

Research Article

ANTIOXIDANT CAPACITIES FROM DIFFERENT POLARITIES EXTRACTS OF CUCURBITACEAE LEAVES USING FRAP, DPPH ASSAYS AND CORRELATION WITH PHENOLIC, FLAVONOID, CAROTENOID CONTENTIRDA FIDRIANNY^{1*}, AGUNG DARMAWATI¹, SUKRASNO¹¹School of Pharmacy, Bandung Institute of Technology, Indonesia. Email: irda@fa.itb.ac.id, irdafidrianny@gmail.com

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ABSTRACT

Objectives: The objectives of this research were to study antioxidant capacity from different polarities extracts of Cucurbitaceae leaves using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) and correlation of total phenolic, flavonoid and carotenoid content in different polarities extracts of Cucurbitaceae leaves with DPPH and FRAP antioxidant capacities.

Methods: Extraction was performed by reflux using different polarities solvents. The extracts were vaporated using rotavapor. Chromatogram pattern on each extracts were observed by thin layer chromatography (TLC). Then antioxidant capacities were tested using DPPH and FRAP assays. Determination of total phenolic, flavonoid and carotenoid content were performed by spectrophotometry UV-visible and its correlation with FRAP and DPPH antioxidant capacities were analyzed by Pearson method.

Results: LA3 (ethanolic extract of *Luffa acutangula* leaves) had the highest DPPH scavenging capacity with IC₅₀ 73 ppm, while SE2 (ethyl acetate extract of *Sechium edule* leaves) had the highest FRAP capacity with EC₅₀ 759 ppm. SE2 contained the highest total phenolic (4.01 g GAE/100 g), MC1 (n-hexane extract of *Momordica charantia* leaves) had highest flavonoid content (14.37 g QE/100 g) and highest carotenoid 19.53 g BET/100 g.

Conclusions: There were positively high correlation between total phenolic content in *Sechium edule* leaves extracts with their antioxidant activity using FRAP and DPPH assays. FRAP capacities in *Sechium edule* leaves extracts had positively high correlation with their DPPH scavenging capacities.

Keywords: Antioxidants, FRAP, DPPH, Cucurbitaceae leaves, Flavonoid, Phenolic, Carotenoid

INTRODUCTION

Antioxidant was known to have beneficial effects on the prevention or progression of diseases related to oxidative stress on account of their high antioxidant activity. Phenolic compounds are commonly found in plants, and they have been reported to have multiple biological effects, including antioxidant activity [1].

Many studies had revealed that phenolic content in plants could be correlated to their antioxidant activities. Plants contained phenolic and polyphenol compounds can act as antioxidant [2].

Some of antioxidant methods such as FRAP (Ferric Reducing Antioxidant Power) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were used to predict antioxidant capacity of fresh fruits, beverages and food [1].

In previous study [1] [3] [4] [5] revealed that DPPH and FRAP methods could be used to determine antioxidant activity in many plants extracts.

The previous study [3] [5] [6] [7] [8] showed antioxidant activities of some plants including Cucurbitaceae. The objective of this research were to study antioxidant capacities of different polarities extracts (n-hexane, ethyl acetate and ethanol) from five species of Cucurbitaceae (*Cucumis sativus*, *Sechium edule*, *Luffa acutangula*, *Cucurbita moschata* and *Momordica charantia*) leaves using antioxidant testing FRAP and DPPH assays and correlations of their 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 five species of Cucurbitaceae, methanol, ethanol. All other reagents were analytical grades.

Preparation of sample

Leaves from five species Cucurbitaceae that were collected: cucumber *Cucumis sativus* (CS) from Cangkuang -Bandung, chayote *Sechium edule* (SE) from Cikajang-Garut, ridged gourd *Luffa acutangula* (LA) from Banjaran-Bandung, pumpkin *Cucurbita moschata* (CM) from Cikajang-Garut and bitter melon *Momordica charantia* (MC) from Banjaran-Bandung 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 five n-hexane extracts (namely CS1, SE1, LA1, CM1 and MC1), five ethyl acetate extracts (CS2, SE2, LA2, CM2 and MC2) and five ethanolic extracts (CS3, SE3, LA3, CM3 and MC3).

FRAP capacity

Preparation of FRAP solution were adopted from Benzi [9]. FRAP solution were prepared in acetate buffer pH 3.6. Each extracts 50 µg/mL were 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 by using spectrophotometer UV-Vis Hewlett Packard 8435. Acetate buffer was used as a blank and FRAP solution 50 µg/mL was used as standard. Antioxidant capacity of each extracts were determined based on increasing in Fe (II) - TPTZ absorbance by calculating percentage of antioxidant capacity [9].

DPPH scavenging capacity

Preparation of DPPH solution were adopted from Blois [10] with minor modification. Each extracts 50 µg/mL was pipetted into DPPH

solution concentration 50 µg/mL (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 517 nm by 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 extracts. Antioxidant activity of each extracts were determined based on the reduction of DPPH absorbance by calculating percentage of antioxidant activity [11].

Total phenolic determination

Total phenolic content were measured using the modified Folin-Ciocalteu method adapted from Pourmorad [12]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extracts. 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 percentage of total gallic acid equivalents per 100 g extract (g GAE /100 g).

Total flavonoid determination

Total flavonoid content was measured using adapted method from Chang *et al* [13]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extracts. Standard solutions of quercetin with concentration 40-160 µg/mL were used to obtain a standard curve. The total flavonoid content was reported as percentage of total quercetin equivalents per 100 g extract (g QE/100 g).

Total carotenoid determination

Total carotenoid content was measured using the modified carotene method adapted from Thaipong *et al* [1]. Each extracts were diluted in n-hexane.

The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extracts. 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 percentage of total beta carotene equivalents per 100 g extract (g BET/100 g).

Statistic

Each sample analysis was performed in triplicate. All results presented were the means (\pm SD) 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 (LSD) 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 different polarities leaves extracts from five species of Cucurbitaceae using FRAP and DPPH assays

The antioxidant capacities using FRAP and DPPH assays of different polarities leaves extracts from five species of Cucurbitaceae were shown in Table 1, Table 2, Table 3. In FRAP method, antioxidant capacities in the range of 0.27 - 4.54 %. SE2 leaves extract (ethyl acetate extract of *Sechium edule* leaves) had the highest FRAP capacity (4.54 %), while the lowest capacity (0.27 %) was given by CS1 leaves extract.

In the DPPH method, free radical scavenging capacities of different polarities leaves extracts from five species of Cucurbitaceae ranged from 1.64 - 41.46 %. LA3 (ethanolic extract of *Luffa acutangula* leaves) had the highest DPPH radical scavenging capacity (41.46%),

while CM3 leaves extract (1.64%) had the lowest DPPH antioxidant capacity.

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

Sample	FRAP capacity (%)	DPPH scavenging capacity (%)
CS1	0.27 \pm 0.02 ^a	14.73 \pm 1.14 ^a
SE1	1.26 \pm 0.02 ^b	9.64 \pm 1.13 ^b
LA1	2.13 \pm 0.04 ^c	4.84 \pm 0.01 ^c
CM1	0.28 \pm 0.35 ^a	11.98 \pm 0.3 ^d
MC1	4.15 \pm 0.20 ^d	3.01 \pm 0.97 ^e
Ascorbic acid	7.39 \pm 0.25	97.14 \pm 0.10
P value	< 0.05	< 0.05

Note: a - e = means within a column with the same letter were not significantly different ($p=0.05$)

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

Sample	FRAP capacity (%)	DPPH scavenging capacity (%)
CS2	0.82 \pm 0.07 ^a	19.19 \pm 1.29 ^a
SE2	4.54 \pm 0.35 ^b	36.74 \pm 0.44 ^b
LA2	3.54 \pm 0.46 ^c	39.11 \pm 1.43 ^c
CM2	0.89 \pm 0.02 ^a	3.96 \pm 0.01 ^d
MC2	1.67 \pm 0.06 ^d	6.57 \pm 0.09 ^d
Ascorbic acid	7.39 \pm 0.25	97.14 \pm 0.10
P value	< 0.05	< 0.05

Note: a - e = means within a column with the same letter were not significantly different ($p=0.05$)

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

Sample	FRAP capacity (%)	DPPH scavenging capacity (%)
CS3	1.63 \pm 0.18 ^a	8.10 \pm 0.70 ^a
SE3	1.69 \pm 0.07 ^a	21.97 \pm 0.34 ^b
LA3	0.43 \pm 0.02 ^b	41.46 \pm 0.69 ^c
CM3	1.37 \pm 0.15 ^a	1.64 \pm 0.49 ^d
MC3	1.27 \pm 0.27 ^c	11.66 \pm 1.16 ^e
Ascorbic acid	7.39 \pm 0.25	97.14 \pm 0.10
P value	< 0.05	< 0.05

Note: a - e = means within a column with the same letter were not significantly different ($p=0.05$)

EC₅₀ of FRAP capacity and IC₅₀ of DPPH scavenging capacity

The EC₅₀ of FRAP capacities and IC₅₀ of DPPH scavenging capacities in different polarities extracts from five species of Cucurbitaceae leaves using DPPH and FRAP assays were shown in Fig 1 and Fig 2. EC₅₀ of FRAP capacities of each extracts were compared to EC₅₀ ascorbic acid standard, while IC₅₀ of DPPH scavenging capacities were compared to IC₅₀ ascorbic acid standard. The lowest EC₅₀ or IC₅₀ means had the highest antioxidant capacity.

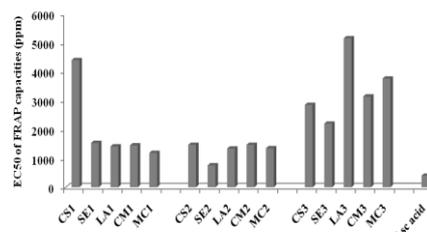


Fig. 1: EC₅₀ of FRAP capacities in different polarities leaves extracts from five species of Cucurbitaceae

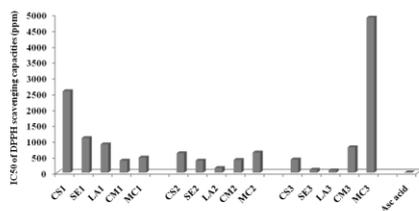


Fig. 2: IC50 of DPPH scavenging capacities in different polarities leaves extracts from five species of Cucurbitaceae

Total phenolic in different polarities leaves extracts from five species of Cucurbitaceae

The total phenolic content among the different polarities extracts were expressed in term of gallic acid equivalent using the standard curve equation $y = 0.004 x + 0.144$, $R^2 = 0.989$. The total phenolic content in different polarities leaves extracts from five species of Cucurbitaceae showed different result ranged from 0.36 to 4.01 g GAE/100 g. SE2 leaves extract (ethyl acetate leaves extract of *Sechium edule*) had the highest phenolic content (4.01 g GAE/100 g) (Fig 3).

Total flavonoid in different polarities leaves extracts from five species of Cucurbitaceae

The total flavonoid content among the different polarities extracts were expressed in term of quercetin equivalent using the standard curve equation $y = 0.006 x + 0.016$, $R^2 = 0.980$. The total flavonoid content in different polarities leaves extracts from five species of Cucurbitaceae showed different result in the range of 0.76 - 14.37 g QE/100 g (Fig 4). MC1 (n-hexane extract of *Momordica charantia* leaves) had the highest total flavonoid content (14.37 g QE/100 g) and the lowest (0.76 g QE/100 g) for MC3 leaves extract.

Total carotenoid in different polarities leaves extracts from five species of Cucurbitaceae

The total carotenoid content among the different polarities extracts were expressed in term of beta carotene equivalent using the standard curve equation $y = 0.019 x + 0.048$, $R^2 = 0.993$. The total carotenoid content in different polarities leaves extracts from five species of Cucurbitaceae showed different result in the range of 0.04 - 19.53 g BET/100 g (Fig 5). The highest carotenoid content (19.53 g BET/100 g) for MC1 leaves extract, while the lowest carotenoid (0.04 g BET/100 g) for CS3 leaves extract.

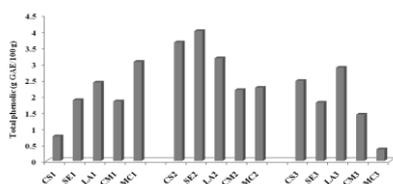


Fig 3: Total phenolic content in different polarities Cucurbitaceae leaves extracts

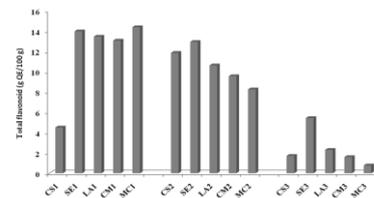


Fig. 4: Total flavonoid content in different polarities Cucurbitaceae leaves extracts

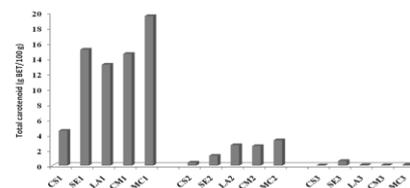


Fig. 5: Total carotenoid content in different polarities Cucurbitaceae leaves extracts

Correlations between total phenolic, flavonoid, carotenoid content and FRAP capacities, DPPH scavenging capacities, in different polarities leaves extracts from five species of Cucurbitaceae

Pearson's correlation coefficient was positively high if $0.68 \leq r \leq 0.97$ [1]. The highest and positive correlation between total phenolic content and FRAP capacity ($r = 0.982$, $p < 0.01$) was given by sample SE, followed by sample MC ($r = 0.806$, $p < 0.01$) (Table 4). The highest and positive correlation between total phenolic content and DPPH scavenging activity ($r = 0.888$, $p < 0.01$) for sample LA, followed by sample SE ($r = 0.875$, $p < 0.01$)

DISCUSSION

In previous study [2] [3] [5] [6] demonstrated that some of tropical plants including Cucurbitaceae leaves had antioxidant capacity using various antioxidant testing assays.

There were no study regarding antioxidant capacity of three different polarities extracts (which were n-hexane, ethyl acetate and ethanol) of leaves from five species of Cucurbitaceae using DPPH and FRAP assays.

DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 517 nm, respectively. Colors of DPPH would be changed when the free radicals were scavenged by antioxidant [14] [15]. FRAP is $FeCl_3$ that combined with 2,4,6-tripyridyltriazine (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 is depend 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.

Table 4: Pearson's correlation coefficient of total phenolic, flavonoid, carotenoid of leaves extract from five species of Cucurbitaceae and FRAP capacities, DPPH scavenging capacities

	Total Phenolic	Total Flavonoid	Total Carotenoid	DPPH CS	DPPH SE	DPPH LA	DPPH CM	DPPH MC
FRAP CS	0.490 ^{ns}	-0.362 ^{ns}	-0.838 ^{**}	-0.683 [*]				
FRAP SE	0.982 ^{**}	0.286 ^{ns}	-0.561 ^{ns}		0.931 ^{**}			
FRAP LA	0.320 ^{ns}	0.764 [*]	0.236 ^{ns}			-0.104 ^{ns}		
FRAP CM	-0.475 ^{ns}	-0.946 ^{**}	-0.943 ^{**}				-0.954 ^{**}	
FRAP MC	0.806 ^{**}	0.895 ^{**}	0.994 ^{**}					-0.868 ^{**}
DPPH CS	0.283 ^{ns}	0.910 ^{**}	0.196 ^{ns}					
DPPH SE	0.875 ^{**}	-0.062 ^{ns}	-0.815 ^{**}					
DPPH LA	0.888 ^{**}	-0.734 [*]	-0.991 ^{**}					
DPPH CM	0.255 ^{ns}	0.860 ^{**}	0.996 ^{**}					
DPPH MC	-0.972 ^{**}	-0.977 ^{**}	-0.873 ^{**}					

Note: FRAP = FRAP capacity, DPPH = DPPH scavenging capacity, CS = sample CS, SE = sample SE, LA = sample LA, CM = sample CM, MC =

sample MC, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

FRAP capacity of sample MC had the highest and positive correlation with total flavonoid ($r = 0.895$, $p < 0.01$), followed by sample LA ($r = 0.764$, $p < 0.05$). Sample CS had the highest and positive correlation between DPPH scavenging capacity and total flavonoid ($r = 0.910$, $p < 0.01$), followed by sample CM ($r = 0.860$, $p < 0.01$).

The highest and positive correlation between total carotenoid content and FRAP capacity ($r = 0.994$, $p < 0.01$) was given by sample MC and the highest and positive correlation with DPPH scavenging capacity ($r = 0.996$, $p < 0.01$) was given by sample CM..

In the present study, the highest DPPH scavenging capacity was given by sample LA3 (ethanolic extract of *Luffa acutangula*), followed by sample LA2 and SE2, while the highest FRAP capacity was given by sample SE2 (ethyl acetate extract of *Sechium edule*). Ethanolic extract of *Luffa acutangula* leaves (LA3) had DPPH scavenging capacity 41.46 %, while study by Sharma et al [6] demonstrated that methanolic extract of *Luffa cylindrica* leaves had DPPH scavenging capacity 85.18 %.

The previous study [8] showed that ethanolic leaves extract, water seed and leaves extracts of *Sechium edule* had antioxidant capacities by DPPH method. The other study [7] 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. Study by Amira [5] exposed that DPPH scavenging capacity of water extract of *Momordica charantia* fruits was higher than its acetone and methanol extracts. Amira [5] also showed that DPPH scavenging capacity of water fruits extracts was 98.29 % and 38.92 % for FRAP capacity.

The FRAP capacities among n-hexane leaves extract demonstrated that SE1, LA1 and MC1 were significantly different from each other ($p < 0.05$). CS1 and CM1 were not significantly different from each other and both of them were significantly different with SE1, LA1 and MC1 ($p < 0.05$). In DPPH scavenging capacities showed that all of samples (CS1, SE1, LA1, CM1 and MC1) were significantly different from each other ($p < 0.05$).

Statistical analysis of FRAP capacities among ethyl acetate leaves extract indicated that SE2, LA2 and MC2 were significantly different from each other ($p < 0.05$). CS2 and CM2 were not significantly different from each other and both of them were significantly different with SE2, LA2 and MC2 ($p < 0.05$). The DPPH scavenging capacities exposed that all of samples (CS2, SE2, LA2, CM2 and MC2) were significantly different from each other ($p < 0.05$).

The FRAP capacities among ethanolic leaves extract showed that SE3, LA3 and MC3 were significantly different from each other ($p < 0.05$). CS3 and CM3 were not significantly different from each other and both of them were significantly different with SE3, LA3 and MC3 ($p < 0.05$). Statistical analysis of DPPH scavenging capacities demonstrated that all of samples (CS3, SE3, LA3, CM3 and MC3) were significantly different from each other ($p < 0.05$).

EC₅₀ of FRAP capacity is concentration of sample or standard that can exhibit 50 % of FRAP capacity, while IC₅₀ of DPPH scavenging capacity is concentration of sample or standard that can inhibit 50 % of DPPH scavenging capacity. The lowest EC₅₀ or IC₅₀ means had the highest antioxidant capacity. EC₅₀ or IC₅₀ were used to determine antioxidant capacity of sample compared to standard. Sample that had EC₅₀ or IC₅₀ < 50 ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with EC₅₀ or IC₅₀ > 150 ppm [10].

SE2 (ethyl acetate extract of *Sechium edule* leaves) had the lowest EC₅₀ of FRAP capacity (759 ppm), while ascorbic acid standard gave EC₅₀ of FRAP capacity 418 ppm. Its showed that antioxidant capacity of SE2 was a half of potency of ascorbic acid using FRAP assays. In the DPPH method, free radical scavenging activities of different polarities extracts from five species of Cucurbitaceae ranged from 73 to 4885 ppm. LA3 (ethanolic extract of *Luffa acutangula* leaves) had the lowest IC₅₀ of DPPH radical scavenging capacity 73 ppm that was strong antioxidant, followed by SE3 with IC₅₀ of DPPH scavenging capacity 94 ppm, while ascorbic acid standard gave IC₅₀ of DPPH scavenging capacity 6 ppm and its exposed that antioxidant capacity

of LA3 was one-tenth of potency of ascorbic acid using DPPH method. In the present study ethanolic extract of *Cucumis sativus* leaves (CS3) had IC₅₀ of DPPH scavenging capacity was 416 ppm, while in the previous study [3] used seeds part and showed that IC₅₀ of DPPH scavenging capacity of methanolic extract of *Cucumis sativus* seeds was 1.25 ppm. Ethanolic extract of *Momordica charantia* leaves (MC3) had IC₅₀ of DPPH scavenging capacity was 4885 ppm, while study by Patel et al [16] exposed that IC₅₀ of DPPH scavenging capacity were 120 ppm and 182 ppm for alcohol fruits extract and water fruits extract of *Momordica charantia* respectively. Research by Lu et al [7] had studied fruits of 16 cultivars of *Momordica charantia* (A-P) and showed that water fruits extract of cultivar N had the lowest IC₅₀ of DPPH scavenging capacity (181 ppm). In this study exposed that IC₅₀ of DPPH scavenging capacity ethanolic extract of *Cucurbita moschata* leaves (CM3) was 800 ppm, while Xia [17] revealed that ethanol extract of *Cucurbita moschata* fruits was 18.8 ppm.

The presence of total phenolic might contribute to antioxidant activity [2]. Phenolic acid might contributed in antioxidant activity and cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid [18]. The data in Table 4 exposed that there were positively high correlation between total phenolic content in *Sechium edule* leaves extracts and antioxidant capacities using two methods FRAP and DPPH assays ($p < 0.01$). Based on this data it could be concluded that antioxidant capacities in *Sechium edule* leaves extracts by FRAP and DPPH assays might be estimated indirectly by determining their total phenolic content. In this study demonstrated that total phenolic content in *Momordica charantia* leaves extract had highly and positive correlation with their FRAP capacity. This results was similar with study by Amira [5] revealed that antioxidant capacity of *Momordica charantia* fruit extracts by FRAP assays had high correlation with its total phenolic content. Total phenolic in *Cucumis sativus* leaves extracts had no correlation with its DPPH scavenging capacities. It was similar with previous study exposed that there was no correlation between antioxidant capacity of *Cucumis sativus* fruits extracts with their total phenolic [3].

Pearson's correlation coefficient above showed that total flavonoid in *Cucumis sativus* and *Cucurbita moschata* leaves extracts had highly and positive correlation with their antioxidant capacities by DPPH assays, while *Luffa acutangula* and *Momordica charantia* leaves extracts had highly and positive correlation with their antioxidant capacities by FRAP assays.

Flavonoid not always be phenolic compounds its depending on position of OH in flavonoid. Phenolic compound included tannins, flavonoid, phenolic acid and other compounds. Flavonoid that have OH in A ring and or B ring is included phenolic groups. Phenolic acid had the lower antioxidant capacity than flavonoid [18]. Flavonoid would give higher antioxidant capacity which had OH in ortho C 3',4', OH in C3, oxo function in C4, 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 [18]. Based on the data correlation of total phenolic, flavonoid in five species Cucurbitaceae leaves extracts and their antioxidant capacities above it can predicted that many flavonoids in Cucurbitaceae leaves were flavonoid that had no OH in ortho C3',4', OH in C3, oxo function in C4, double bond at C2 and C3. There were predicted that flavonoid in Cucurbitaceae leaves 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.

Total carotenoid in *Momordica charantia* leaves extracts had highly and positive correlation with their antioxidant capacities by DPPH assays, while *Cucumis sativus* leaves extracts had highly and positive correlation with their antioxidant capacities by FRAP assays.

Carotenoid had antioxidant capacity by scavenging free radical. More double bonds in carotenoid would give higher scavenging free radical capacity [19]. Carotenoid that consisted of maximum 7 double bonds gave lower scavenging radical free capacity than more

double bonds [20]. In previous study by Kobayashi and Sakamoto [21] stated that increasing in lipophilicity of carotenoid would increase scavenging radical capacity. 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) [22]. Beta carotene was used as standard because of it had conjugation double bonds due to its ability to scavenge free radicals [23]. Based on the above data, it could be seen that many carotenoid in MC1 leaves extracts (that had the highest carotenoid) was lower than 7 double bonds, that had no or low antioxidant capacity. FRAP and DPPH methods had different mechanism reaction. Mechanism of DPPH that was electron transfer assays [24] and FRAP was redox assays. So the results of this study showed that FRAP capacity not always linear with DPPH scavenging capacity. 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 had reducing power to reduce Fe (III) to Fe (II) and this sample will be oxidized. The Pearson's correlation coefficient of different polarities leaves extracts from five species of Cucurbitaceae indicated that only in *Sechium edule* leaves extracts had positively and high correlation between FRAP capacities and DPPH scavenging capacities. It could be seen that antioxidant capacities in *Sechium edule* leaves extracts by FRAP assays were linear with DPPH assays.

CONCLUSION

To assess the antioxidant capacity of sample, variety of methods must be used in parallel, because different methods often give different results. The ethanolic extracts of *Luffa acutangula* leaves and *Sechium edule* leaves had the lowest IC₅₀ of DPPH scavenging capacities that were strong antioxidant. The ethyl acetate leaves extracts of *Sechium edule* had the lowest EC₅₀ of FRAP capacities. The positively high correlation between total phenolic content with DPPH and FRAP capacities was given by *Sechium edule* leaves extracts. Antioxidant capacity using FRAP and DPPH assays in *Sechium edule* leaves extracts might be estimated indirectly by using total phenolic content. Phenolic compounds were the major contributor in antioxidant capacity in *Sechium edule* leaves extracts. There were not all of FRAP capacities in leaves extracts from five species of Cucurbitaceae linear with DPPH scavenging capacities. *Luffa acutangula* and *Sechium edule* leaves extracts may be exploited as biopreservatives in food applications as well as for health supplements or functional food, to alleviate oxidative stress.

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