

EVALUATION OF ANTIOXIDANT CAPACITIES, FLAVONOID, PHENOLIC, CAROTENOID CONTENT FROM VARIOUS EXTRACTS OF FOUR KINDS BRASSICA HERBS

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ABSTRACT

Objective: The objectives of this research were to study antioxidant capacity from various extracts of Brassica herbs 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 extracts of Brassica herbs with DPPH and FRAP antioxidant capacities.

Methods: Extraction was performed by reflux using different polarity 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 DPPH and FRAP antioxidant capacities were analyzed by Pearson method.

Results: D2 (ethyl acetate extract of Chinese kale herbs) had the highest DPPH scavenging capacity with IC₅₀ 8.36 ppm, while A2 (ethyl acetate extract of Chinese white cabbage herbs) had the highest FRAP capacity with EC₅₀ 1126 ppm. D2 contained the highest total flavonoid (23.08 g QE/100 g), C2 (ethyl acetate extract of Chinese cabbage herbs) had the highest phenolic content (7.40 g GAE/100 g) and B1 (n-hexane extract of choy sum had the highest carotenoid 41.04 g BET/100 g).

Conclusions: There were positively and high correlation between total phenolic and total flavonoid content in Chinese kale herbs extracts with their antioxidant activity using FRAP and DPPH assays. FRAP capacities in Chinese white cabbage, choy sum and Chinese kale herbs extracts had linier result with DPPH scavenging capacities.

Keywords: Antioxidants, DPPH, FRAP, Brassica herbs, Flavonoid, Phenolic, Carotenoid.

INTRODUCTION

Antioxidant was known to have beneficial effects to reduce the risk of many degenerative diseases that related to oxidative stress. 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 including vegetables contained phenolic and polyphenol compounds can act as antioxidant [2] [3].

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, beverages and food [1]. In previous study [1] [4] [5] revealed that DPPH and FRAP methods could be used to determine antioxidant activity in many plants extracts. The previous study [6] [7] [8] [9] [10] showed Brassica vegetables had antioxidant capacities by using DPPH, FRAP and ABTS assays. The objective of this research were to study antioxidant capacities of various extracts (n-hexane, ethyl acetate and ethanol) from four kinds of Brassica (*Brassica rapa* L.cv. group Pak choi, *Brassica rapa* L. cv. group Caisin, *Brassica rapa* L.cv. group Chinese, *Brassica oleracea* L.cv. group Chinese Kale) herbs 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, herbs from four kindsof Brassica, methanol, ethanol. All other reagents were analytical grades.

Preparation of sample

Herbs of four kinds Brassica were collected from Lembang-Bandung that were Chinese white cabbage (*Brassica rapa* L.cv. group Pak choi

) namely as sample A, choy sum (*Brassica rapa* L.cv.group Caisin) as sample B, Chinese cabbage (*Brassica rapa* L.cv.group Chinese) as sample C and Chinese kale (*Brassica oleracea* L.cv.group Chinese Kale) as sample D 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 four n-hexane extracts (namely A1, B1, C1 and D1), four ethyl acetate extracts (A2, B2, C2 and D2) and four ethanolic extracts (A3, B3, C3 and D3).

Determination of DPPH scavenging capacity

Preparation of DPPH solution were adopted from Blois [11] 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 516 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 [12].

Determination of FRAP capacity

Preparation of FRAP solution were adopted from Benzi [13]. FRAP solution were prepared in acetate buffer pH 3.6. Each extracts 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 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. Analysis was done in triplicate for standard and each extracts. Antioxidant capacity of each extracts were determined based on increasing in Fe (II) - TPTZ absorbance by calculating percentage of antioxidant capacity [13].

Determination of total flavonoid content

Total flavonoid content was measured using adapted method from Chang *et al* [14]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extracts. Standard solutions of quercetin with concentration 40-100 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).

Determination of total phenolic content

Total phenolic content were measured using the modified Folin-Ciocalteu method adapted from Pourmorad [15]. 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).

Determination of total carotenoid content

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 10-80 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).

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 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 various herbs extracts from four kinds of Brassica using DPPH and FRAP assays

The antioxidant capacities using DPPH and FRAP assays of various herbs extracts from four kinds of Brassica were shown in Table 1, Table 2, Table 3.

In the DPPH method, free radical scavenging capacities of various herbs extracts from four kinds of Brassica ranged from 50.56 – 85.79 %. D2 (ethyl acetate extract of Chinese kale herbs) had the highest DPPH radical scavenging capacity (85.79 %), while B1 (n-hexane extract of choy sum herbs) had the lowest DPPH antioxidant capacity (50.56 %).

In FRAP method, antioxidant capacities in the range of 0.46 – 19.56 %. D3 herbs extract (ethanolic extract of Chinese kale herbs) had the highest FRAP capacity (19.56 %), while the lowest capacity (0.46 %) was given by B1 herbs extract.

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

Sample	DPPH scavenging capacity (%)	FRAP capacity (%)
A1	53,56 ± 0,005 a	0,80 ± 0,07 a
B1	50,56 ± 0,01 b	0,46 ± 0,10 b
C1	52,55 ± 0,01 c	1,00 ± 0,34 c
D1	55,27 ± 0,16 d	2,57 ± 0,10 d
Ascorbic acid	97.14 ± 0.10	7.39 ± 0.25
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: DPPH scavenging capacities and FRAP capacities of ethyl acetate herbs extracts

Sample	DPPH scavenging capacity (%)	FRAP capacity (%)
A2	79,24 ± 0,10 a	16,32 ± 0,37 a
B2	80,59 ± 0,07 b	12,29 ± 0,17 b
C2	75,59 ± 0,02 c	1,17 ± 0,24 c
D2	85,79 ± 0,08 d	18,54 ± 0,20 d
Ascorbic acid	97.14 ± 0.10	7.39 ± 0.25
P value	< 0.05	< 0.05

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

IC₅₀ of DPPH scavenging capacity and EC₅₀ of FRAP capacity

The IC₅₀ of DPPH scavenging capacities and EC₅₀ of FRAP capacities in various extracts from four kinds of Brassica herbs using DPPH and FRAP assays were shown in Fig 1 and Fig 2. IC₅₀ of DPPH scavenging capacities were compared to IC₅₀ ascorbic acid standard, while EC₅₀ of FRAP capacities of each extracts were compared to EC₅₀ ascorbic acid standard. The lowest EC₅₀ or IC₅₀ means had the highest antioxidant capacity.

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

Sample	DPPH scavenging capacity (%)	FRAP capacity (%)
A3	82,56 ± 0,07 a	12,98 ± 0,21 a
B3	72,74 ± 0,02 b	10,92 ± 1,18 b
C3	75,08 ± 0,21 c	8,47 ± 0,08 c
D3	73,21 ± 0,24 b	19,56 ± 0,18 d
Ascorbic acid	97.14 ± 0.10	7.39 ± 0.25
P value	< 0.05	< 0.05

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

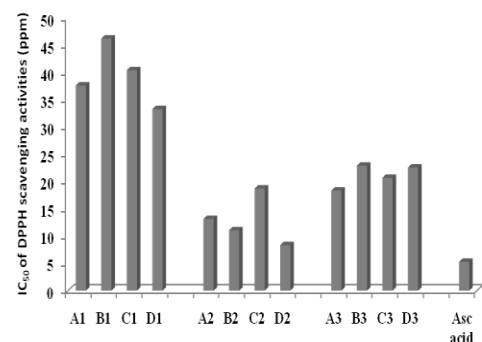


Fig. 1: IC₅₀ of DPPH scavenging capacities in various herbs extracts from four kinds of Brassica

Total flavonoid in various herbs extracts from four kinds of Brassica

The total flavonoid content among the various extracts were expressed in term of quercetin equivalent using the standard curve equation $y = 0.006x + 0.016$, $R^2 = 0.980$. The total flavonoid content in various herbs extracts from four kinds of Brassica showed different result in the range of 1.62 – 23.08 g QE/100 g (Fig 3). D2 (ethyl acetate extract of Chinese kale herbs) had the highest total flavonoid content (23.08 g QE/100 g) and the lowest (1.62 g QE/100 g) for C1 herbs extract.

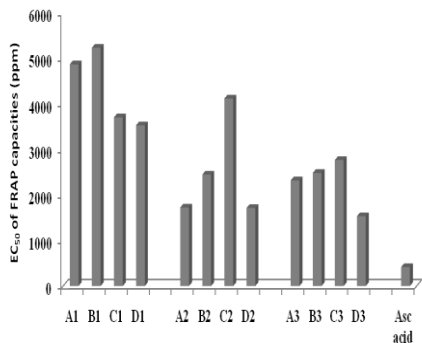


Fig. 2: EC₅₀ of FRAP capacities in various herbs extracts from four kinds of Brassica

Total phenolic in various herbs extracts from four kinds of Brassica

The total phenolic content among the various 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 various herbs extracts from four kinds of Brassica showed different result ranged from 0.86 to 7.40 g GAE/100 g. C2 herbs extract (ethyl acetate herbs extract of Chinese cabbage) had the highest phenolic content (7.40 g GAE/100 g) (Fig 4).

Total carotenoid in various herbs extracts from four kinds of Brassica

The total carotenoid content among the various 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 various herbs extracts from four kinds of Brassica showed different result in the range of 0.38 – 41.04 g BET/100 g (Fig 5). The highest carotenoid content (41.04 g BET/100 g) for B1 herbs extract, while the lowest carotenoid (0.38 g BET/100 g) for C3 herbs extract.

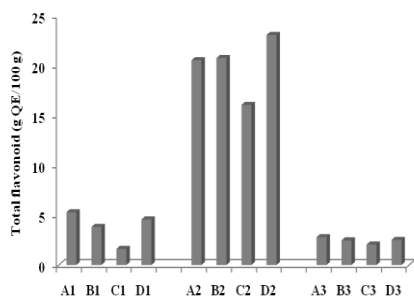


Fig. 3: Total flavonoid content in various Brassica herbs extracts

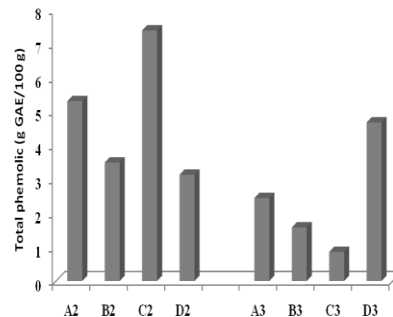


Fig. 4: Total phenolic content in various Brassica herbs extracts

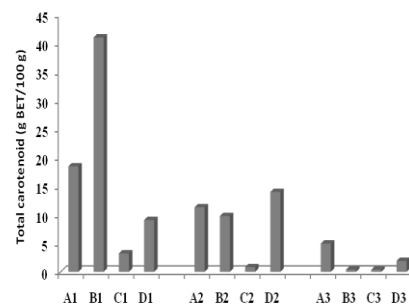


Fig. 5: Total carotenoid content in various Brassica herbs extracts

Correlations between total phenolic, flavonoid, carotenoid content and DPPH scavenging capacities, FRAP capacities in various herbs extracts from four kinds of Brassica

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 DPPH scavenging activity ($r = 0.946$, $p < 0.01$) for sample B, followed by sample A ($r = 0.782$, $p < 0.05$). The highest and positive correlation between total phenolic content and FRAP capacity ($r = 0.962$, $p < 0.01$) was given by sample D, followed by sample A ($r = 0.935$, $p < 0.01$) (Table 4).

Pearson’s correlation coefficient between total flavonoid form various extracts of four kinds of Brassica and their antioxidant capacities demonstrated that only total flavonoid in sample D (Chinese kale herbs extracts) had positively high correlation with DPPH scavenging capacities ($r = 0.755$, $p < 0.05$).

The correlation between total carotenoid and their antioxidant capacities exposed that almost all of Brassica herbs extracts sample had negative correlation with DPPH scavenging capacities and FRAP capacities.

Table 4: Pearson’s correlation coefficient of total flavonoid, phenolic, carotenoid of herbs extract from four kinds of Brassica and DPPH scavenging capacities, FRAP capacities

	Total Flavonoid	Total Phenolic	Total Carotenoid	FRAP A	FRAP B	FRAP C	FRAP D
DPPH A	0,284 ^{ns}	0,782*	-0,927**	0.952**			
DPPH B	0,652 ^{ns}	0,946**	-0,887**		0.988**		
DPPH C	0,539 ^{ns}	0,605 ^{ns}	-0,983**			0.500 ^{ns}	
DPPH D	0,755*	0,730**	0,311 ^{ns}				0.889**
FRAP A	0,563 ^{ns}	0,935**	-0,768*				
FRAP B	0,534 ^{ns}	0,889**	-0,946**				
FRAP C	-0,459 ^{ns}	-0,386 ^{ns}	-0,628**				
FRAP D	0,372 ^{ns}	0,962**	-0,159**				

Note: FRAP = FRAP capacity, DPPH = DPPH scavenging capacity, A = sample A, B = sample B, C = sample C, D = sample D, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

DISCUSSION

In previous study [6] [7] [8] [9] [10] revealed that Brassica vegetables had antioxidant capacity. There were no study regarding antioxidant capacity of three various polarities extracts (which were n-hexane, ethyl acetate and ethanol) of herbs from four kinds of Brassica using DPPH and FRAP assays.

DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 516 nm, respectively. Colors of DPPH would be changed when the free radicals were scavenged by antioxidant [16] [17]. 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. In the present study, the highest DPPH scavenging capacity was given by sample D2 (ethyl acetate extract of Chinese kale herbs), followed by sample A3 and B2, while the highest FRAP capacity was given by sample D3 (ethanolic herbs extract of Chinese kale). Ethanolic extract of Chinese white cabbage herbs (A3) had DPPH scavenging capacity 82.56 %, while study by Yee et al [10] demonstrated that methanolic extract of Chinese white cabbage had DPPH scavenging capacity 97 %. Yee et al [10] also revealed that methanolic extract of Chinese white cabbage had higher antioxidant capacity than Chinese cabbage. It was similar with the present study which showed DPPH scavenging capacity of ethanolic extract of Chinese white cabbage herbs (82.56 %) was higher than ethanolic extract of Chinese cabbage herbs (75.08 %). In contrast, water extract of Chinese cabbage had the higher DPPH scavenging capacities than water extract of Chinese white cabbage was showed by Amin [18]. Samec et al [7] exposed that Chinese cabbage juice had the higher DPPH scavenging capacity and FRAP capacity than Chinese white cabbage juice. The previous study showed that antioxidant capacity of methanolic extract of Chinese cabbage was higher than methanolic extract of Chinese white cabbage [19]. The DPPH scavenging capacities among n-hexane herbs extracts were significantly different from each other ($p < 0.05$). The same result was exposed by ethyl acetate and ethanol extracts. In FRAP capacities among n-hexane herbs extracts were significantly different from each other ($p < 0.05$) and the same result was showed by ethyl acetate and ethanol extracts.

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 that compared to standard. Sample that had IC_{50} or $\text{EC}_{50} < 50$ ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with IC_{50} or $\text{EC}_{50} > 150$ ppm [11].

In the DPPH method, antioxidant capacities of various extracts from four kinds of Brassica ranged from 8.36 to 46.26 ppm. D2 (ethyl acetate extract of Chinese kale herbs) had the lowest IC_{50} of DPPH radical scavenging capacity 8.36 ppm, while ascorbic acid standard gave IC_{50} of DPPH scavenging capacity 5.29 ppm. Based on value of IC_{50} of DPPH scavenging capacity it can be concluded that all of sample extracts of Brassica in this study can be categorized as very strong antioxidant. A2 (ethyl acetate extract of Chinese white cabbage herbs) had the lowest EC_{50} of FRAP capacity (1126 ppm) while ascorbic acid standard gave EC_{50} of FRAP capacity 418 ppm. Its showed that potency of ascorbic acid was around three times of potency of A2 using FRAP assays.

The presence of total phenolic might contribute to antioxidant capacity [2]. Phenolic acid might contributed in antioxidant capacity and cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid [20]. The previous study [10] showed that total phenolic content in methanolic extract of Chinese white cabbage (96 mg GAE/100 g) was higher than Chinese cabbage (67 mg GAE/100 g). It was similar with present study which exposed that total phenolic in ethanolic extract of Chinese white cabbage

(2.45 g GAE/100 g) was higher than ethanolic extract of Chinese cabbage (0.86 g GAE/100 g). In contrast, Samec [7] found that total phenolic content in Chinese cabbage juice was higher than Chinese white cabbage juice.

The data in Table 4 exposed that there were positively high correlation between total phenolic content in Chinese white cabbage, choy sum and Chinese kale herbs extracts and antioxidant capacities using two methods FRAP and DPPH assays. Based on this data it could be concluded that antioxidant capacities in Chinese white cabbage, choy sum and Chinese kale herbs extracts with FRAP and DPPH assays might be estimated indirectly by determining their total phenolic content. The previous study [9] revealed that total phenolic content in *Brassica oleracea encephala* had positive and strong correlation with its FRAP capacity. The result was similar with the present study that exposed total phenolic in Chinese kale (*Brassica oleracea* L.cv.group) had highly and positive correlation with FRAP capacity ($r = 0.962$, $p < 0.01$).

In this study found that total flavonoid content in ethanolic extract of Chinese white cabbage was higher than ethanolic extract of Chinese cabbage. It was not similar with the previous study [7] which demonstrated that total phenolic in Chinese white cabbage juice was lower than Chinese cabbage juice.

Pearson's correlation coefficient in Table 4 above showed that total flavonoid in almost all of sample in this study had no correlation with their antioxidant capacities by DPPH and FRAP assays. Only total flavonoid in Chinese kale herbs extracts that had highly and positive correlation with their antioxidant capacities by DPPH assays ($r = 0.755$, $p < 0.05$).

Phenolic compound included tannins, flavonoid, phenolic acid and other compounds. Flavonoid not always be phenolic compounds its depending on position of OH in flavonoid. 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 [20]. 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 [20]. Generally it could be seen in Fig 3 that total flavonoid in ethyl acetate extracts higher than total flavonoid in ethanolic extracts, but antioxidant capacities of ethyl acetate extracts was almost similar with ethanolic extracts. Based on the data above it can predicted that many flavonoids in ethyl acetate extracts of Brassica herbs 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 ethanolic extracts of Brassica herbs were flavonoid that had OH in position which can influence antioxidant capacities. The previous study [6] exposed that common flavonoid that were found in Brassica such as quercetin, kaempferol and isorhamnetin. The three of flavonoids had many OH in the position that can exhibit their antioxidant capacity. Based on the data above, it can be concluded that ethyl acetate extracts of Brassica in this study contained only little amount of quercetin, kaempferol and isorhamnetin.

Study by Ahmed [9] revealed that it had no significant correlation between total carotenoid in *Brassica oleracea encephala* with FRAP capacity. It was similar with the present study which showed that total carotenoid in Chinese kale (*Brassica oleracea* L.cv.group Chinese kale) had negative correlation with its FRAP capacity and DPPH scavenging capacity. The data Pearson's correlation between total carotenoid and their antioxidant capacities demonstrated that almost all of sample of Brassica herbs had highly negative correlation with antioxidant capacities using DPPH and FRAP assays, it means higher total carotenoid of sample will give lower antioxidant capacities. Carotenoid had antioxidant capacity by scavenging free radical. More double bonds in carotenoid would give higher scavenging free radical capacity [21]. Carotenoid that consisted of maximum 7 double bonds gave lower scavenging radical free capacity than more double bonds [22]. In previous study by Kobayashi and Sakamoto [23] 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) [24]. Beta carotene was used as standard because of it had conjugation double bonds due to its ability to scavenge free radicals [25]. Based on the above data, it could be seen that many carotenoid in B1 herbs extracts (that had the highest carotenoid) contained 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 [26] and FRAP was redox assays. So the results of this study showed that FRAP capacity was 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 four kinds of Brassica herbs indicated that sample A, B and D had positively and high correlation between DPPH scavenging capacities and FRAP capacities. It could be seen that antioxidant capacities of sample A, B and D by DPPH and FRAP assays gave linear result.

CONCLUSION

To assess the antioxidant capacity of sample, variety of methods must be used in parallel, because different methods could give different results. All of herbs extracts of Brassica had IC₅₀ of DPPH scavenging capacities < 50 ppm that were very strong antioxidant. The positively and high correlation between total phenolic and total flavonoid content with DPPH scavenging capacities was given by Chinese kale herbs extracts.

Antioxidant capacity using DPPH assays in Chinese kale herbs extracts might be estimated indirectly by using total phenolic and total flavonoid content.

Phenolic and flavonoid compounds were the major contributor in antioxidant capacity in Chinese kale herbs extracts. Antioxidant capacities of Chinese white cabbage, choy sum and Chinese kale gave linear result by DPPH and FRAP assays. Chinese kale herbs extracts may be exploited as a source of beneficial compounds for human health to alleviate oxidative stress.

REFERENCES

1. Thaipong K, Boonprakob U, Crosby K, Zevallos LC, Byrne DH. Comparison of FRAP, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. *J Food Comp Anal* 2006; 19: 669-675.
2. Ling, LT and Palanisamy, UD. Review: Potential antioxidants from tropical plants, In: Valdez, B., editor, Food industrial processes-methods, Kuala Lumpur: In Tech; 1999. p.64-72.
3. Lindley MG. The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. *Trends in Food Sci Technol* 1998; 9: 336-340.
4. Souri E, Amin G, Farsan H, Barazandeh TM. Screening antioxidant activity and phenolic content of 24 medicinal plants extracts. *DARU J Pharm Sci* 2008; 16: 83 -87
5. Luis A, Gil N, Amaral ME, Duarte AP. Antioxidant activities of extract from *Acacia melanoxylon*, *Acacia dealbata*, *Olea europaea* and alkaloids estimation. *Int J Pharm Pharm Sci* 2012; 4: 225-231.
6. Cartea ME, Fransisco M, Soengas P, Velasco P. Phenolic compounds in Brassica vegetables. *Molecules* 2011; 16: 251-280.
7. Samec D, Zegarac JP, Bogovic M, Habjanic K, Gruz J. Antioxidant potency of white (*Brassica oleracea* L. var. capitata) and Chinese (*Brassica rapa* L. var. var. pkinensis (Lour.)) cabbage: The influence of development stage, cultivar choice and seed selection. *Scientia Horticulturae* 2011; 128: 78-83.
8. Soengas P, Sotelo T, Velasco P, Cartea ME. Antioxidant properties of Brassica vegetables In: Functional plant science and biotechnology, Pontevedra: Global Science Books; 2011. 43-55
9. Ahmed S, Beigh SH. Ascorbic acid, carotenoids, total phenolic content and antioxidant activity of various genotypes of *Brassica oleracea encephala*. *Journal of Medical and Biological Sciences* 2009; 3: 1-8
10. Yee LW, Ikram EHK, Jalil AMM, Ismail A. Antioxidant capacity and Phenolic Content of Selected Commercially Available Cruciferous Vegetables. *Mal J Nutr* 2007; 13: 71-80
11. Blois MS. Antioxidant determination by the use of stable free radicals. *Nature* 1958; 181: 1199-2000.
12. Bedaway AA. Characteristics of antioxidant isolated from some plants sources, Cairo: Shubin El-Kom; 2010, p. 1-11.
13. Benzi IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* 1996; 239: 70-76.
14. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. *J Food Drug Anal* 2002; 10: 178-182
15. Pourmorad F, Hosseinimehr SJ, Shahabimajid N. Antioxidant activity, phenol and flavonoid content of some selected Iranian medicinal plants. *Afr J Biotechnol* 2006; 5(11): 1142-1145.
16. Li XC, Wang XZ, Chen DF, Chen SZ. Antioxidant activity and mechanism of protochatechuic acid in vitro. *J Funct Food Health Dis* 2011; 1: 232-244.
17. Apak R, Kubilay G, Birsen D, Mustava O, Saliha EC, Burcu B et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* 2007; 12: 1496-1547
18. Amin I, Lee WY. Effect of different blanching times on antioxidant properties in selected cruciferous vegetables. *J Sci Food Agric* 2005; 98: 2314-2320.
19. Jagdish S, Upadhyay AK, Singh S, Rai M. Total phenolic content and free radical scavenging activity of Brassica vegetables. *J Food Sci Tech- Mysore* 2009; 46: 595-597.
20. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* 2002; 13: 572 -584.
21. Foote, CS. Free radicals in biology. 3rd ed. New York: Academic Press; 1976.
22. Beutner S, Bloedorn B, Hoffmann T, Martin HD. Synthetic singlet oxygen quenchers. *Methods Enzymol* 2000; 319: 226-241.
23. Kobayashi M, Sakamoto Y. Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. *Biotech Lett* 1999; 21: 265-269.
24. Müller L, Fröhlich K, Böhm V. Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (aTEAC), DPPH assay and peroxy radical scavenging assay. *Food Chem* 2011; 129: 139-148
25. Charles DJ. Antioxidant properties of spices herbs and other. London: John Wiley; 2013.
26. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. *J Agric Food Chem* 2005; 53: 1841 -1856.