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Research Article

DPPH and ABTS Scavenging Activities of Oolong Tea from Three Regions in Indonesia

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ABSTRACT

The aim of this research were to determine antioxidant activity from various extracts of oolong tea from three different regions using two methods of antioxidant testing which were DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and correlation of total phenolic, flavonoid and carotenoid content in various extracts of oolong tea with IC_{50} of DPPH and IC_{50} of ABTS antioxidant activities. Extraction was conducted by reflux using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH and ABTS assays, determination of total phenolic, flavonoid and carotenoid content were performed by UV-visible spectrophotometry and its correlation with IC_{50} of DPPH and IC_{50} of ABTS scavenging activities were analyzed by Pearson's method. All of extracts of oolong tea from three regions were categorized as very strong antioxidant by DPPH and ABTS methods. Phenolic compounds in all of extracts of oolong tea were the major contributor in IC_{50} of DPPH and IC_{50} of ABTS scavenging activities. All of extracts of oolong tea had linear result in DPPH and ABTS assays.

Keywords: Antioxidant, DPPH, ABTS, oolong tea, phenolic, flavonoid, carotenoid

INTRODUCTION

Oxidative stress can cause many diseases, which can be prevented by antioxidant. Phenolic compounds are commonly found in plants, and they have been revealed to have multiple biological effects, including antibacterial and antioxidant activity[1-3]. Previous study[4-6] demonstrated that phenolic and flavonoid content could be correlated to their antioxidant activities. Plants including tea contained phenolic and polyphenol compounds[7-9]. DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power) and ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) can be used to predict antioxidant activity of vegetables, fruits and food[9-11]. Previous research[8,9,11,12] revealed that DPPH, FRAP and ABTS methods could be used to determine antioxidant activity in many plants extracts. The previous study[12-14] reported that tea had antioxidant activities by using ABTS, DPPH, and FRAP assays. Tea leaves contained many phenolic compound such as epigallocatechin gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), gallocatechin (GC), epicatechin (EC) and catechin, which can act as antioxidant[12]. The aim of this research were to determine antioxidant activities of different polarities extracts (n-hexane, ethyl acetate and ethanol) of oolong tea from three different regions in Indonesia using antioxidant testing DPPH and ABTS assays, and correlations of their antioxidant activities with total phenolic, flavonoid and carotenoid content.

MATERIALS AND METHODS

Materials

DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt), gallic acid, quercetin, beta carotene was purchased from Sigma-Aldrich (MO, USA), three organs of oolong tea, ethanol. All other reagents were analytical grades.

Preparation of sample

Oolong tea were collected from three different regions were: Halimun mountain, Bayah-Banten namely as BA, Cigudek- Mas mountain, Bogor –West Java as sample WJ and Kepahiang-Bengkulu as sample BE, 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 different polarity solvents. Extraction using n-hexane was repeated three times. The remaining residue was then extracted three times by using ethyl acetate. Finally the remaining residue was extracted three times using ethanol. So totally there were nine extracts: three n-hexane extracts (namely BA1, WJ1 and BE1), three ethyl acetate extracts (BA2, WJ2 and BE2) and three of ethanolic extracts (BA3, WJ3 and BE3).

DPPH scavenging activity

Preparation of DPPH solution was adopted from Blois[15] with minor modification. Various concentration of each extract were pipetted into DPPH solution 50 µg/ml (volume 1:1) to initiate the reaction for obtaining a calibration curve. The absorbance was measured after 30 minutes incubation at wavelength 515 nm by using

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spectrophotometer UV-Vis Beckman Coulter DU 720. Methanol was used as a blank. DPPH solution 50 µg/ml was used as control. Ascorbic acid was used as standard. Analysis was done in triplicate for standard and each extract. Antioxidant activity of each extract by DPPH method was determined by calculating percentage of antioxidant activity using reduction of DPPH absorbance[16]. IC_{50} of DPPH scavenging activity of each extract can be calculated using its calibration curve.

ABTS scavenging activity

Preparation of ABTS solution was adopted from Li *et al.*[17] method with minor modification. ABTS diammonium salt solution 7.6 mM in aquadest and potassium persulfate solution 2.5 mM in aquadest were prepared. Each solution was left in dark room for 12 hours. The two solutions were mixed with 30 minutes incubation, left the mixture in refrigerator for 24 hours, then diluted in ethanol. Various concentration of each extract were pipetted into ABTS solution 50 µg/ml (volume 1:1) to initiate the reaction for obtaining a calibration curve. The absorbance was read at wavelength 734 nm using spectrophotometer UV-Vis Beckman Coulter DU 720. Ethanol (95%) was used as a blank, ABTS solution 50 µg/ml as control and ascorbic acid as standard. Analysis was done in triplicate for standard and each extract. Antioxidant capacity of each extract by ABTS method was determined by calculating percentage of antioxidant activity using reduction of ABTS absorbance[16]. IC_{50} of ABTS scavenging activity of each extract can be calculated using its calibration curve.

Total phenolic content (TPC)

Total phenolic content were adapted from Pourmorad[4] using the modified Folin-Ciolcalteu method. The absorbance was read at wavelength 765 nm. Analysis was done in triplicate for each extract. Gallic acid standard solution (40-165 µg/ml) was used to obtain a calibration curve. Total phenolic content was reported as percentage of total gallic acid equivalent per 100 g extract (g GAE /100 g).

Total flavonoid content (TFC)

Total flavonoid content was measured using modified method from Chang *et al*[18]. The absorbance was read at wavelength 415 nm. Analysis was done in triplicate for each extract. Quercetin standard solution (36-120 µg/ml) was used to obtain a calibration curve. The total flavonoid content was reported as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Total carotenoid content (TCC)

Total carotenoid content was measured using the modified carotene method adapted from Thaipong *et al*[10]. Each extract was diluted in n-hexane. The absorbance was read at wavelength 470 nm. Analysis was done in triplicate for each extract. Beta carotene standard solution (15-55 µg/ml) was used to obtain a calibration curve. The total carotenoid content was reported as percentage of total beta carotene equivalent per 100 g extract (g BE/100 g).

Statistical Analysis

Each sample analysis was performed in triplicate. All results presented are means (± standard deviation) of at least three independent experiments. Statistical analysis using ANOVA with a statistical significance level set at $p < 0.05$ and post-hoc Tukey procedure was carried out with SPSS 16 for Windows. Correlation between the total phenolic, flavonoid, carotenoid content and antioxidant activities, and correlation between two antioxidant activity methods were performed using the Pearson's method.

RESULTS

IC_{50} of DPPH and IC_{50} of ABTS scavenging activity

The IC_{50} of DPPH and IC_{50} of ABTS scavenging activities in various extracts from three different regions of oolong tea using DPPH and ABTS assays were shown in Fig 1 and Fig 2. IC_{50} of DPPH and IC_{50} of ABTS scavenging activities of each extract were compared to IC_{50} ascorbic acid as standard. The lowest value of IC_{50} means had the highest antioxidant activity.

TPC in various extracts of oolong tea

TPC among the various extracts were exposed in term of gallic acid equivalent using the standard curve equation $y = 0.0048\ x + 0.00257$, $R^2 = 0.9989$. The TPC in various extracts from three different regions of oolong tea exposed different result in the range of 2.10 – 83.06 g GAE/100 g. Ethanolic extract of oolong tea from Bengkulu (BE3) expressed the highest phenolic content (83.06 g GAE/100 g) (Fig 3) and the lowest was given by n-hexane extract of oolong tea from Banten (BA1).

TFC in various extracts of oolong tea

TFC among the various extracts were demonstrated in term of quercetin equivalent using the standard curve equation $y = 0.0069\ x - 0.019$, $R^2 = 0.9983$. The TFC in various extracts from three different regions of oolong tea showed different result ranged from 2.22 to 8.13 g QE/100 g (Fig 4). Ethyl acetate extract of oolong tea from Banten (BA2) had the highest total flavonoid content (8.13 g QE/100 g).

TCC in various extracts of oolong tea

TCC among the various extracts were expressed in term of beta carotene equivalent using the standard curve equation $y = 0.0121x - 0.0084$, $R^2 = 0.9998$. The TCC in various extracts from three different regions of oolong tea gave different result in the range of 0.20 – 32.14 g BE/100 g (Fig 5). The highest carotenoid content (32.14 g BE/100 g) was given by n-hexane extract of oolong tea from Banten (BA1), while the lowest carotenoid (0.20 g BE/100 g) for ethanolic extract of oolong tea from Bengkulu (BE3).

Correlations between total phenolic, flavonoid, carotenoid content in various extracts of oolong tea and IC_{50} of DPPH, IC_{50} of ABTS scavenging activities

Pearson's correlation coefficient between TPC in various extracts of oolong tea and their antioxidant activities exposed that TPC in all of extracts (BA, WJ and BE) had negative and high significant correlation with IC_{50} of DPPH scavenging activities ($r = -0.857$; $r = -0.850$; $r = -0.832$, $p<0.01$) and IC_{50} of ABTS scavenging activities ($r = -0.850$; $r = -0.880$; $r = -0.809$, $p<0.01$). TFC in all of extracts had no significant correlation with their IC_{50} of DPPH scavenging activities and IC_{50} of ABTS

scavenging activities and TCC in all of extracts had positive correlation with their IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities (Table 1).

DISCUSSION

The previous research[19-22] reported that tea leaves had antioxidant capacity. Some research showed that antioxidant activity of green tea > oolong tea > black tea[22,23], but other research demonstrated that oolong tea had higher antioxidant activity than the green ones [11]. There were no study regarding antioxidant activity of various extracts (which were n-hexane, ethyl acetate and ethanol) of oolong tea from three different regions in Indonesia using DPPH and ABTS assays.

DPPH free radicals dissolve in methanol give characteristic absorption at wavelength 516 nm and ABTS free radicals dissolve in ethanol have absorption at λ 734 nm. Colors of DPPH and ABTS would be changed when the free radicals were scavenged by antioxidant[17]. DPPH would be changed from purple to yellow color, while ABTS changed from turquoise to white color. The IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities in various extracts of oolong tea from three regions using DPPH and ABTS assays were shown in Fig 1 and Fig 2. The IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities in various extracts compared to IC_{50} of ascorbic acid standard. The lowest value of IC_{50} means had the highest antioxidant activity. IC_{50} were used to determine antioxidant capacity of sample was compared to standard. Sample which had IC_{50} lower than 50 μg/ml was a very strong antioxidant, 50-100 μg/ml was a strong antioxidant, 101-150 μg/ml was a medium antioxidant, while a weak antioxidant with IC_{50} greater than 150 μg/ml[15]. In the present study demonstrated that IC_{50} of DPPH and IC_{50} of ABTS scavenging activities of various extracts of oolong tea from three different regions ranged from 0.31 to 14.27 μg/ml and 0.02 to 5.29 μg/ml, respectively. Based on value of IC_{50} of DPPH and IC_{50} of ABTS scavenging capacity it can be concluded that all of extracts of oolong tea from three regions (Banten, West Java and Bengkulu) can be classified as very strong antioxidant. The lowest IC_{50} of DPPH was given by ethyl acetate extract of oolong tea from West Java (WJ2) 0.31 μg/ml, while IC_{50} of DPPH of ascorbic acid was 0.11 μg/ml. It exposed that potency of ascorbic acid was around three times potency of WJ2 using DPPH method. Ethanolic extract of oolong tea from Banten (BA3) had the lowest IC_{50} of ABTS scavenging activities (0.02 μg/ml) while ascorbic acid standard showed IC_{50} of ABTS scavenging activity 0.09 μg/ml. It demonstrated that antioxidant potency of BA3 was around four times of potency of ascorbic acid using ABTS assay. In the present study exposed that IC_{50} of DPPH scavenging activity of ethanolic extract of oolong tea from Banten, West Java and Bengkulu were 0.87, 0.37, 1.37 μg/ml, respectively, while their IC_{50} of ABTS scavenging activity were 0.02, 0.14, 0.09 μg/ml, respectively. Previous research revealed that antioxidant activity of methanol –water infusion of white tea, green tea, black tea orthodox, black tea CTC from Malawi - Africa, by ABTS assay were 38.4, 55.0, 34.0, 25.5 mM TE (mM Trolox equivalents) respectively, while by ORAC assays were 47, 61, 62 and 32 mM TE, respectively[19]. Study by Lee[20] expressed that tetraglycosides of quercetin and tetraglycosides of kaempferol had EC_{50} of DPPH 30.5 and 487.2 μM. Venditti[11] stated that oolong tea, green tea and black tea in hot water had antioxidant activity 17.5, 12.5, 7.5 mM TEAC by using ABTS method, while in cold water gave 12, 10, 5 mM TEAC. This result exposed that green tea was not always have higher antioxidant activity compared to oolong tea. ABTS scavenging activity of white tea in cold water was significantly higher than the hot ones ($p<0.05$). Black tea had no significant different between in hot water and cold water, while oolong tea demonstrated that ABTS scavenging activity of hot water was significantly higher than the cold ones. Previous study by Zielinski[14] exhibited that infusion of four clusters of Brazillian teas had percentage of DPPH scavenging activities of cluster 1, 2, 3 and 4 were 16.29, 46.52, 72.36, 46.91 %, respectively, while by FRAP method showed 1023, 4999, 11537, 4463 μmol TE/l, respectively. Evans [22] stated that FRAP capacity of green tea infusion was 2.5 fold greater than black tea. Extraction was performed by preparation infusion water in the ranged of 20 -90°C. Increasing in temperature could increase antioxidant potential 4 to 9.5 fold by FRAP assay. Previous research exposed that antioxidant activity of water extract of green tea higher than oolong tea and black tea by DPPH method[21]. Methanol extract of tea leaves from lowland which was classified as shoots, young leaves and mature leaves, had IC_{50} of DPPH scavenging activity 26, 30, 37 μg/ml respectively, while their FRAP capacity 55.6, 54.5, 21.3 mg GAE/g, respectively. IC_{50} of DPPH scavenging activity of methanol extract of young leaves form lowland 30 μg/ml which was lower than from highland 35 μg/ml. Methanol extract of microwaved young leaves had IC_{50} of DPPH scavenging activity 13 μg/ml which was lower than its hot water extract, while its FRAP capacity 126 mg GAE/g was higher than hot water extract 123 mg GAE/g[12]. Yang[13] revealed that ethanol-water (7:3) extract of tea flower showed IC_{50} of DPPH scavenging activity 47.6 μg/ml, while IC_{50} of DPPH of chloroform fraction, ethyl acetate fraction, n-butanol fraction and water fraction were 59.6, 11.1, 45.9, 183.6 μg/ml, while ascorbic acid had 8.8 μg/ml. Methanol extract of freeze dried leaves of twelve tea clones of Iran were studied by Gonbad[24] and the result reported that tea clone Iran 100 gave the lowest IC_{50} of DPPH scavenging activity (218 μg/ml). Total phenolic content (TPC) and Total flavonoid content (TFC) might be related with antioxidant activity. Study by Satoh[21] exposed that TPC in water extract of green tea was higher than oolong tea and black tea, it was similar with previous research by Roginsky[23]. TPC in water extract of black tea 80.5-134.9 mg/g of dry matter and TFC in water extract of green tea 87.5-106.2 mg/g of dry matter[7]. Hot water and cold water extracts of oolong tea had TPC 6 mM GAE and 5.7 mM GAE which was

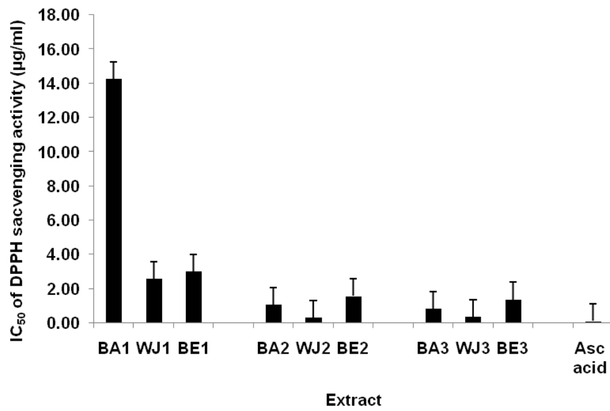

Fig 1: IC_{50} of DPPH scavenging activities in various extracts of oolong tea from three regions

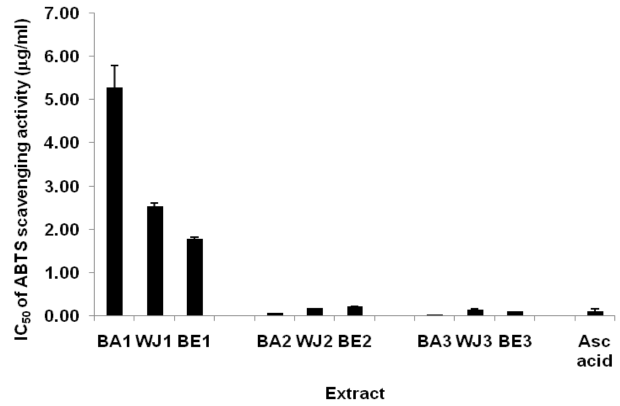

Fig 2: IC_{50} of ABTS scavenging activities in various extracts of oolong tea from three regions

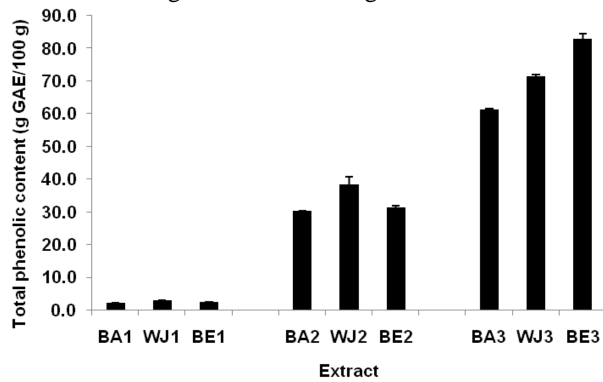

Fig 3: Total phenolic content in various extracts of oolong tea

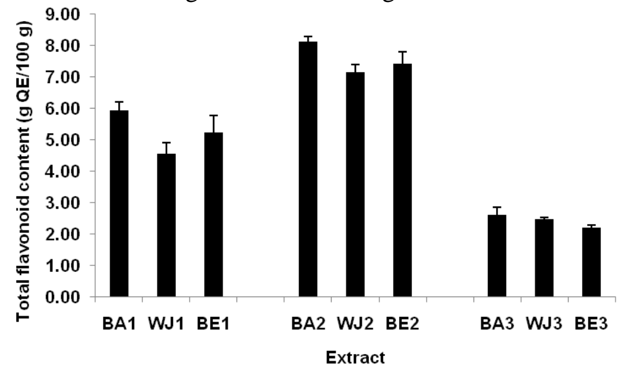

Fig 4: Total flavonoid content in various extracts of oolong tea

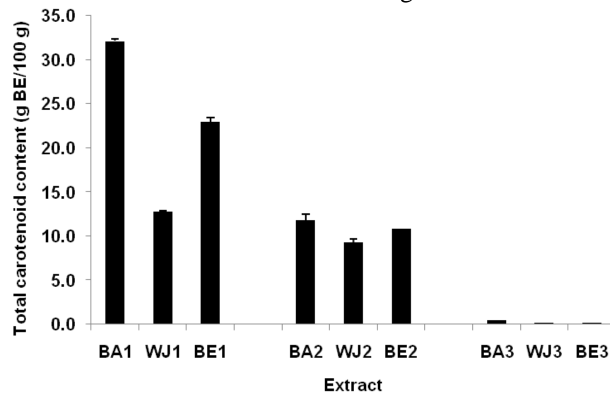

Fig 5: Total carotenoid content in various extracts of oolong tea

higher than green tea and black tea, but it was lower than white tea 6.3 mM GAE and 7.9 mM GAE[11]. Previous research by Chan[12] reported that TPC in methanol extract of microwaved young leaves 20.55 mg GAE/100 g was higher than TPC in hot water extract 19.12 mg GAE/100 g. The methanol extract of young leaves from lowland had TPC 7280 mg GAE/100 g which was lower than highland 7586 mg GAE/100 g, while TPC in methanolic extract of shoots, young leaves and mature leaves from lowland were 7666, 7280 and 5836 mg GAE/100 g. Research by Gonbad[24] demonstrated that methanol extract of freeze dried leaves of tea clone Iran 100 gave the highest TPC 8.44 mg GAE/g and TFC 5.40 mg RE/g compared to the other clones. Carloni[19] revealed that TPC in methanol –water infusion of white tea, green tea, black tea orthodox, black tea CTC were 18.1, 23.6, 14.9, 10.7 mM GAE, respectively, and TFC in white tea 4.75 mM CE was lower than black tea orthodox 6.60 mM CE. Infusion of four clusters of Brazillian teas showed that TPC (1157 mg GAE/l) and TFC (187 mg CE/l) in cluster 3 were higher than the other clusters[14]. Pearson's correlation coefficient was positively high if $0.61 \leq r \leq 0.97$[10] and negatively high if $-0.61 \leq r \leq -0.97$. Sample which had the lowest IC_{50} of DPPH and IC_{50} of ABTS scavenging activity had the highest antioxidant activity. So negatively and high correlation will be given in good correlation between IC_{50} of DPPH or IC_{50} of ABTS with TPC, TFC and TCC. It means increasing in TFC, TPC and TCC caused increasing in antioxidant activities, which was expressed by lower IC_{50} of DPPH scavenging

Table 1. Pearson's correlation coefficient of IC_{50} of DPPH scavenging activities, IC_{50} of ABTS scavenging activities and total phenolic, flavonoid, carotenoid content in various extracts of oolong tea

	TPC	TFC	TCC	IC_{50} ABTS BA	IC_{50} ABTS WJ	IC_{50} ABTS BE
IC_{50} DPPH BA	-0.857**	0.134 ns	0.938**	0.992**		
IC_{50} DPPH WJ	-0.850**	-0.084 ns	0.685*		0.983**	
IC_{50} DPPH BE	-0.832**	0.202 ns	0.917**			0.981**
IC_{50} ABTS BA	-0.850**	0.119 ns	0.933**			
IC_{50} ABTS WJ	-0.880**	-0.570 ns	0.720*			
IC_{50} ABTS BE	-0.809**	0.150 ns	0.911**			

IC_{50} DPPH = IC_{50} DPPH scavenging activity, IC_{50} ABTS = IC_{50} ABTS scavenging activity, BA = oolong tea from Banten, WJ = oolong tea from West Java, BE = oolong tea from Bengkulu, ns = not significant, * = significant at $p < 0.05$, ** = significant at $p < 0.01$

activity and or IC_{50} of ABTS scavenging activity. The data in Table 1 exhibited that TPC in all of oolong tea extracts (Banten, West Java and Bengkulu) had negative and high correlation with IC_{50} of DPPH scavenging activities (r = -0.857; r = -0.850; r = -0.832, p<0.01) and IC_{50} of ABTS scavenging activities (r = -0.850; r = -0.880; r = -0.809, p<0.01), TCC in all of extracts had positive and high correlation with their IC_{50} of DPPH and IC_{50} of ABTS, while TFC in all of extracts had no correlation with IC_{50} of DPPH and IC_{50} of ABTS scavenging activities. It was similar with the previous study by Venditti[11] which exposed that TPC in water extract of oolong tea had good correlation with their ABTS scavenging activities (r^2 = 0.8487, p<0.01) and DMPD (N,N-dimethyl-p-phenylenediamine dihydrochloride) scavenging activities (r^2 = 0.9807, p<0.01). Previous research[24] stated that IC_{50} of DPPH scavenging activities in methanol extract of freeze dried leaves of twelve tea clones of Iran had good correlation with their TPC (r = 0.85, p<0.01) and TFC (r = 0.88, p<0.01). Research by Carloni[19] reported that TPC in methanol –water infusion of white tea, green tea, black tea orthodox, black tea CTC had good correlation with their ABTS scavenging activities (r^2 = 0.871, p<0.01). Phenolic groups were included flavonoid, phenolic acid, tannins, qoumarine and quinone. Flavonoid compound will be included in phenolic groups if have OH in A ring and or B ring. Flavonoid had greater antioxidant activity than phenolic acid [25]. Flavonoid which had OH in ortho C 3',4', OH in C3, oxo function in C4, double bond at C2 and C3 have high antioxidant activity. The OH with ortho position in C3'-C4' had the highest influence to antioxidant activity of flavonoid. The flavonoid aglycones would give higher antioxidant activity than flavonoid glycosides[25]. It could be seen in Fig 4 that TFC in ethyl acetate extract of oolong tea from West Java (WJ2) 7.16 g QE/100 g was similar with TFC in ethyl acetate extract of oolong tea from Bengkulu (BE2) 7.43 g QE/100 g, but IC_{50} of DPPH scavenging activity of WJ2 (0.31 µg/ml) was smaller than BE2 (1.58 µg/ml). Based on this data it can be supposed that many flavonoids in BE2 had OH in C5, C7, or C3' only, or C4' only, or C3 only without oxo function in C4, that had no and low influence in antioxidant activities. In contrast, almost all of flavonoid in WJ2 were flavonoid that had OH in position which can influence high antioxidant capacities. TPC in ethyl acetate extract of oolong tea form Bengkulu (BE2) 31.31 g GAE/100 g was smaller than with its ethanol extract (BE3) 83.06 g GAE/100 g, but IC_{50} of DPPH scavenging activity of BE2 (1.58 µg/ml) was similar with IC_{50} of DPPH scavenging activity of BE3 (1.37 µg/ml). It can be supposed that many phenolic compounds in BE2 which had influence high antioxidant capacities, whereas in BE3 only a little phenolic compounds with high antioxidant activities. Carotenoid have antioxidant capacity by scavenging free radical. Higher scavenging free radical capacity will be given by more double bonds in carotenoid[26]. Carotenoid which contained more than 7 double bonds would have higher scavenging radical capacity[27]. Beta carotene was used as standard because of it had conjugation double bonds which had ability to scavenge free radicals[28]. Study by Kobayashi[29] expressed that increasing in lipophilicity of carotenoid would increase scavenging radical capacity, it means give the lower IC_{50} of DPPH scavenging capacity. TCC in ethyl acetate extract of oolong tea from West Java (WJ2) 9.30 g BE/100 g was higher than TCC in ethanolic extract of oolong tea from West Java (WJ3) 0.24 g BE/100 g, but IC_{50} of DPPH and IC_{50} of ABTS scavenging activities of WJ2 similar with WJ3 (0.31 and 0.37 µg/ml respectively, and 0.16 and 0.14 µg/ml respectively). It might be predicted that many carotenoid in WJ2 contained maximum 7 double bonds, whereas many carotenoid in WJ3 consisted of more than 7 double bonds which had high antioxidant activities. ABTS and DPPH methods had the same mechanism reactions, which was electron transfer assays[30]. In previous study[11] stated that ABTS scavenging activities of water extract of oolong tea had positive correlation with its DMPD scavenging activities (r^2 = 0.8487, p<0.01). It was similar with the present study which all of extracts of oolong tea from three regions had positively high correlation between IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities. So the results showed that IC_{50} of DPPH scavenging activities in all of extracts sample were linear with their IC_{50} of ABTS scavenging activities.

CONCLUSION

Antioxidant activity of sample should be measured by different methods in parallel, because various methods could give different results. All of extracts of oolong tea

from three regions (Banten, West Java and Bengkulu) were very strong antioxidant, using DPPH and ABTS assays. TPC in all of extracts had negative and high correlation with IC_{50} of DPPH and IC_{50} of ABTS scavenging activities. Phenolic compounds in oolong tea extracts from three regions were the major contributor in IC_{50} of DPPH scavenging activity and IC_{50} of ABTS scavenging activity. There were linear correlation between IC_{50} of DPPH scavenging activities and IC_{50} of ABTS scavenging activities of all of extracts sample. Oolong tea from Banten, West Java and Bengkulu may be exploited as natural antioxidant sources to alleviate oxidative stress.

REFERENCES

1. Xu BJ, Chang SK. Total phenolic content and antioxidant properties of eclipse black beans (*Phaseolus vulgaris* L.) as affected by processing methods. J Food Sci 2008; 73(2): H19-27.
2. Mashkor IMAAL. Phenolic content and antioxidant activity of fenugreek seeds extract. Int J Pharmacogn Phytochem Res 2014; 6(4): 841-844.
3. Mokbel MS, Hashinaga F. Antibacterial and antioxidant activities of banana (*Musa*, AAA cv. Cavendish) fruits peel. Am J Biochem Biotechnol 2005; 1(3): 125-131.
4. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid content of some selected Iranian medicinal plants. Afr J Biotechnol 2006; 5(11): 1142-1145.
5. Win M, Hamid A, Baharin B, Anwar FS, Pak-Dek MS. Phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour. Pak J Bot 2011; 43(3): 1635-1642.
6. Fidrianny I, Johan Y, Sukrasno. Antioxidant activities of different polarity extracts from three organs of makrut lime (C*itrus hystrix* DC) and correlation with total flavonoid, phenolic, carotenoid content, Asian J Pharm Clin Res 2015; 8(4): 239-243.
7. Khokhar S, Magnusdottir SG. Total phenol, catechin, and caffeine contents of teas commonly consumed in the United kingdom. J Agric Food Chem 2002; 50(3): 565-570.
8. Pellegrini N, Serafini M, Colombi B, Rio DD, Salvatore S. Total antioxidant capacity of plant food, beverages and oils consumed in Italy assessed by three different in vitro assays. J Am Coll Nutr 2003; 133: 2812-2819.
9. Fidrianny I, Aristya T, Hartati R. Antioxidant capacities of various leaves extracts from three species of legumes and correlation with total flavonoid, phenolic, carotenoid content, Int. J. Pharmacogn Phytochem Res 2015; 7(3): 628-634.
10. Thaipong K, Boonprakob U, Crosby K, Zevallos LC, Byrne DH. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J Food Comp Anal 2006; 19: 669-675.
11. Venditti E, Bacchetti T, Tiano L, Carloni P, Greci L, Damian E. Hot vs. cold water steeping of different teas: Do they affect antioxidant activity? Food Chem 2010; 119: 1597–1604.
12. Chan EWC, Lim YY, Chew YL. Antioxidant activity of *Camellia sinensis* leaves and tea from a lowland plantation in Malaysia. Food Chem 2007; 102: 1214–1222.
13. Yang Z, Tu Y, Baldermann S, Dong F, Xu Y, Watanabe N. Isolation and identification of compounds from the ethanolic extract of flowers of the tea (*Camellia sinensis*) plant and their contribution to the antioxidant capacity. LWT - Food Sci Technol 2009; 42: 1439–1443.
14. Zielinski AAF, Haminiuk CWI, Alberti A, Nogueira A, Demiate IM, Granato D. A comparative study of the phenolic compounds and the in vitro antioxidant activity of different Brazilian teas using multivariate statistical techniques. Food Res Int 2014; 60: 246–254.
15. Blois MS. Antioxidant determination by the use of stable free radicals. Nature 1958; 181: 1199-2000.
16. Bedawey AA. Characteristics of antioxidant isolated from some plants sources, Shibin El-Kom: Cairo; 2010. p. 1-11.
17. 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.
18. 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.
19. Carloni P, Tiano L, Padella L, Bacchetti T, Customu C, Kay A, Damiani E. Antioxidant activity of white, green and black tea obtained from the same tea cultivar. Food Res Int 2013; 53: 900–908.
20. Lee VSY, Chen CR, Liao YW, Tzen JTC, Chang CI. Structural determination and DPPH radical-scavenging activity of two acylated flavonoid tetraglycosides in oolong Tea (*Camellia sinensis*). Chem Pharm Bull 2008; 56(6): 851—853.
21. Satoh E, Tohyama N, Nishimura M. Comparison of the antioxidant activity of roasted tea with green, oolong, and black teas. Int J Food Sci Nutr. 2005; 56(8): 551-559.
22. Evans SL. Antioxidant potential of green and black tea determined using the ferric reducing power (FRAP) assay. Int J Food Sci Nutr 2000; 51(3): 181-8.
23. Roginsky V, Barsukova T, Hsu CF, Kilmartin PA. Chain-breaking antioxidant activity and cyclic voltammetry characterization of polyphenols in a range of green, oolong, and black teas. J Agric Food Chem 2003; 51(19): 5798-5802.
24. Gonbad RA, Afzan A, Karimi E, Sinniah UR, Swamy MK. Phytoconstituents and antioxidant properties among commercial tea (*Camellia sinensis* L.) clones of Iran. Electron J Biotechnol 2015; 18: 433–438.
25. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: Chemistry, metabolism and structure-activity relationships. J Nutr Biochem 2002; 13: 572 - 584.

26. Foote, CS. Free radicals in biology. 3rd ed. New York: Academic Press; 1976
27. Beutner S, Bloedorn B, Hoffmann T, Martin HD. Synthetic singlet oxygen quenchers. Methods Enzymol 2000; 319: 226-241.
28. Charles DJ. Antioxidant properties of spices shells and other. London: John Willey; 2013.
29. Kobayashi M, Sakamoto Y. Singlet oxygen quenching ability of astaxanthin esters from the green alga *Haematococcus pluvialis*. Biotech Lett 1999; 21: 265-269.
30. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005; 53: 1841 -1856.