58.62 µg/ml, respectively. The previous research by Shan[9] demonstrated that IC_{50} of DPPH scavenging activity of ethanol modified supercritical carbon dioxide ($SC\text{-}CO_2$) extract of bitter gourd fruit and 85 % ethanol extract by Soxhlet classical solvent extraction (CSE) were 270 µg/ml and 310 µg/ml,

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 organs extracts of bitter gourd

	Pearson's correlation coefficient (r)								
	TPC	TFC	TCC	IC_{50} DPPH LV		IC_{50} DPPH PE	IC_{50} DPPH FR		
IC_{50} DPPH LV	-0.639*	-0.961**	-0.381						
IC_{50} DPPH PE	-0.992**	-0.082	0.575						
IC_{50} DPPH FR	-0.669*	0.960**	0.185						
EC_{50} FRAP LV	-0.924**	-0.723*	0.097	0.883**					
EC_{50} FRAP PE	-0.863**	-0.493	0.178			0.907**			
EC_{50} FRAP FR	-0.995**	0.526	-0.595				0.679*		

IC_{50} DPPH = IC_{50} DPPH scavenging capacity, EC_{50} FRAP = EC_{50} FRAP capacity, LV = leaves extract, PE = pedicel extract, FR = fruit extract, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

respectively. Lu[25] studied regarding sixteen cultivar of bitter gourd reported that water extract of cultivar N had the lowest IC_{50} of DPPH scavenging activity (181 µg/ml) and the same result in its methanolic extract which showed cultivar N gave the lowest IC_{50} of DPPH (246 µg/ml). Previous study[10] revealed that water fruit extract of bitter gourd (IC_{50} of DPPH 129.94 µg/ml) had higher antioxidant than its ethanolic fruit extract (IC_{50} of DPPH 156.78 µg/ml). Patel *et al.*[5] exposed that IC_{50} of DPPH scavenging capacity were 120 µg/ml and 182 µg/ml for alcohol fruits extract and water fruits extract of *Momordica charantia* respectively. Study by Hamissou[14] reported that percentage of DPPH scavenging activity of water fruit extract of bitter gourd was 82.05 % which was expressed as ascorbic acid. Ghosh[26] expressed that IC_{50} of DPPH scavenging activity of water fruit extract of bitter gourd (bitter melon) which was extracted at 35 ^{o}C was 90 µg/ml, while in the present study found that ethanolic fruit extract of bitter gourd had IC_{50} of DPPH 58.62 µg/ml. The previous study[8] stated that percentage of DPPH radical scavenging activity of ethanolic ripe fruit, ripe seed, unripe fruit, unripe seed extracts were 45.95, 21.14, 15.49, 8.32 %, respectively, while study by Fidrianny[19] demonstrated that percentage of DPPH of scavenging activity of ethanolic leaves extract of *Cucumis sativus*, *Sechium edule*, *Luffa acutangula*, *Cucurbita moschata* and *Momordica charantia* were 8.10, 21.97, 41.46, 1.64, 11.66 %, respectively. The other study [4] showed that DPPH scavenging capacity of methanolic extract of *Momordica charantia* fruits was higher than its water extract and the green fruits of *Momordica charantia* had higher DPPH scavenging capacity than the yellow fruits. Previous research by Amira[21] exposed that DPPH scavenging capacity of water extract of *Momordica charantia* fruits was higher than its acetone and methanol extracts. DPPH scavenging capacity of water fruits extracts was 98.29 % and 38.92 % for FRAP capacity[21]. The present study showed that EC_{50} of FRAP capacity of ethyl acetate and ethanolic fruit extract of bitter gourd were 259.21 and 331.44 µg/ml, respectively. It was different with the previous research [20] which reported that EC_{50} of FRAP capacity of ethyl acetate and ethanolic fruit extracts of bitter gourd were 754.86 and 931.63 µg/ml, respectively. Study by Ozusaglam[8] exposed that antioxidant capacity using FRAP and CUPRAC methods found that FRAP and CUPRAC capacity of ripe fruit extract was higher than its ripe seed, unripe fruit and unripe seed extracts. The previous research[19] expressed that percentage of FRAP capacity of ethanolic leaves extract of *Cucumis sativus*, *Sechium edule*, *Luffa acutangula*, *Cucurbita moschata* and *Momordica charantia* were 1.63, 1.69, 0.43, 1.37, 1.27 %, respectively, while in the present study stated that EC_{50} of FRAP capacity of ethanolic extract of leaves, pedicel and fruit extracts of bitter gourd (*Momordica charantia*) were 276.54, 381.98, 331.44 µg/ml, respectively.

Antioxidant capacity might be related with the presence of total phenolic content, included phenolic acid [22,27]. Cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid[28]. Study by Wu[10] revealed that TPC in water fruit extract of bitter gourd (wild bitter melon) 5.16 g GAE/100 g was lower than TPC in its ethanol extract 6.88 g GAE/100 g. Ozusaglam[8] exposed that TPC in ethanolic ripe fruit and seed extracts were 2.345 and 0.936 g GAE/100 g, respectively. The previous study[19] regarding Cucurbitaceae leaves showed that TPC in ethanolic leaves extract of *Cucumis sativus*, *Sechium edule*, *Luffa acutangula*, *Cucurbita moschata* and *Momordica charantia* (from Banjaran-Bandung) were 2.47, 1.79, 2.88, 1.43, 0.36 g GAE/100 g, respectively, while in the present study which found that TPC in ethanolic leaves, pedicel and fruit extracts of bitter gourd (*Momordica charantia*) from Cipatat-West Bandung were 2.09, 1.61, 1.58 g GAE/100 g, respectively. The result showed that the ethanolic leaves extract of bitter gourd from different region gave different TPC. The previous study by Hamissou[14] exhibited that TPC in water fruit extract of bitter gourd was 1.328 g GAE/100 g fresh weight which was higher than TPC in water fruit extract of zucchini (*Cucurbita pepo*) 0.867 g GAE/100 g fresh weight.

In Figure 3 it could be seen that TPC in n-hexane leaves extract (LE1) 1.14 g GAE/100 g was similar with TPC in

n-hexane pedicel extract (PE1)1.16 g GAE/100 g, but IC_{50} of DPPH scavenging activity of LE1 51.66 µg/ml which was classified as strong antioxidant, was lower than IC_{50} of DPPH of PE1 116.87 µg/ml medium antioxidant. Based on this data it can predicted that many phenolic compounds in LE1 had high antioxidant capacity, while in PE1 contained many phenolic compounds with low antioxidant activity. The previous study[9] stated that percentage of flavonoid in bitter gourd fruit which was extracted by ethanol modified supercritical carbon dioxide ($SC\text{-}CO_2$) extraction method (96.14%) was higher than extracted by Soxhlet classical solvent extraction (CSE) using 85 % ethanol (91.42%). Wu[10] reported that TFC in ethanolic fruit extract of bitter gourd (4.4 g RE/100 g) was lower than TFC in its water extract (6.2 g RE /100 g). The present study exhibited that TFC in ethanolic extract of leaves, pedicel and fruit of bitter gourd (*Momordica charantia*) were 2.11, 0.32, 0.64 g GAE/100 g, respectively, while Fidrianny[19] demonstrated that TFC in ethanolic leaves extract of *Cucumis sativus*, *Sechium edule*, *Luffa acutangula*, *Cucurbita moschata* and *Momordica charantia* were 1.70, 5.42, 2.30, 1.59, 0.76 g GAE/100 g, respectively. Flavonoid, tannins, phenolic acid, coumarin and quinone were included in phenolic compound. Flavonoid is not always included in phenolic compound, except it has OH in A ring and or B ring. Flavonoid had higher antioxidant capacity than phenolic acid[28].

Flavonoid which has OH in ortho C-3'-C-4', OH in C-3, oxo function in C-4, double bond at C-2 and C-3 would give high antioxidant capacity. The highest influence in antioxidant capacity of flavonoid was given by ortho OH position in C-3'-C-4'. The flavonoid aglycones would give higher antioxidant capacity than flavonoid glycosides[28]. In the Figure 4 it could be seen that TFC in ethyl acetate fruit extract of bitter gourd (FR2) 2.20 g QE/100 g was similar with TFC in ethanolic leaves extract (LV3) 2.11 g QE/100 g, but IC_{50} of DPPH scavenging activity of LV3 77.10 µg/ml which was categorized as strong antioxidant, was lower than IC_{50} of DPPH of FR2 102.46 µg/ml medium antioxidant. Based on this data it can be supposed that many flavonoid in LV3 had OH in ortho position C-3'-C-4', OH in C-3, oxo function in C-4, double bond at C-2 and C-3 which can influence high antioxidant activity, while FR2 contained many flavonoid in other position which had low antioxidant capacity.

TFC in n-hexane leaves extract (LE1) 4.08 g QE/100 g was similar with TFC in ethyl acetate pedicel extract (PE2) 4.08 g/100 g, but PE2 had EC_{50} of FRAP 259 µg/mlwas lower than LV1 300 µg/ml. It can be supposed that many flavonoid in PE2 had reduction potential lower than reduction potential of Fe (III)/Fe (II), so many Fe (II) would be formed and gave blue color complex with TPTZ.

The present study showed that TCC in ethanolic extract of leaves, pedicel and fruit of bitter gourd were 0.29, 0.07, 0.11 g BE/100 g, respectively. It was similar with the previous study[19] which found that TCC in ethanolic leaves extract of *Cucumis sativus*, *Sechium edule*, *Luffa acutangula*, *Cucurbita moschata* and *Momordica charantia* were 0.04, 0.60, 0.09, 0.07, 0.11 g BE/100 g, respectively.

Carotenoid had antioxidant capacity by scavenging free radical. More double bonds in carotenoid would give higher scavenging free radical capacity[29]. Carotenoid which contained more than 7 double bonds would give higher scavenging radical capacity[30]. Beta carotene was used as standard because of it had conjugation double bonds which could scavenge free radicals[31]. In previous study[32] exposed that increasing in lipophilicity of carotenoid would increase scavenging radical capacity, it means give the lower IC_{50} of DPPH scavenging capacity. Figure 5 expressed that TCC in n-hexane pedicel extract of bitter gourd (PE1) 0.35 g BE/100 g was similar with TCC in ethyl acetate pedicel extract (PE2) 0.35 g BE/100 g, but IC_{50} of DPPH scavenging capacity of PE2 13.43 µg/ml which was categorized as very strong antioxidant, was lower than IC_{50} of DPPH of PE1 116.87 µg/ml medium antioxidant. Based on this data it can be predicted that PE2 contained many carotenoid which had more than 7 double bonds, while PE1 consisted of many carotenoid with maximum 7 double bonds which had low antioxidant activity. Lycopene was effective in reducing Fe (III) to Fe (II), because of it has 11 conjugated double bonds. Carotenoid such as phytoene, phytofluene, neurosporene that contain 3, 5 and 9 conjugated double bonds respectively, did not show significant capacity to reduce Fe (III)[13]. TCC in PE2 was similar with PE1, but EC_{50} of FRAP capacity of PE2 259 µg/ml was lower than EC_{50} of FRAP capacity of PE1 359 µg/ml. It can be predicted that many carotenoid in PE2 had potential reduction lower than potential reduction of Fe (III)/Fe (II) so it could reduce Fe (III) to Fe (II).

Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$ [11] 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 TPC, TFC and TCC with IC_{50} DPPH or EC_{50} of FRAP will be given in negatively and high correlation. It means increasing in TFC, TPC and TCC could give 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 demonstrated that the negatively high correlation between TPC in all of sample extract (leaves, pedicel and fruit) with IC_{50} of DPPH scavenging activities ($r = -0.639$, $p<0.05$; $r = -0.992$, $p<0.01$; $r = -0.669$, $p<0.05$, respectively) and EC_{50} of FRAP capacity ($r = -0.924$; $r = -0.863$; $r = -0.995$, $p<0.01$, respectively). Based on the data it can be concluded that increasing in TPC would increase antioxidant activity which showed by lower IC_{50} of DPPH or EC_{50} of FRAP and phenolic

compounds were the major contributor in antioxidant activity of leaves, pedicel and fruit extracts of bitter gourd by DPPH and FRAP methods.

In the previous research by Fidrianny[19], Pearson's correlation coefficient was investigated which was different with the present study. Previous study[19] determined the correlation between TPC and percentage of DPPH scavenging activity or FRAP capacity, so the good correlation will be exposed in parallel position, increasing in TPC would give increasing in percentage of scavenging activity of DPPH or capacity of FRAP. TPC in leaves extract of bitter gourd had positively high correlation with its percentage of FRAP, but it had negatively high correlation with its percentage of DPPH scavenging capacity[19].

DPPH and FRAP methods had different mechanism reaction. Mechanism of DPPH that was electron transfer assays[33] and FRAP was redox assays[17]. All of extracts sample (except n-hexane pedicel extract, n-hexane fruit extract and ethyl acetate fruit extract) were categorized as strong and very strong antioxidant. TPC in all of extracts had negatively high correlation with their IC_{50} of DPPH scavenging capacity and EC_{50} of FRAP capacity. There were positive and high correlation between IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities. It means 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. All of extracts sample (except n-hexane pedicel extract, n-hexane fruit extract and ethyl acetate fruit extract) were categorized as strong and very strong antioxidant. The negatively and high correlation between TPC with IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP capacities were given by all of organs extracts of bitter gourd. Phenolic compounds in all of organs extracts of bitter gourd were the major contributor in IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP capacities. DPPH and FRAP methods gave linear result in antioxidant activity of all of organs extracts of bitter gourd. Leaves, pedicel and fruit extracts of bitter gourd (*Momordica charantia*) may be exploited as natural antioxidant sources.

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