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Original Article

IN VITRO ANTIOXIDANT ACTIVITIES, TOTAL FLAVONOID, PHENOLIC AND CAROTENOID CONTENT FROM VARIOUS EXTRACTS OF FOUR SPECIES *ASTERACEAE* HERB

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ABSTRACT

Objectives: The objectives of this research were to study antioxidant activities from various extracts of Asteraceae herbs using two methods of antioxidant assays which were DPPH (2,2-diphenyl-1-picrylhidrazyl) and FRAP (Ferric Reducing Antioxidant Power); and correlation of total flavonoid, phenolic, and carotenoid content in various extracts of Asteraceae herbs with DPPH antioxidant activities and FRAP antioxidant capacities.

Methods: Extraction was performed by reflux apparatus using different polarity solvents. The extracts were evaporated using the rotary evaporator. Antioxidant capacities were tested using DPPH and FRAP assays. Determination of total flavonoid, phenolic, and carotenoid content was performed by spectrophotometer UV-visible and their correlation with DPPH antioxidant activities and FRAP antioxidant capacities were analyzed by Pearson's method.

Results: Methanolic extract of *Bidens pilosa* herbs (BP3) had the highest DPPH scavenging activity with IC_{50} 76.25 µg/ml, while ethyl acetate extract of *B. pilosa* herbs (BP2) had the highest FRAP capacity with EC_{50} 33.50 µg/ml. Ethyl acetate extract of *B. pilosa* (BP2) had the highest total flavonoid (14.66 g QE/100 g), BP3 had the highest phenolic content (7.61 g GAE/100 g), and ethyl acetate extract of *Sonchus arvensis* (SA2) had the highest carotenoid content (11.92 g BE/100 g).

Conclusions: There was a positively high correlation between total phenolic with their antioxidant activity using FRAP and DPPH assays. The FRAP capacities in *Artemisia vulgaris*, *Bidens pilosa*, *Ageratum conyzoides,* and *Sonchus arvensis* herbs extracts had linear result with DPPH scavenging activities.

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

INTRODUCTION

Many degenerative diseases are related with oxidative stress. Antioxidant is known to inhibit and reduce oxidative stress. Phenolic compounds are commonly found in plants, and they have been demonstrated to have multiple biological effects, including antioxidant activity [1, 2]. 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 [1, 3, 4].

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 vegetables, fruits, beverages, and food [2, 5]. Previous studies [2, 5-7] revealed that DPPH and FRAP methods could be used to determine antioxidant activity in many plants extracts. The previous studies [1, 5, 6, 8, 9] exhibited that Asteraceae had antioxidant capacities by using DPPH, FRAP, and ABTS assays.

The objectives of this research were to study antioxidant activities of various extracts (n-hexane, ethyl acetate, and methanol) from four species of Asteraceae (*Artemisia vulgaris*, *Bidens pilosa*, *Ageratum conyzoides*, and *Sonchus arvensis*) herbs using 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-picrylhidrazyl), gallic acid, quercetin, beta carotene purchased from Sigma-Aldrich (MO, USA), ferric chloride, herbs from four species of Asteraceae, methanol. All other reagents were analytical grades.

Preparation of sample

Herbs from four species of Asteraceae: *A. vulgaris* as sample AV from Lembang, *B. pilosa* as sample BP, *A. conyzoides* as sample AC, and *S. arvensis* as sample SA from Cimahi, were thoroughly washed with tap water, sorted while wet, cut, dried, and grinded into powder.

Extraction

Three hundred grams of powdered samples were extracted by reflux apparatus using increasing polarity of solvents. The extraction using n-hexane was repeated three times. The remaining residue was then extracted three times with ethyl acetate. Finally the remaining residue was extracted three times with methanol. So there were four n-hexane extracts (AV1, BP1, AC1, and SA1), four ethyl acetate extracts (AV2, BP2, AC2, and SA2) and four methanolic extracts (AV3, BP3, AC3, and SA3).

Determination of DPPH scavenging activity

Preparation of DPPH solution was adopted from Blois [10] with minor modification. Each extract 50 µg/ml was pipetted into DPPH solution 50 µg/ml (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was read at wavelength 515 nm by using spectrophotometer UV-Vis Hewlett Packard 8435. Methanol was used as a blank. DPPH solution 50 µg/ml and methanol (1:1) 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 [11].

Determination of FRAP capacity

Preparation of FRAP solution was adopted from Benzi [12]. The 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 by using spectrophotometer UV-Vis Hewlett Packard 8435. Acetate buffer was used as a blank and FRAP solution 50 µg/ml and methanol (1:1) 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 [12].

Determination of total flavonoid content

Total flavonoid content was measured using an adapted method from Chang *et al* [13]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Standard solutions of Quercetin 25-150 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 was measured using the modified Folin-Ciolcalteu method adapted from Pourmorad [14]. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Standard solutions of gallic acid 40-200 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* [2]. Each extract was diluted in n-hexane. The absorbance was read at the wavelength 470 nm. Analysis was done in triplicate for each extract. Standard solutions of beta carotene 15-45 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 BE/100 g).

Statistical analysis

Analysis of each sample was performed in triplicate. All results presented were the means±SD of at least three independent experiments. Statistical analysis (ANOVA with a statistical significance level set at $p<0.05$ and post-hoc Tukey procedure) was carried out with SPSS 17.0 for Windows. Correlations between the total flavonoid, phenolic, and carotenoid content with antioxidant capacities were made using the Pearson's method ($p<0.01$).

RESULTS

Antioxidant capacities of various herb extracts from four species of Asteraceae using DPPH and FRAP assays

The antioxidant activities and capacities using DPPH and FRAP assays of various herb extracts from four species of Asteraceae were shown in table 1, table 2, and table 3. In the DPPH method, free radical scavenging activities of various herb extracts from four species of Asteraceae ranged from 1.48 to 36.54%. Ethyl acetate extract of *B. pilosa* herb (BP2) had the highest DPPH radical scavenging activity (36.54%), while n-hexane extract of *Sonchus arvensis* herb (SA1) had the lowest DPPH antioxidant activity (1.48%). Using FRAP method, antioxidant capacities in the range of 4.21 to 68.09%. Ethyl acetate extract of *B. pilosa* herb (BP2) had the highest FRAP capacity (68.09%), while the lowest capacity (4.21%) was given by n-hexane extract of *B. pilosa* herb (BP1).

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

Sample	DPPH scavenging activity (%)	FRAP capacity (%)
AV1	2.63±0.37 [a]	6.85±0.03 [a]
BP1	3.84±0.05 [b]	4.21±0.01 [b]
AC1	2.56±0.19 [a]	11.25±0.04 [c]
SA1	1.48±0.14 [c]	7.76±0.01 [d]
Ascorbic acid	95.59±0.03 [d]	92.62±0.10 [e]

Note: a–e = means within a column with the different letter were significantly different ($p<0.05$)

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

Sample	DPPH scavenging activity (%)	FRAP capacity (%)
AV2	9.74±0.28 [a]	21.33±0.05 [a]
BP2	36.54±0.14 [b]	68.09±0.04 [b]
AC2	15.42±0.12 [c]	27.89±0.15 [c]
SA2	7.40±0.17 [d]	17.44±0.02 [d]
Ascorbic acid	95.59±0.03 [e]	92.62±0.10 [e]

Note: a–e = means within a column with the different letter were significantly different ($p<0.05$)

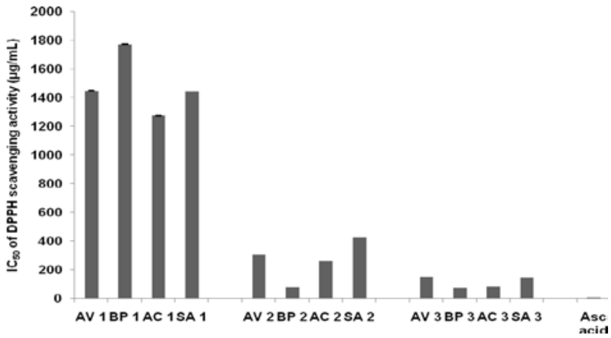
Table 3: DPPH scavenging activities and FRAP capacities of methanolic herb extracts

Sample	DPPH scavenging activity (%)	FRAP capacity (%)
AV3	27.97±0.10 [a]	39.28±0.03 [a]
BP3	36.50±0.18 [b]	65.80±0.10 [b]
AC3	26.84±0.09 [c]	50.56±0.04 [c]
SA3	27.35±0.09 [d]	47.31±0.02 [d]
Ascorbic acid	95.59±0.03 [e]	92.62±0.10 [e]

Note: a–e = means within a column with the different letter were significantly different ($p<0.05$)

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

The IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities in various extract from four species of Asteraceae herbs using DPPH and FRAP assays were shown in fig. 1 and fig. 2. The half maximum inhibitory concentration (IC_{50}) of DPPH scavenging activities was compared to IC_{50} ascorbic acid standard, while EC_{50} of FRAP capacities of each extracts was compared to EC_{50} ascorbic acid standard. The lowest EC_{50} or IC_{50} means to have the highest antioxidant capacity.

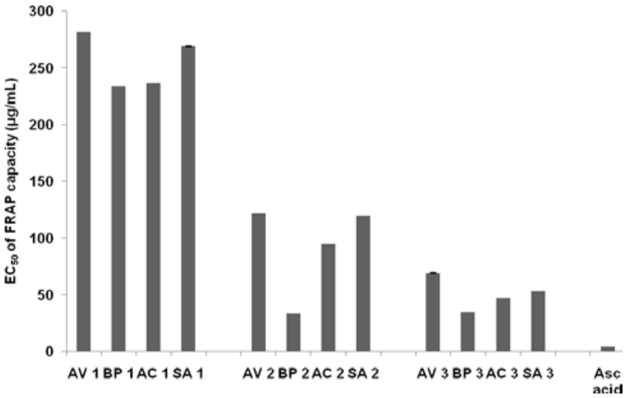


n=3

Fig. 1: IC_{50} of DPPH scavenging activities in various herb extracts from four species of Asteraceae

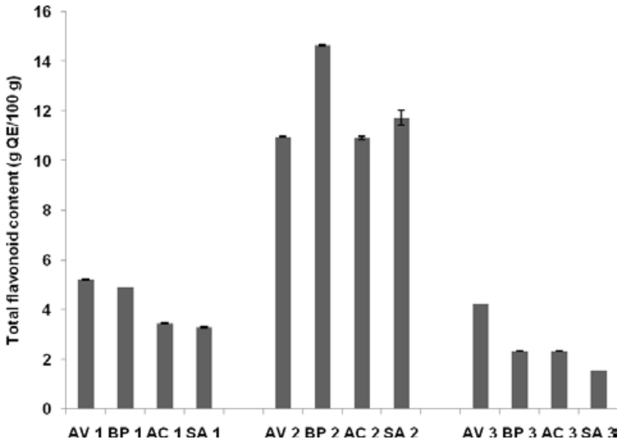
Total flavonoid in various herb extracts from four species of Asteraceae

The total flavonoid content among the various extracts was expressed in term of quercetin equivalent using the standard curve equation $y = 0.005x+0.004$, $R^2= 0.998$. The total flavonoid content in various herb extracts from four species of Asteraceae showed different results within the range of 1.57 to 14.66 g QE/100 g (fig. 3). Ethyl acetate extract of *B. pilosa* herbs (BP2) had the highest total flavonoid content (14.66 g QE/100 g) and methanolic extract of *S. arvensis* (SA3) had the lowest (1.57 g QE/100 g).



n=3

Fig. 2: EC_{50} of FRAP capacities in various herb extracts from four species of Asteraceae



n=3

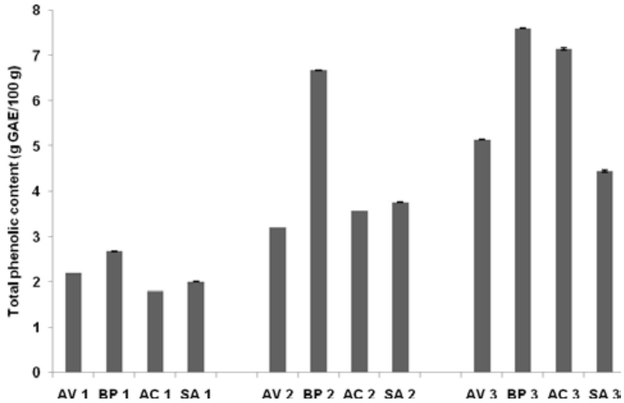
Fig. 3: Total flavonoid content in various Asteraceae herb extracts

Total phenolic in various herb extracts from four species of Asteraceae

The total phenolic content among the various extracts was expressed in term of gallic acid equivalent using the standard curve equation y = 0.004x+0.039, R^2= 0.998. The total phenolic content in various herb extracts from four species of Asteraceae showed different result ranged from 1.80 to 7.61 g GAE/100 g. Methanolic extract of *B. pilosa* herbs (BP3) had the highest phenolic content (7.61 g GAE/100 g) (fig. 4).

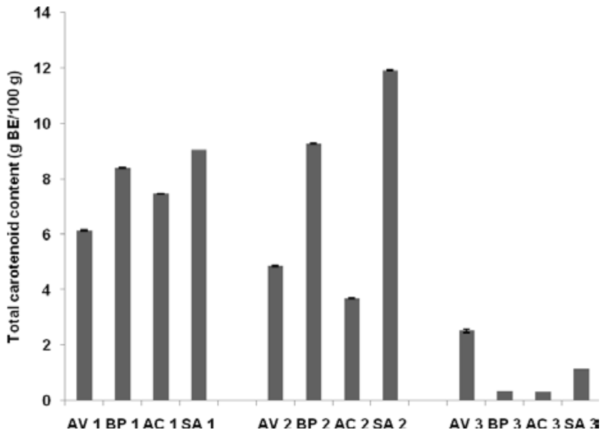
Total carotenoid in various herb extracts from four species of Asteraceae

The total carotenoid content among the various extracts was expressed in term of beta carotene equivalent using the standard curve equation y = 0.012 x-0.039, R^2= 0.988. The total carotenoid content in various herb extracts from four species of Asteraceae showed the different result in the range of 0.32 to 11.92 g BE/100 g (fig. 5). Ethyl acetate extract of *S. arvensis* herbs (SA2) had the highest carotenoid content (11.92 g BE/100 g), while methanolic extract of *A. conyzoides* herbs (AC3) had the lowest carotenoid content (0.32 g BE/100 g).



n=3

Fig. 4: Total phenolic content in various Asteraceae herb extracts



n=3

Fig. 5: Total carotenoid content in various Asteraceae herb extracts

Table 4: Pearson's correlation coefficient of total flavonoid, phenolic, carotenoid of herb extracts from four species of Asteraceae and DPPH scavenging activities, FRAP capacities

	Total flavonoid	Total phenolic	Total carotenoid	FRAP AV	FRAP BP	FRAP AC	FRAP SA
DPPH AV	-0.375 ns	0.997 **	-0.996 **	0.982**			
DPPH BP	0.319 ns	0.984 **	-0.418 ns		1.000**		
DPPH AC	-0.086 ns	0.974 **	-1.000**			0.992**	
DPPH SA	-0.446 ns	0.853 **	-0.887 **				1.000**
FRAP AV	-0.196 ns	0.993 **	-0.994 **				
FRAP BP	0.348 ns	0.978 **	-0.39 ns				
FRAP AC	-0.208 ns	0.994 **	-0.993 **				
FRAP SA	-0.43 ns	0.862 **	-0.879 **				

Note: FRAP = FRAP capacity, DPPH = DPPH scavenging activity, AV = sample AV, BP = sample BP, AC = sample AC, SA = sample SA, ns = not significant, * = significant at $p<0.05$, ** = significant at $p<0.01$

Correlations between total flavonoid, phenolic, carotenoid content with DPPH scavenging activities, and FRAP capacities in various herb extracts from four species of Asteraceae

Pearson's correlation coefficient was positively high if $0.68 \le r \le 0.97$ [2]. The highest and positive correlation between total phenolic content and DPPH scavenging activity (r = 0.997, p<0.01) was given by sample AV, followed by sample BP (r = 0.984, p<0.01). The highest and positive correlation between total phenolic content and FRAP capacity (r = 0.994, p<0.01) was given by sample AC, followed by sample AV (r = 0.993, p<0.01) (table 4). Pearson's correlation coefficient between total flavonoid form various extracts of four species of Asteraceae and their antioxidant capacities demonstrated that there was no significant correlation with DPPH scavenging activities and FRAP capacities. The correlation between total carotenoid and their antioxidant capacities demonstrated that almost all of Asteraceae herb extracts sample had negative correlation with DPPH scavenging activities and FRAP capacities.

DISCUSSION

The previous study [5-6, 9, 15-16] revealed that Asteraceae had antioxidant capacity. There were no studies regarding antioxidant capacity of three various polarities extracts (which were n-hexane, ethyl acetate, and methanol) of herbs from four species of Asteraceae using DPPH and FRAP assays.

The DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 515-520 nm. Colors of DPPH would be changed when the free radicals were scavenged by antioxidant [17, 18]. Reagent of FRAP is $FeCl_3$ that combined with TPTZ in acetate buffer pH 3.6. Fe (III) will be reduced to Fe (II). Complex Fe (II)-TPTZ gives blue color and show characteristic absorption at wavelength 593 nm. Intensity of blue color depends on amount of Fe (III) that is reduced to Fe (II). If a sample reduces Fe (III) to Fe (II), at the same time it will be oxidized, so that sample can act as antioxidant. Sample will act as antioxidant in FRAP assays if sample had reduction potential lower than reduction potential of Fe (III)/Fe (II) which was 0.77 V, so the sample had the reducing power to reduce Fe (III) to Fe (II) and this sample will be oxidized.

In the current study, the highest DPPH scavenging activity was given by sample BP2, followed by sample BP3 and AV3 (methanolic extract of *A. vulgaris* herbs), while the highest FRAP capacity was given by sample BP2, followed by sample BP3 and AC3. Methanolic extract of *A. vulgaris* using reflux extraction had DPPH scavenging activity 27.97%, while the previous study by Erel [16] stated that DPPH scavenging activity of methanolic extract of *A. vulgaris* by Soxhlet extraction was 43.38%. Study by Muchuweti [9] revealed that DPPH scavenging activities of methanolic extract of *B. pilosa* decreased from 80% at 0 minute to 60% at 30 minutes.

The DPPH scavenging activities among n-hexane herb extracts were significantly different from one another (p<0.05). The same result was exposed by ethyl acetate and methanol extracts. In FRAP capacities among n-hexane herb extracts were significantly different from one another (p<0.05) and the same result was showed by ethyl acetate and methanol extracts.

The half maximum inhibitory concentration of DPPH scavenging activity is the concentration of the sample or standard that can inhibit 50% of DPPH scavenging activity, while EC_{50} of FRAP capacity is the concentration of the sample or standard that can exhibit 50% of FRAP capacity. The lowest IC_{50} or EC_{50} means had the highest antioxidant capacity. The IC_{50} or EC_{50} were used to determine antioxidant capacity of a sample that compared to standard. Sample that has IC_{50} or EC_{50} less than 50 µg/ml is a very strong antioxidant, 50-100 µg/ml is a strong antioxidant, 101-150 µg/ml is a medium antioxidant, while IC_{50} or EC_{50} greater than 150 µg/ml is a weak antioxidant [10].

In the DPPH method, antioxidant activities of various herb extracts from four species of Asteraceae ranged from 76.25 to 1772.05 µg/ml. Methanolic extract of *B. pilosa* herbs (BP3) had the lowest IC_{50} of DPPH radical scavenging activity 76.25 µg/ml, followed by BP2 80.15 µg/ml, and AC3 83.52 µg/ml, while ascorbic acid standard gave IC_{50} of DPPH scavenging activity 7.36 µg/ml. Based on the value of IC_{50} of DPPH scavenging activity it could be concluded that BP3, BP2, and AC3 could be categorized as strong antioxidants. The current study showed that IC_{50} of DPPH scavenging activities of AV3 (methanolic extract of *A. vulgaris* herbs) was 152.51 µg/ml. Study by Karabegovic [15] revealed that methanolic extract of *A. vulgaris* that was extracted by maceration, ultrasonic extraction, Soxhlet extraction had IC_{50} 22.2, 26.5, and 28.1 µg/ml, respectively, while *A. campestris* had IC_{50} 19.8, 20.6, and 28.1 µg/ml, respectively. Ethyl acetate extract of *B. pilosa* herbs (BP2) had the lowest EC_{50} of FRAP capacity (33.50 µg/ml) while ascorbic acid standard gave EC_{50} of FRAP capacity 4.41 µg/ml. It showed that potency of ascorbic acid was around eight times as much as the potency of BP2 using FRAP assays. Study by Deba [6] demonstrated that IC_{50} of DPPH scavenging activity of essential oil from leaves and flower of *B. pilosa* Linn. var radiata were 47.5 and 49.7 µg/ml, respectively, while aqueous flower and leaves extracts had IC_{50} 172 and 61 µg/ml, respectively. Ethyl acetate fraction of *B. pilosa* had IC_{50} of DPPH scavenging activity 43.53 µg/ml [19]. These results were in contrast with the current study which revealed that IC_{50} DPPH scavenging activity of ethyl acetate extract of *B. pilosa* was 80.15 µg/ml. Patil [5] reported that methanolic extract of *Ageratum conyzoides* had IC_{50} of DPPH scavenging activity 22,500 µg/ml and EC_{50} of FRAP capacity 4,480 µg/ml, while the current study showed that the methanolic extract of *A. conyzoides* had IC_{50} 83.52 µg/ml and EC_{50} 47.14 µg/ml. The methanolic herb extract of *A. conyzoides* had IC_{50} of DPPH scavenging activity 65,300 µg/ml [20] and 25 µg/ml [8]. Research by Xia [1] exhibited that methanolic extract *S. arvensis* had IC_{50} of DPPH and ABTS scavenging activities of 15.92 and 55.52 µg/ml, respectively which were lower than *S. oleraceus*, *S. asper, S. uliginosus, S. brachyotus,* and *S. lingianus.*

The presence of total phenolic might contribute to antioxidant capacity [3]. Phenolic acid might contributed in antioxidant capacity and cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid [21]. The previous study [15] showed that total phenolic content in methanolic extract of *A. vulgaris* using Soxhlet extraction 12.34 g GAE/100 g was lower than *A. campestris* 12.81 g GAE/100 g. It was in contrast with the current study which exposed that total phenolic in methanolic extract of *A. vulgaris* using reflux extraction was 5.15 g GAE/100 g. Research by Erel [16] demonstrated that total phenolic in methanolic extract of *A. vulgaris* was 217.46 mg/l and *A. campestris* 201.4 mg/l. The current study showed total phenolic content in methanolic extract of *B. pilosa* was 7.61 g GAE/100 g, while Muchuweti [9] exposed that methanolic extract of *B. pilosa* contained total phenolic content of 110.28 g GAE/100 g. Previous study revealed that total flavonoid and total phenolic content in ethanolic herb extract of *B. pilosa* were 20.90 g rutin equivalent/100 g and 9.53 g GAE/100 g. Ethyl acetate fraction of *B. pilosa* had the highest antioxidant activity using DPPH, ABTS, and FRAP assays compared to that of ethanolic extract, petroleum ether fraction, butanol fraction and water fraction [19]. Study by Xia [1] exhibited that total phenolic content in methanolic extract of *S. arvensis* (38.8 g GAE/100 g*)* was higher than total phenolic in *S. oleraceus*, *S. asper, S. uliginosus, S. brachyotus,* and *S. lingianus,* while total flavonoid in *S. oleraceus* (14.85 g rutin equivalent/100 g) was higher than that of the others. Total phenolic in methanolic extract *S. arvensis* (42 g GAE/100 g) was higher than that of chloroform fraction, ethyl acetate fraction, and n-hexane fraction [22].

The data in table 4 exposed that there was positively high correlation between total phenolic content in all of herb samples and antioxidant capacities using two methods FRAP and DPPH assays. Based on this data it could be concluded that antioxidant capacities in *A. vulgaris*, *B. pilosa*, *A. conyzoides* and *S. arvensis* herb extracts with FRAP and DPPH assays might be estimated indirectly by determining their total phenolic content. The previous study [9] exposed that *B. pilosa* contained ferulic acid, caffeic acid, and p-coumaric acid which could act as hydrogen donors and/or reducing agents, but there was no significant correlation between its total phenolic content with DPPH scavenging activity and FRAP capacity. Wu [19] demonstrated that total flavonoid and total phenolic content in butanol fraction of *B. pilosa* had positively high correlation with its DPPH, ABTS scavenging activities and FRAP capacities. Pearson's correlation coefficients in table 4 above showed that total flavonoid in all of herb samples in this study had no correlation with their antioxidant capacities by DPPH and FRAP assays.

Phenolic compound included tannins, flavonoid, phenolic acid and other compounds. Flavonoid will be included in phenolic groups if it has OH in A ring and/or B ring. Phenolic acid had lower antioxidant capacity than flavonoid [21]. 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 [21]. Generally it could be seen in fig. 3 that total flavonoid in ethyl acetate extracts were higher than total flavonoid in methanolic extracts, but IC_{50} of DPPH scavenging activities and FRAP capacities of methanolic extracts were lower than ethyl acetate extracts. It means antioxidant activity of methanolic extracts was stronger than that of ethyl acetate extracts. Based on the data above it can be predicted that many flavonoids in ethyl acetate extracts of Asteraceae herbs had -OH in other position, for 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 methanolic extracts of Asteraceae herbs were flavonoid that had OH in position which can influence antioxidant capacities. *Sonchus arvensis* contained apigenin-7-glucuronide and luteolin-7-glucoside, which could act as antioxidant [1]. Total flavonoid in methanolic extract of *S. arvensis* 1.57 g QE/100 g was lower than that of *A. vulgaris* 4.24 g QE/100 g. The half maximuml inhibitory concentration value of DPPH scavenging activities of methanolic extract of *S. arvensis* was 144.33 µg/ml and similar with methanolic extract of *A. vulgaris* 152.51 µg/ml. Its means that might be apigenin-7-glucuronide and luteolin-7-glucoside influenced antioxidant activity of methanolic extract of *S. arvensis*.

Study by Khan [22] demonstrated that total phenolic and total flavonoid had high and positive correlation with IC_{50} of DPPH scavenging activities of methanolic extract in *S arvensis* that were R^2= 0.892, $p<0.05$ and R^2 = 0.981, $p<0.01$, respecitively. While with ABTS assays it was showed that no correlation with total phenolic and total flavonoid in methanolic extract.

The data Pearson's correlation between total carotenoid and their antioxidant capacities demonstrated that almost all of sample of Asteraceae herbs had highly negative correlation with antioxidant capacities using DPPH and FRAP assays; it means higher total carotenoid of the sample will give lower antioxidant capacities.

Carotenoid had antioxidant capacity by scavenging free radical. More double bonds in carotenoid would give higher free radical scavenging capacity [23]. Carotenoid that consisted of above 7 double bonds gave higher free radical scavenging activity than 7 double bonds [24]. Previous study by Kobayashi and Sakamoto [25] stated that increase in lipophilicity of carotenoid would increase free radical scavenging capacity. Lycopene was effective to reduce Fe (III), because 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) [26]. Beta carotene was used as standard because it had conjugation double bonds due to its ability to scavenge free radicals [27].

The FRAP and DPPH methods had different mechanisms reaction. Mechanism of DPPH that was electron transfer assays [28] and FRAP was redox assays. So the results of the two methods not always linear. The Pearson's correlation coefficient of four species of Asteraceae herbs indicated that all of samples (AV, BP, AC, and SA) had positively high correlation between DPPH scavenging activities and FRAP capacities. It could be seen that antioxidant activities of sample AV, BP, AC and SA gave linear result by DPPH and FRAP assays.

CONCLUSION

To assess the antioxidant capacity of sample, variety of methods must be used in parallel, because different methods could give different results. Methanolic extract of *B. pilosa* and *A. conyzoides* had IC_{50} of DPPH scavenging activities less than 100 µg/ml that means as strong antioxidants. The positive and high correlation between total phenolic with DPPH scavenging activities and FRAP capacities was given by all of herb extracts. Antioxidant capacity using DPPH and FRAP assays in all of herb extracts might be estimated indirectly by using total phenolic content. Phenolic compounds were the major contributor in antioxidant capacity in all of herb extracts. Antioxidant capacities of *A. vulgaris*, *B. pilosa*, *A. conyzoides*, and *S. arvensis* gave linear result by DPPH and FRAP assays. *Bidens pilosa* and *A. conyzoides* may be exploited as sources of beneficial compounds for human health to alleviate oxidative stress.

CONFLICT OF INTERESTS

Declared None

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