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Original Article

ANTIOXIDANT CAPACITIES FROM DIFFERENT POLARITIES EXTRACTSOF THREE KINDS GINGERUSINGDPPH, FRAPASSAYS ANDCORRELATION WITH PHENOLIC, FLAVONOID, CAROTENOID CONTENT

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ABSTRACT

Objectives: The objectives of this research were to study antioxidant capacityfrom different polarities extracts of three kinds ginger 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 three kinds gingerwith DPPH and FRAP antioxidant capacities.

Methods: Extraction was performed by reflux using different polarities solvents. The extracts were vaporated using rota vapor. Then antioxidant capacities were tested using DPPH and FRAP assays. Determination of total phenolic, flavonoid and carotenoid content were performed by spectrophotometry UV-Vis and its correlation with FRAP and DPPH antioxidant capacities were analyzed by Pearson method.

Results: EG3(ethanol extract of elephant ginger rhizomes) had the highest DPPH scavenging capacity with IC_{50} 0.26 ppmandEG3had the highest FRAP capacity also with EC_{50}91.90 ppm.SG1(n-hexane extract of small ginger) contained the highest total phenolic (14.56 g GAE/100 g), EG2(ethyl acetate extract of elephant ginger rhizomes) had highest flavonoid content (7.5 gQE/100 g) andEG3 had the highest carotenoid 0.95 g BET/100 g.

Conclusions: There were positively high correlation between total phenolic content in small ginger rhizomes extracts with their antioxidant activity using DPPH and FRAP assays. DPPH scavenging capacities in small ginger rhizomes extracts had positively high correlation with their FRAP capacities.

Keywords: Antioxidants,FRAP, DPPH, Three kinds ginger,flavonoid, Phenolic, Carotenoid.

INTRODUCTION

Antioxidant has ability of mobilizing protective effects against oxidative stress on account of their high antioxidant activity [1]. Phenolic compounds are commonly found in plants, and they have been reported 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 which have antioxidant activity[1][3].

Some of antioxidant methods such as DPPH (2,2-diphenyl-1 picrylhydrazyl) and FRAP (Ferric Reducing Antioxidant Power) were widely used to predict antioxidant capacity of fresh fruits, beverages and food[2]. In previous study [2] [4] [5]revealed that DPPH and FRAP methods could be used to determine antioxidant activity in many plants extracts. The previous study[4] [6] [7][8] 9][10]showed antioxidant activities of some plants including ginger. The objective of this research were to study antioxidant capacities of different polarities extracts (n-hexane, ethyl acetate and ethanol) from three kinds ginger(small ginger, elephant ginger and red ginger) rhizomes using antioxidant testing DPPH and FRAP assays and correlations of their capacitieswith 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 were purchased from Sigma-Aldrich (MO,USA),ferric chloride, methanol, ethanol. All other reagents were analytical grades.

Preparation of sample

Rhizomesfrom three kinds ginger(*Zingiberofficinale*)that were collectedfrom Nakrak-Sukabumi,West Java-Indonesia that were:small ginger (SG)*Zingiberofficinale*var. amarum, elephant ginger(EG) *Zingiberofficinale*var. Officinarum and red ginger(RG) *Zingiberofficinale*var. Rubrum were thoroughly washed with tap water, wet sortation, cut,driedand 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 residuewas extracted three times with ethanol.So there were three n-hexane extracts (namely SG1, EG1, RG1), three ethyl acetate extracts (SG2, EG2, RG2) and threeethanolic extracts (SG3, EG3, RG3).

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 517nm 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].

FRAP capacity

Preparation of FRAPsolution 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.Acetatebuffer 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].

Total phenolic determination

Total phenolic content were 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 extracts. Standard solutions of gallic acid with concentration 60-150µg/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* [15]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extracts. Standard solutions of quercetin with concentration 40-160µg/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*[2].Each extracts were diluted in acetone. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extracts. Standard solutions of beta carotene with concentration10-40µg/mL were used to obtain a standard curve. The total carotenoid content was reported as percentage of total beta carotene equivalents per 100 gextract (g BET/100 g).

Statistic

Each sample analysis 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 $p < 0.05$ 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 madeusing the Pearson method ($p < 0.01$).

RESULTS

Antioxidant capacities of different polarities rhizomes extracts fromthree kinds ginger using DPPH and FRAP assays

The antioxidant capacities using DPPH and FRAP assays of different polarities rhizomes extracts from three kinds ginger were shown in Table 1, Table 2, Table 3. In DPPH method, antioxidant capacities in the range of 70.17–90.91 %.SG1rhizomes extract (n-hexane extract of small ginger rhizomes) had the highest DPPHradical scavenging capacity (90.91%), while the lowest antioxidant capacity (70.17 %) was given by SG3rhizomes extract.

In the FRAP method, free radical scavenging capacities of different polarities rhizomes extracts from three kinds ginger ranged from 5.03 - 11.75 %. EG3(ethanolic extract of elephant ginger rhizomes) had the highest FRAP capacity (11.75%), while SG3rhizomes extract (5.03%) had the lowest FRAP capacity.

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

The IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP capacities in different polarities extracts from three kinds ginger using DPPH and FRAP assays were shown in Fig 1 and Fig 2. Both of IC_{50} of DPPH scavenging capacities and EC_{50} of FRAP capacities of each extracts were compared to ascorbic acid as standard. The lowest IC_{50} or EC_{50} mean shad the highest antioxidant capacity.

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

Sample	DPPH scavenging capacitiy (%)	FRAP capacity (%)
SG1	90.91 ± 0.14 a	9.88 ± 1.67 a
EG1	83. 6 ± 1.60 b	7.71 ± 1.08ab
RG1	80.63 ± 0.62 c	7.13 ± 0.34 c
Ascorbic acid	98.49 ± 0.33	39.65 ± 0.28
P value	< 0.05	< 0.05

Note: a –c = 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 rhizomes extracts

Sample	DPPH scavenging capacitiy(%)	FRAP capacity(%)
SG2	86.29 ± 0.82 a	7.27 ± 0.43 a
EG2	84.07 ± 0.42 b	6.30 ± 0.74ab
RG2	82.32 ± 1.17 c	6.11 ± 0.34 b
Ascorbic acid	98.49 ± 0.33	39.65 ± 0.28
P value	< 0.05	< 0.05

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

Table 3: DPPH scavenging capacities and FRAP capacities of ethanolicrhizomes extracts

Sample	DPPH scavenging capacitiy(%)	FRAP capacity(%)
SG3	70.17 ± 1.03 a	5.03 ± 0.94 a
EG3	70.32 ±1.04 a	11.75 ± 0.32 b
RG3	75.77 ± 0.67 b	9.61 ± 0.53 c
Ascorbic acid	98.49 ± 0.33	39.65 ± 0.28
P value	< 0.05	< 0.05

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

Total phenolic in different polarities rhizomes extracts from three kinds ginger

The total phenolic content among the different polarities extracts were expressed in term of gallic acid equivalent using the standard curve equation $y = 0.0044 x + 0.031$, $R^2 = 0.993$. The total phenolic content in different polarities rhizomes extracts from three kinds ginger showed different result ranged from 3.64 to 14.56 g GAE/100 g. SG1rhizomes extract (n-hexane rhizomes extract of small ginger) had the highest phenolic content (14.56 g GAE/100g) (Fig 3).

Total flavonoid indifferent polaritiesrhizomes extracts from three kinds ginger

The total flavonoid content among the different polarities extracts were expressed in term of quercetin equivalent using the standard curve equation y = 0.00761355x + 0.00491857, R^2= 0.998. The total flavonoid content indifferent polaritiesrhizomes extracts from three kinds gingershowed different result in the range of 1.33–7.50 g QE/100 g (Fig 4). SG2 (ethyl acetate extract of small ginger rhizomes) had the highest total flavonoid content (7.50 g QE/100 g) and the lowest (1.33 g QE/100 g) for SG1rhizomes extract.

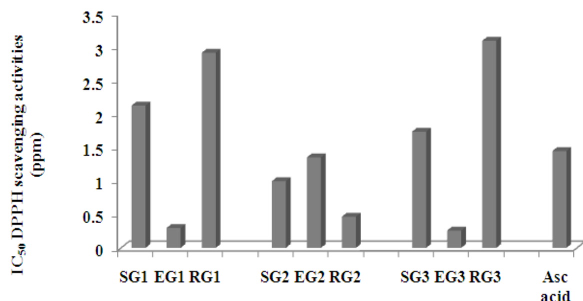

Fig. 1: IC$_{50}$ of DPPH scavenging capacities indifferent polarities rhizomes extracts from three kinds ginger

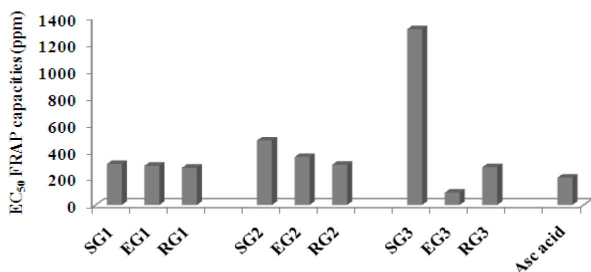

Fig. 2: EC$_{50}$ of FRAP capacities indifferent polaritiesrhizomes extractsfromthree kinds ginger

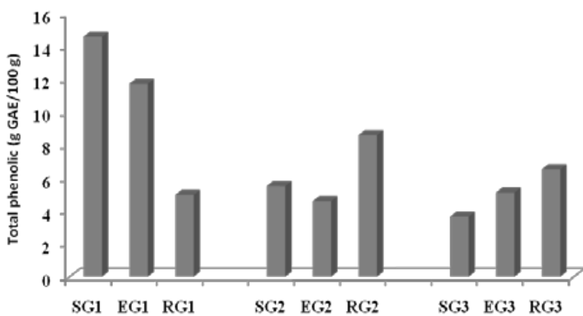

Fig. 3: Total phenolic content in different polarities extracts of three kinds ginger

Total carotenoid in different polaritiesrhizomes extracts from three kinds ginger

The total carotenoid content among the different polarities extracts were expressed in term of beta carotene equivalent using the standard curve equation y = 0.02764x - 0.00324857, R^2 = 0.999. The total carotenoid content in different polarities rhizomes extracts from three kinds ginger showed different result in the range of 0.24 – 0.95 g BET/100 g (Fig 5). The highest carotenoid content (0.95 g BET/100 g) for SG2rhizomes extract, while the lowest carotenoid (0.24 g BET/100 g) for SG1 and RG1rhizomes extract.

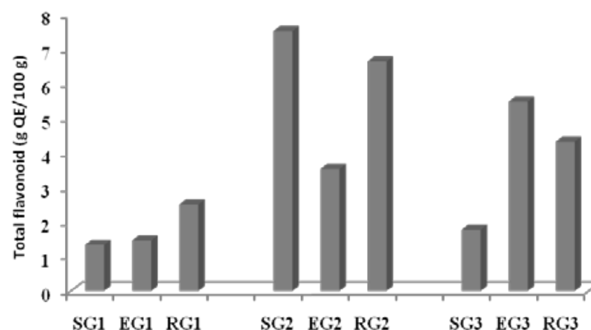

Fig. 4: Total flavonoid content in different polarities extracts of three kinds ginger

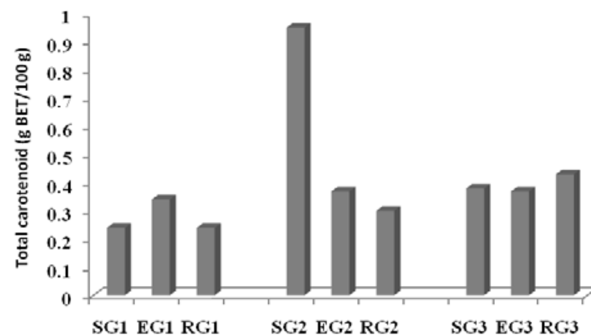

Fig. 5: Total carotenoid content in different polaritiesextractsof three kinds ginger

Correlations between total phenolic, flavonoid, carotenoid content and DPPH scavenging capacities, FRAP capacities, in different polarities rhizomes extracts from three kinds ginger

Pearson's correlation coefficient was positively high if 0.68 ≤ r ≤ 0.97 [2]. The positive correlation between total phenolic content and DPPH scavenging activity (r = 0.773, p<0.05) was given by sample SG(Table 4), while the positive correlation between total phenolic content and FRAP capacity (r = 0.894, p<0.01) for sample SG also.

Table 4: Pearson's correlation coefficient of total phenolic, flavonoid, carotenoid of rhizomes extract from three kinds gingerand DPPH scavenging capacities, FRAP capacities

	Total Phenolic	Total Flavonoid	Total Carotenoid	DPPH SG	DPPH EG	DPPH RG
DPPH SG	0,773*	0,231^{ns}	0,403^{ns}			
DPPH EG	0,611^{ns}	0,455^{ns}	0,602^{ns}			
DPPH RG	0,384^{ns}	0,588^{ns}	0,790*			
FRAP SG	0,894**	-0,052^{ns}	0,052^{ns}	0,858**		
FRAP EG	-0,388^{ns}	-0.604^{ns}	-0,705*		-0,862**	
FRAP RG	-0,365^{ns}	-0,644^{ns}	-0,757*			-0,849**

Note: DPPH = DPPH scavenging capacity, FRAP = FRAPcapacity, SG = sample SG, EG = sample EG, RG = sample RG,ns = not significant, * = significant at p < 0.05, ** = significant at p < 0.01 Sample RG had positive correlation between DPPH scavenging capacity and total carotenoid content (r = 0.790, p<0.05).

DISCUSSION

In previous study[3][4] demonstrated that some of tropical plants includingthree kinds gingerhad antioxidant capacity usingvarious antioxidant testing assays. There were no study regarding antioxidant capacity of three different polarities extracts (which were n-hexane, ethyl acetate and ethanol) ofrhizomesfrom three kinds gingerusing DPPH and FRAP assays.

DPPH is stable free radicals which dissolve in methanol or ethanol, and its colors show characteristic absorption at wavelength 517nm, 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 pH3.6. 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 thatsample can act as antioxidant.

In the present study, the highest DPPH scavenging capacity was given by sample SG1 (n-hexane extract of small ginger), followed by sample SG2 and EG2.Ethanolic extract of small ginger (SG3), elephant ginger (EG3) and red ginger (RG3) had DPPH scavenging capacity 70.17 %, 70.32 % and 75.77 %, respectively, while study by Stoilovaet al [18] demonstrated that ethanolic extract of *Zingiberofficinale*rhizomes had DPPH scavenging capacity 90.1 %. The previous study [19] showed that water extract of gingerhad antioxidant capacities 79 % by DPPH method. The other study [20]exposed that DPPH scavenging capacity of methanolic extract of ginger rhizomes (68.3 %) washigher than ethanolic extract (27.2%). In this study n-hexane extract of small ginger (SG1), elephant ginger (EG1) and red ginger (RG1) had DPPH scavenging capacity 90.91 %, 83.6 % and 80.63 % respectively, while study by Sattar[20] revealed that n-hexane extract of ginger had antioxidant activity 49.2 % by DPPH method.Study by Ghasemzadeh[21] exposed that DPPH scavenging capacities of methanolic rhizomes extract of *Zingiberofficinale* variety HaliaBentong was 51.41 % and variety Halai Bara 58.22 %. Ghasemzadeh[21] also demonstrated that DPPH scavenging activities of stem of ginger (HaliaBentong 32. 85 % and Halia Bara 31.45 %) were lower than their leaves 51.12 %, 56.36 % and rhizomes 51.41 %, 58.22 %, respectively.

The highest FRAP capacitiy was given by EG3 (ethanolic extract of elephant ginger rhizomes), followed by SG1 (n-hexane extract of small ginger rhizomes) and RG3 (ethanolic extract of red ginger rihizomes). Ethanolic extract of small ginger, elephant ginger and red ginger had FRAP capacity 5.03 %, 11.75 % and 9.61 %, respectively. In previous study [21] exposed that FRAP capacities of methanolic rhizomes extract of two varieties ginger (HaliaBentong and Halia Bara) were 680 μmol Fe II/g extract and 767 μmol Fe II/g extract respectively and compared to ascorbic acid with FRAP capacity 3107 μmol Fe II/g extract. FRAP capacities of stem from HaliaBentong and Halia Bara varieties (376 μmol Fe II/g extract and 368 μmol Fe II/g extract) were lower than their FRAP leaves capacities (537 μmol Fe II/g extract and 579 μmol Fe II/g extract)and FRAP rhizome capacities.Study by Maizura[19] stated that water extract of ginger had FRAP capacity 26.2 μmol Fe II/g extract.

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 IC50 or EC_{50} meanshadthe highest antioxidant capacity.IC_{50} orEC_{50} were used to determine antioxidant capacity of sample compared to standard. Sample that had IC_{50} < 50 ppm, it was very strong antioxidant, 50-100 ppm strong antioxidant, 101-150 ppm medium antioxidant, while weak antioxidant with EC_{50} or IC_{50}> 150 ppm [11].

EG3 (ethanolicextract of elephant ginger rhizomes) had the lowest IC_{50} of DPPH scavenging activity (0.26 ppm), while ascorbic acid standard gave IC_{50} of DPPH scavenging capacity 1.45 ppm.All of extracts (n-hexane, ethyl acetate and ethanol) of three kinds ginger (small ginger, elephant ginger and red ginger) had the IC_{50} in the range of 0.26-3.1 ppm. Based on the classified of antioxidant potency by Blois [11], it could be classified as very strong antioxidant.In the present study ethanolic extract of small ginger (SG3), elephant ginger (EG3) and red ginger rhizomes (RG3) had IC_{50} of DPPH scavenging capacities was 1.74, 0.26 and 3.1 ppm respectively, while in the previous study [18] showed that IC_{50} of DPPH scavenging capacity of ethanolic extract of ginger rhizomes was 0.64 ppm.

Different polarities extracts from three kinds gingerhad FRAP capacities ranged from 92 to 1313 ppm. EG3 (ethanolic extract of elephant ginger rhizomes) had the lowest EC_{50} ofFRAPcapacity 92 ppm,while ascorbic acid standard gave EC_{50} of FRAP capacity 203 ppm and its exposed thatantioxidant capacity of EG3 was two times of potency of ascorbic acid using FRAPmethod.

The presence of total phenolic might contribute to antioxidant activity [3]. Phenolic acid might contributed in antioxidant activity and cinnamic acid had higher antioxidant capacity than phenyl acetic acid and benzoic acid [22]. In present study total phenolic of ethanolic extract of small ginger, elephant ginger and red ginger were 3.63 g GAE/100 g, 5.1 g GAE/100 g, 6.52 g GAE/100 g. Its different with previous study [20] revealed that total phenolic of ethanolic extract of ginger was 11.2 mg gallic acid/g extract.Study by Maizura[19] exposed that water extract of ginger contained 101.56 mg GAE/100 g extract.

Total phenolic of n-hexane extract in present study were 14.56 g GAE/100 g, 11.7 g GAE/100 g and 4.96 g GAE/100 g for small ginger, elephant ginger and red ginger respectively, while research by Sattar[20] showed that total phenolic content in n-hexane of ginger was 13.5 mg gallic acid/g extract or the same as 1.35 g GAE/100 g.

Total flavonoid of ethanolic extract in the present study exposed that elephant ginger had the highest total flavonoid (5.47 g QE/100 g) compared to small ginger (1.77 g QE/100 g) and red ginger (4.31 g QE/100 g). It was different with previous study [20] revealed that total flavonoid in ethanolic extract of ginger was 5.33 mg catechin/g extract and methanolic extract was 8.34 mg catechin/g extract. Ghasemzadeh[21] demonstrated that total flavonoid in methanolic extracts were 3.66 mg gallic acid/g and 4.21 mg gallic acid/g for HaliaBentong and Halia Bara ginger varieties respectively.

The data in Table 4 exposed that there were positively high correlation betweentotal phenolic content in small ginger rhizomes extracts and antioxidant capacities using two methods DPPH (0.773, $p<0.05$) and FRAP assays (0.894, $p<0.01$). Based on this data it could be concluded that antioxidant capacitiesin small ginger rhizomes extracts byDPPH and FRAP assays might be estimated indirectly by determining their total phenolic content.In this study demonstrated that only total phenolic content in small ginger rhizomes extract had highly and positive correlation withtheir DPPH scavenging capacity and FRAP capacity, while total phenolic in elephant ginger and red ginger had no correlation with their DPPH and FRAP capacities.Study by Maizura[19] stated that total phenolic in mixture of kesum (*Polygenus minus*), ginger (*Zingiberofficinale*) and turmeric (*Curcuma longa*) had highly and positive correlation with DPPH scavenging capacities and FRAP capacities. The same result exposed that total flavonoid in those mixture had positive and high correlation with FRAP and DPPH capacities.

Flavonoid that have OH in A ring and or B ring is included phenolic groups. Flavonoid had the higher antioxidant capacity than phenolic acid[22]. Flavonoid which had OH in ortho C 3',4', OH in C3, oxo function in C4, double bond at C2 and C3 would give higher antioxidant capacity. The OH with ortho position in C3'-C4' had the highest influence to antioxidant capacity of flavonoid. The flavonoid glycosides would give lower antioxidant capacity than flavonoid aglycones[22].Fig 4 showed that total phenolic in SG2 (ethyl acetate extracts of small ginger rhizomes) was the higher than the other extracts, but its DPPH scavenging capacities (86.29 %) was lower than SG1 (90.91 %). Based on this data it can predicted that many flavonoids in ethyl acetate extract of small gingerwere 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 ethyl acetate of small ginger 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 red ginger rhizomes extracts had highly and positive correlation with their antioxidant capacities by DPPH assays (0.790, $p<0.05$) and had negative correlation with their antioxidant capacities by FRAP assays (-0.757, $p<0.05$).Carotenoid with more double bonds would give higher scavenging free radical capacity[23]. Carotenoid that consisted of 7 double bonds gave lower scavenging

radical free capacity than more double bonds [24]. In previous study by Kobayashi and Sakamoto[25] 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, neurosporenethatconsistedof3, 5 and 9 conjugated double bonds respectively, did not show significant capacity to reduce Fe (III) [26]. Beta carotene was used as standard because of it had conjugation double bonds doe to its ability to scavenge free radicals [27]. Based on the above data, it could be seen that many carotenoid in small ginger ethyl acetate 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 [28]and FRAP was redox assays. The results of this study showed that DPPH scavenging capacity not always linear with FRAP 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 rhizomes extracts from three kinds ginger indicated that only small ginger rhizomes extracts had positively and high correlation between DPPH scavenging capacities and FRAP capacities. It could be seen that antioxidant capacities in small ginger rhizomes extracts by DPPH assays were linear with FRAP 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 ethanolextracts of elephant ginger rhizomes had the lowestIC_{50} of DPPH scavenging capacitiesand EC_{50} of FRAP capacities that were very strong antioxidant. The positively high correlation between total phenolic content with DPPH and FRAP capacities was given by small ginger rhizomes extracts.

Antioxidant capacity using FRAP and DPPH assays in small ginger rhizomes extracts might be estimated indirectly by using total phenolic content. Phenolic compounds were the major contributor in antioxidant capacity in small ginger rhizomes extracts. There were not all of DPPH scavenging capacities in rhizomes extracts from three kinds ginger linear with FRAP capacities. Small ginger, elephant ginger and red ginger rhizomes extracts may be exploited as natural antioxidant in food applications as well as for health supplements or functional food, to alleviate oxidative stress.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Jain N, Goyal S, J. Ramawat KGEvaluation of antioxidant properties and total phenolic content of medicinal plants used in diet therapy during postpartum healthcare in Rajasthan. Int PharmSci 2011;3(3):248-53.
2. Thaipong K, Boonprakob U, Crosby K, Zevallos LC, J. Byrne DHComparison of FRAP, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Comp Anal 2006;19:669-75.
3. Ling LT, D. U, Valdez B. Palanisamy, Potential antioxidants from tropical plants, In:editor, Food industrial processes-methods, Kuala Lumpur:In Tech;. p1999. 64-72.
4. Souri E, Amin G, Farsan H, M. T, J. U. Barazandeh antioxidant activity and phenolic content of 24 medicinal plats extracts. DARSci 2008;16:83-7.
5. Luis A, Gil N, Amaral ME, P. A, J. Duarte activities of extract from Acacia melanoxylon, Acacia dealbata, Oleaeuropaeaand alkaloids estimation. Int PharmSci 2012;4:225-31.
6. Ali BH, Blunden G, Tanira MO, Nemmar A. Some phytochemical, pharmacological and toxicological properties of ginger (Zingiber officinale Roscoe):a review of recent research. Food and chemical toxicology:an international journal published for the British Industrial Biological Research Association 2008;46(2):409-20.
7. Masuda Y, Kikuzaki H, Hisamoto M, Nakatani N. Antioxidant properties of gingerol related compounds from ginger. BioFactors (Oxford, England) 2004;21(1-4):293-6.
8. Tahtawy RHM, Bastawesy AM, Monem MGA, Zekry ZK, Mehdar HA, M. M, et al. Merzabani activity of the volatile oils of Zingiberofficinale (ginger). BioFactors (Oxford, England) 2011;1(1):1-8.
9. Huang W-y, Zhang H-c, Liu W-x, Li C-y. Survey of antioxidant capacity and phenolic composition of blueberry, blackberry, and strawberry in Nanjing. Journal of Zhejiang University Science B 2012;13(2):94-102.
10. Agbor GA, Moumbegna P, Oluwasola EO, Nwosu LU, Njoku R-CC, Kanu S, et al. Antioxidant capacity of some plant foods and beverages consumed in the Eastern Region of Nigeria. African journal of traditional, complementary, and alternative medicines:AJTCAM / African Networks on Ethnomedicines 2011;8(4):362-9.
11. S. M. Blois determination by the use of stable free radicals. Nature 1958;181:1199-2000.
12. Bedawey AA Characteristics of antioxidant isolated from some plants sources, Cairo:Shibin 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. Pourmorad F, Hosseinimehr SJ, N. Shahabimajd activity, phenol and flavonoid content of some selected Iranian medicinal plants. AfrJBiotechnol 2006;5(11):1142-5.
15. Chang CC, Yang MH, Wen HM, C. J, J. Chern of total flavonoid content in propolis by two complementary colorimetric methods. Drug Anal 2002;10:178-82.
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, Güçlü K, Demirata B, Ozyürek M, Celik SE, Bektaşoğlu B, et al. Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. Molecules (Basel, Switzerland) 2007;12(7):1496-547.
18. Stoilova TB, Dutseva EA, Pashkovskaia AA, Sychev SV, Koval'chuk SI, Sobko AA, et al. [Ion channels of various types induced in lipid membranes by gramicidin A derivatives carrying a cationic sequence at their C-termini]. Bioorg Khim 2007;33(5):511-9.
19. Maizura M, Aminah A, M. WW, Res J. Aida phenolic content and antioxidant activity of kesum (Polygonum minus), ginger (Zingiberofficinale) and turmeric (Curcuma longa) extract. Int Food. BioFactors (Oxford, England) 2011;18:529-34.
20. Sattar NA, Hussain F, T., Bangladesh J. Iqbal activities of Z. officinaleRoscoe and A allughasRoscoe Zingiberaceae RhizomesSci Ind Res 2013;48(2):115-8.
21. Ghasemzadeh A, Jaafar HZE, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (Zingiber officinale Roscoe). Molecules (Basel, Switzerland) 2010;15(6):4324-33.
22. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants:chemistry, metabolism and structure-activity relationships. The Journal of nutritional biochemistry 2002;13(10):572-84.
23. Foote CS. Free radicals in biology1976.
24. Beutner S, Bloedorn B, Hoffmann T, Martin HD. Synthetic singlet oxygen quenchers. Methods Enzymol 2000;319:226-41.
25. Kobayashi H, Kitai R, Ido K, Kabuto M, Handa Y, Kubota T, et al. Hemodynamic and metabolic changes following cerebral revascularization in patients with cerebral occlusive diseases. Neurol Res 1999;21(2):153-60.
26. V. Müller L, Fröhlich K, Böhm antioxidant activities of carotenoids measured by ferricreducing antioxidant power (FRAP), ABTS bleaching assay (aTEAC),DPPH assay and peroxyl radical scavenging assay. Food Chem 2011;129:139-48.
27. Charles DJ Antioxidant properties of spices herbs and other. London:John Willey;2013.
28. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005;53(6):1841-56.