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Research Article

ANTIOXIDANT PROFILE AND PHYTOCHEMICAL CONTENT OF THREE KINDS OF LEMON GRASS GROWN IN WEST JAVA-INDONESIA

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ABSTRACT

Objective: The aims of this research were to determine antioxidant activity from various herbs extracts of three kinds of lemon grass using two antioxidant testing methods which were 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) and correlation of total phenolic content (TPC), total flavonoid content (TFC), and total carotenoid contents (TCC) with their inhibitory concentration 50% (IC_{50}) of DPPH and exhibitory concentration 50% (EC_{50}) of FRAP.

Methods: A sample was extracted by reflux method using different polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH and FRAP assays, determination of TPC, TFC, and TCC were carried out by ultraviolet -visible spectrophotometry and correlation with their IC_{50} of DPPH and EC_{50} of FRAP capacities were analyzed by Pearson's method.

Results: The ethanolic herbs extract of *Cymbopogon citratus* (CC) had the lowest IC_{50} of DPPH scavenging activity 2.75 µg/ml and the lowest EC_{50} of FRAP capacity 12.22 µg/ml. Ethanolic herbs extract of *Cymbopogon winterianus* exposed the highest phenolic content and its n-hexane extract presented the highest carotenoid content. Ethyl acetate herbs extract of CC gave the highest flavonoid content. There was significantly negative correlation between TPC in CC herbs extract with their IC_{50} of DPPH and EC_{50} of FRAP.

Conclusions: All herbs extracts from three kinds of lemon grass were categorized as a very strong antioxidant by DPPH method. Phenolic compounds in CC were the major contributor in antioxidant activities by DPPH and FRAP methods. DPPH and FRAP gave linear result in antioxidant activities of herbs extract of three kinds of lemon grass.

Keywords: Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, Ferric reducing antioxidant power, Lemon grass, Three kinds, Herbs.

INTRODUCTION

Phenolic compounds can be found in plants, and they have been reported to have multiple biological effects including antioxidant and antibacterial activities [1,2]. Previous research [3-5] expressed that phenolic and flavonoid contents could be correlated to their antioxidant activities. The antioxidant has many benefits to prevent the excessive of free radical in oxidative stress which can cause many degenerative diseases. Plants included banana, sweet potatoes, lemon grass, tea, and legumes contained phenolic and flavonoid compounds [1,2,5-7].

2,2-diphenyl-1-picrylhydrazy (DPPH), ferric reducing antioxidant power (FRAP), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) methods could be used to determine antioxidant activity in many plants extracts [7-9]. The previous researches [1,3,9,10] exposed that DPPH, ABTS and FRAP can be conducted to evaluate antioxidant activity of fruits, vegetables, and food. Lemon grass was commonly used in cooking, contained many compounds such as luteolin, luteolin glycoside, chlorogenic acid, and caffeic acid which can act as antioxidant [s6,11,12].Lemon grass had antioxidant activities using DPPH, β-carotene bleaching (BCB), and FRAP assays [13].

The objectives of this research were to measure antioxidant activities in different polarity herbs extracts (n-hexane, ethyl acetate, and ethanol) from three kinds of lemon grass grown in West Java-Indonesia using DPPH and FRAP assays, and correlations of total phenolic content (TPC), total flavonoid content (TFC), and total carotenoid content (TCC) with their antioxidant activities.

METHODS

Materials

DPPH, FRAP, gallic acid, quercetin, and beta-carotene were purchased from Sigma-Aldrich (MO, USA), herbs of three kinds of lemon grass. All of other reagents were analytical grades.

Preparation of sample

Herbs of three kinds of lemon grass which were *Cymbopogon citratus*, namely, as CC and *Cymbopogon martini* as CM were collected from Cirebon, West Java-Indonesia, *Cymbopogon winterianus* as CW from Bandung, West Java-Indonesia, were thoroughly washed with tap water, sortation while wet, cut, dried, and grinded into powder.

Extraction

About 300 g of powdered samples were extracted by reflux using different polarity solvents. The extraction using n-hexane was repeated three times. The remaining residue was then extracted three times using ethyl acetate. Finally, the remaining residue was extracted three times using ethanol. Hence, in total, there were nine extracts: three n-hexane extracts (namely CC1, CW1, and CM1), three ethyl acetate extracts (CC2, CW2, and CM2), and three ethanolic extracts (CC3, CW3, and CM3).

Determination of TPC

Folin-Ciolcalteu reagent was used for measuring TPC [14]. The absorbance was read at wavelength 765 nm. The analysis was conducted in triplicate for each extract. A standard solution of gallic acid (55-175 µmg/ml) was used to obtain a calibration curve. TPC was presented as a percentage of total gallic acid equivalent per 100 g extract (g GAE/100 g).

Determination of TFC

Modification of Chang's method [15] was performed in determining TFC. The absorbance was measured at wavelength 415 nm. The analysis was conducted in triplicate for each extract. The standard solution of quercetin (30-120 mg/ml) was used to obtain a calibration curve. The TFC was exhibited as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Determination of TCC

TCC was performed by modified Thaipong's method [9]. Each extract was diluted in n-hexane [7]. The absorbance was read at wavelength 470 nm. The analysis was conducted in triplicate for each extract. The standard solution of beta-carotene (40-80 µmg/ml) was used to obtain a calibration curve. The TCC was figured as percentage of total beta-carotene equivalent per 100 g extract (g BE/100 g).

Inhibitory concentration 50% (IC_{50}) DPPH scavenging activity

The preparation of DPPH solution was conducted using method from Blois [16] with minor modification. Various concentrations 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 read after 30 minutes incubation at wavelength 515 nm by ultraviolet-visible (UV-Vis) spectrophotometer Hewlett Packard 8435. Methanol was used as a blank, DPPH 50 µg/ml as control, and ascorbic acid as standard. The analysis was performed in triplicate for standard and each extract. The antioxidant activity of each extract by DPPH method was performed by calculating percentage of antioxidant activity using reduction of DPPH absorbance [17]. IC_{50} of DPPH scavenging activity of each extract can be determined using its calibration curve.

FRAP capacity

The preparation of FRAPsolution was adopted from Benzie and Strain [18], which was prepared in acetate buffer pH 3.6. Each extract 50 µg/ml was added into FRAP solution 50 µg/ml (1:1) to initiate the reaction. After 30 minutes incubation, the absorbance was observed at wavelength 593 nm using UV-Vis spectrophotometer Hewlett Packard 8435. Acetate buffer was used as a blank, FRAP 50 µg/ml as control, and ascorbic acid as standard. The analysis was conducted in triplicate for standard and each extract. Antioxidant capacity of each extract was determined based on increasing in Fe (II)-tripyridyl triazine (TPTZ) absorbance by calculating the percentage of antioxidant capacity [18].

Statistical analysis

Each sample analysis was performed in triplicate. All of presented results are mean (± standard deviation) of at least three independent experiments. A statistical analysis using analysis of variance (ANOVA) with a statistical significance level set at $p<0.05$ and *post-hoc* Tukey's procedure was conducted with SPSS 16 for Windows. The correlation between the TPC, TFC, TCC and antioxidant activities, and correlation between two antioxidant activity methods were performed using the Pearson's method.

RESULTS

TPC in various herbs extracts of lemon grass

TPC among the various herbs extracts was denoted in term of GAE using the standard curve equation y=0.005 x−0.016, R^2=0.987. The TPC in various herbs extracts from three kinds of lemon grass had different results ranged from 2.01 to 12.53 g GAE/100 g. The highest phenolic content (12.53 g GAE/100 g) for ethanolic herbs extract of CW3 and the lowest was given by n-hexane herbs extract of CC1 2.01 g GAE/100 g (Fig. 1).

TFC in various herbs extracts of lemon grass

TFC among the various herbs extracts were presented in term of QE using the standard curve equation y=0.006 x+0.029, R^2=0.998. The TFC in various herbs extracts from three kinds of lemon grass expressed varied from 2.56 to 12.71 g QE/100 g (Fig. 2). The highest TFC was represented by ethyl acetate herbs extract of CC2 (12.71 g QE/100 g) and the lowest for n-hexane herbs extract of CM1.

TCC in various herbs extracts of lemon grass

TCC among the various herbs extracts were reported in term of beta-carotene equivalent using the standard curve equation y=0.012x−0.178, R^2=0.981. The TCC in various herbs extracts from three kinds of lemon grass gave different results in the range of 0.10-20.36 g BE/100 g (Fig. 3). N-hexane herbs extract of *C. winterinaus* (CW1) showed the highest carotenoid content (20.36 g BE/100 g), while the lowest carotenoid (0.10 g BE/100 g) for ethanolic herbs extract of CC3 and CM3.

Antioxidant activity by DPPH and FRAP methods

The IC_{50} of DPPH scavenging activities and effective concentration 50% (EC_{50}) of FRAP capacities in various herbs extracts from three kinds of lemon grass using DPPH and FRAP assays were shown in Figs. 4 and 5. IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities of each extract were compared to IC_{50} or EC_{50} ascorbic acid as standard. The lowest value of IC_{50} or EC_{50} means had the highest antioxidant activity.

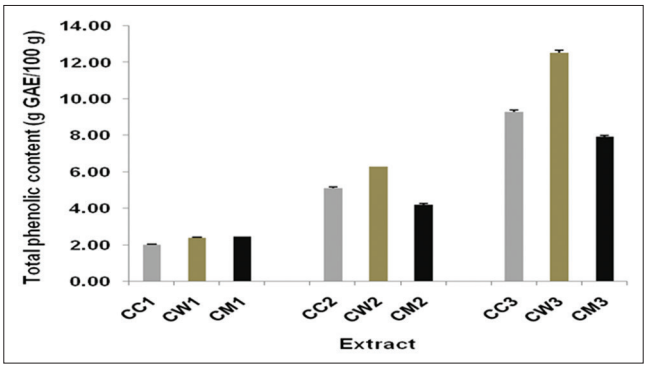

Fig. 1: Total phenolic content in various herbs extracts of lemon grass, n=3

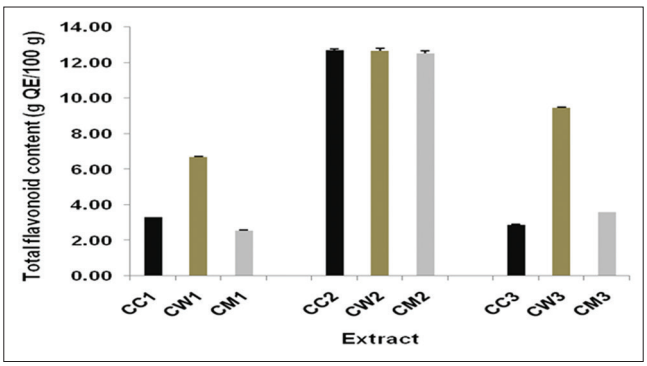

Fig. 2: Total flavonoid content in various herbs extracts of lemon grass, n=3

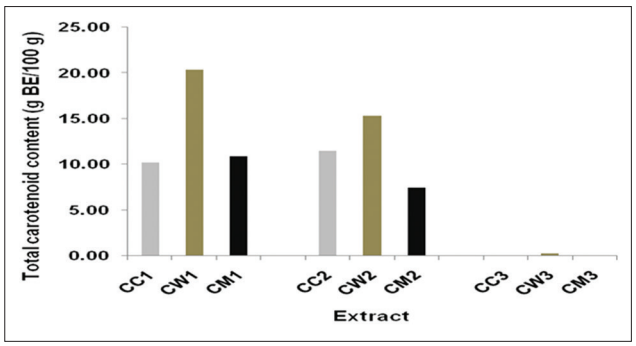

Fig. 3: Total carotenoid content in various herbs extracts of lemon grass, n=3

Correlations between TPC, TFC, TCC in various herbs extracts of three kinds of lemon grass and IC_{50} of DPPH scavenging activities, EC_{50} of FRAP capacities

Pearson's correlation coefficient between TPC in various herbs extracts from three kinds of lemon grass, and their antioxidant activities exhibited that TPC and TFC in CW had significantly negative correlation with their IC_{50} of DPPH scavenging activities (r=−0.818; r=−0.816, p<0.01, respectively) and their EC_{50} FRAP capacities (r=−0.939, p<0.01; r=−0.641, p<0.05, respectively) (Table 1).

DISCUSSION

The previous study [19,20] revealed that lemon grass had antioxidant capacity. Antioxidant activity of leaves, herbs, and essential oil of CC had been determined using DPPH, FRAP and BCB methods [21,22]. There was no research regarding antioxidant activity of various herbs extracts (which were n-hexane, ethyl acetate, and ethanol) from three kinds of lemon grass (CC, CW, and CM) using DPPH and FRAP assays.

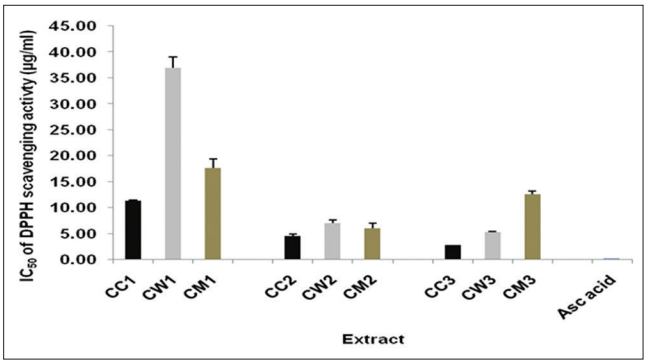

Fig. 4: Inhibitory concentration 50% of 2,2-diphenyl-1-picrylhydrazyl in various herbs extracts of lemon grass, n=3

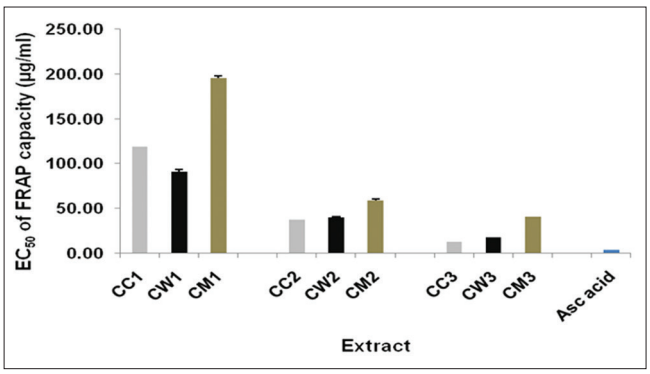

Fig. 5: Exhibitory concentration 50% of ferric reducing antioxidant power capacities in various herbs extracts of lemon grass, n=3

The previous study [3,5,23] exposed that antioxidant activity can be related with the TPC which was included phenolic acid. Cinnamic acid had higher antioxidant activity than benzoic acid [24]. Research by Cheel *et al.* [6] presented that CC contained phenolic compounds such as chlorogenic acid and caffeic acid. The presence of chlorogenic acid and caffeic acid might be contributed in antioxidant activity of the lemon grass herbs extract. The previous studies denoted that TPC in 80:20 (v/v) methanol-water extract of CC 0.1 g GAE/100 g was lower than TPC of *Curcuma longa* (1.22 g GAE/100 g) and *Thea sinensis* (9.74 g GAE/100 g) [25], TPC in water extract of CC (13 GAE) was lower than its TPC in methanolic extract (202.4 GAE) [26]. The study by Hasim *et al*. [27] 30% ethanolic leaves extract of CC showed the highest TPC (50.017 mg GAE/g compared to its 70% ethanolic extract (49.317 mg GAE/g) and 96% ethanolic extract (43.433 mg GAE/g). Essential oil of CC (extracted by distillation assisted by microwaves) had TPC 149.2 mg GAE/100 ml [22]. The lemon grass leaves extract which was extracted using 40% ethanol in 300 minutes 25°C had TPC 6.73 g GAE/100 g. It was similar to this study which reported that TPC in ethanolic extract of CC was 9.29 g GAE/100 g.

The previous research by Akande *et al*. [26] exhibited that TFC in methanol extract and water extract of CC were 80.29 QE and 152.2 QE, respectively. It was different from this study which expressed that TFC in n-hexane, ethyl acetate and ethanol extract of three kinds of lemon grass (CC, CW and CM) ranging from 2.56 to 12.71 g QE/100 g, while their ethanolic herbs extract gave TFC 2.87, 9.45 and 3.60 g QE/100 g, respectively, and TCC 0.10, 0.23 and 0.10 g BE/100 g, respectively.

DPPH free radicals dissolve in methanol give absorption at wavelength 516 nm. Colors of DPPH would be changed when the free radicals were scavenged by antioxidant [23]. Ferric (III) chloride is reagent of FRAP which was combined with TPTZ in acetate buffer pH 3.6. Fe (III) will be reduced to Fe (II). Complex of Fe (II) - TPTZ shows blue color and gave characteristic absorption at wavelength 593 nm. The intensity of blue color depends on amount of Fe (III) which is reduced to Fe (II). IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities in various herbs extracts of three kinds of lemon grass can be seen in Figs. 4 and 5. The IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities in various herbs extracts of lemon grass were compared to IC_{50} or EC_{50} of the ascorbic acid standard. The lowest value of IC_{50} or EC_{50} means had the highest antioxidant activity. The sample which had IC_{50} or EC_{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} or EC_{50} >150 µg/ml [16].

In the previous research stated that 80:20 (v/v) methanol-water extract of CC had higher antioxidant activity by DPPH assay, which was revealed by lower IC_{50} of DPPH (1.14 mg/ml) compared to IC_{50} of DPPH of *Psidium guajava* (2.34 mg/ml) and *Cucurbita pepo* (23.12 mg/ml) [25]. It was contrary with this study which exposed that IC_{50} of DPPH scavenging activity of ethanolic herbs extract of CC was 2.75 µg/ml and categorized as a very strong antioxidant, while in the previous study above [25] IC_{50} of DPPH of 80:20 (v/v) methanol-water extract of CC can be

Table 1: Pearson's correlation coefficient of TPC, TFC, TCC content in various herbs extracts from three kinds of lemon grass with their IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities

Antioxidant parameter	Pearson's correlation coefficient (r)					
	TPC	TFC	TCC	EC_{50} FRAP CC	EC_{50} FRAP CW	EC_{50} FRAP CM
IC_{50} DPPH CC	−0.918**	−0.276 ns	0.583 ns	0.999**		
IC_{50} DPPH CW	−0.818**	−0.816**	0.725*		0.960**	
IC_{50} DPPH CM	−0.232 ns	−0.923**	0.232 ns			0.748**
EC_{50} FRAP CC	−0.929**	−0.252 ns	0.604 ns			
EC_{50} FRAP CW	−0.939**	−0.641*	0.879**			
EC_{50} FRAP CM	−0.811**	−0.488 ns	0.811**			

IC_{50} DPPH: Inhibitory concentration 50% DPPH scavenging activity, EC_{50} FRAP: Exhibitory concentration 50% FRAP capacity, CC: *Cymbopogon citratus*, CW: *Cymbopogon winterianus*, CM: *Cymbopogon martini*, ns: Not significant, *Significant at p<0.05, **Significant at p<0.01, FRAP: Ferric reducing antioxidant power, TPC: Total phenolic content, TFC: Total flavonoid content, TCC: Total carotenoid content, DPPH: 2,2-diphenyl-1-picrylhydrazyl

classified as weak antioxidant. Research by Hasim *et al*. [27] regarding antioxidant activity of CC which was extracted using 30% ethanol, 70% ethanol, and 96% ethanol by maceration method expressed that 70% ethanolic leaves extract of CC gave the lowest IC_{50} of DPPH 79.44 mg/l compared to its 96% ethanol extract 89.64 mg/l and 30% ethanol extract 138 mg/l.

Akande *et al*. [26] demonstrated that different concentrations of DPPH showed variation in the percentage of DPPH scavenging activity. Water extract and methanolic extract of CC which was reacted with DPPH 50 µg/ml gave 69.81% and 69.22%, respectively, in the percentage of DPPH scavenging activity. It was the same as this study, DPPH concentration which was used 50 µg/ml. Essential oil of CC had lower IC_{50} of DPPH scavenging activity (41.7 µg/ml) compared to its methanol leaves extract (55.7 µg/ml), while synthetic antioxidant butylated hydroxytoluene (BHT) had IC_{50} of DPPH 37.7 µg/ml [19]. The previous study by Lu [13] represented that chloroform herbs extract CC gave the lowest antioxidant capacity by FRAP method which its EC_{50} of FRAP capacity 0.09 mmol/g compared to EC_{50} of FRAP capacity of ascorbic acid 4.79 mmol/g and gallic acid 2.17 mmol/g. It was similar to DPPH and BCB assays which revealed that its IC_{50} of DPPH 1998 µg/ml and EC_{50} of BCB capacity 95.9 µg/ml were higher than IC_{50} of DPPH of ascorbic acid 0.60 µg/ml, quercetin 1.00 µg/ml, gallic acid 1.50 µg/ml and EC_{50} of BCB capacity of ascorbic acid 9.6 µg/ml, gallic acid 10.5 µg/ml, and quercetin 25.1 µg/ml. The previous researches exhibited that essential oil of CC which was extracted using distillation assisted by microwaves had IC_{50} of DPPH scavenging activity 44.06 mg Trolox per 100 ml [22], volatile oil of CC which was extracted by hydrodistillation method showed percentage of DPPH scavenging activity 37%, while BHT 54% and IC_{50} of DPPH of volatile oil 3.83% v/v, BHT 4.25% v/v [20]. Sah *et al*. [21] exposed that 40% ethanol leaves extract of CC had the highest antioxidant activity by DPPH method which stated by the lowest IC_{50} of DPPH (200 µg/ml) compared to 20%, 60%, 80% and 100% ethanol leaves extracts. Extraction using 40% ethanol in 300 minutes gave the lowest IC_{50} of DPPH (253 µg/ml) compared to 60, 120, 180 and 240 minutes. This study denoted that extraction using 40% ethanol in 300 minutes 25°C showed the lowest IC_{50} of DPPH (288 µg/ml) compared to 30°, 40°, 50° and 60°C. Then, Sah's research also expressed that leaves extract which was extracted using 40% ethanol in 300 minutes 25°C gave EC_{50} FRAP capacity 129 mg TE/g and percentage of BCB capacity 67%.

Pearson's correlation coefficient was significantly negative if $-0.61 \leq r \leq -0.97$ and significantly positive if $0.61 \leq r \leq 0.97$ [9]. The lowest IC_{50} of DPPH scavenging activity and EC_{50} of FRAP capacity presented the highest antioxidant activity. It means increasing in TFC, TPC and TCC caused increasing in antioxidant activities, which was denoted by lower IC_{50} of DPPH scavenging activity and or EC_{50} of FRAP capacity. Therefore, the good correlation between TPC, TFC, and TCC with IC_{50} of DPPH or EC_{50} of FRAP was significant and negative correlation [28]. This research exposed that TPC in herbs extracts of three kinds of lemon grass (CC, CW and CM) had significantly negative correlation with their EC_{50} of FRAP ($r=-0.929$; $r=-0.939$; $r=-0.811$, $p<0.01$, respectively) and only TPC in herbs extract of CC and CW had negative and significant correlation with their IC_{50} of DPPH ($r=-0.918$; $r=-0.818$, $p<0.01$, respectively). TFC in herbs extract of CW had negative and significant correlation with their IC_{50} of DPPH and EC_{50} of FRAP ($r=-0.816$, $p<0.01$; $r=-0.641$, $p<0.05$, respectively). It could be seen that phenolic compounds in CC were the major contributor in its antioxidant activity by DPPH and FRAP assays. TFC in CM herbs extract had significant and negative correlation with its IC_{50} of DPPH scavenging activity ($r=-0.923$, $p<0.01$). It means flavonoid compounds were the major contributor in antioxidant activity of CM by DPPH assay. In the previous study [21], the correlation coefficient was analyzed between TPC and EC_{50} of FRAP which presented as mg TE/g. Hence, the good correlation would present by positive and significant correlation. It means the higher TPC would give the higher EC_{50} of FRAP (mg TE/g). TPC in 40 % ethanol leaves extract which was extracted in 300 minutes 25°C had significantly positive correlation with its EC_{50} of FRAP ($r=0.995$, $p<0.01$). TPC in this extract also gave positive and significant correlation with their percentage of BCB ($r=0.932$, $p<0.01$).

Coumarine, quinone, tannin, phenolic acid, and flavonoid are included in phenolic groups. Flavonoid compound which has OH in A ring and or B ring will be included in phenolic groups. The flavonoid glycosides would give lower antioxidant activity than flavonoid aglycones [24]. Flavonoid which had ortho di OH at C-3'-C4', OH at C-3, oxo function at C-4, and double bond at C-2 and C-3 have high antioxidant activity. The ortho with di-OH position at C-3'-C-4' had the highest influence to antioxidant activity of flavonoid. Previous researches stated that CC contained luteolin [11], luteolin 6-C-glucosyl-8-C-arabinoside, luteolin 6-C-glucoside, luteolin 7-O-glucoside, luteolin 7-O-neohesperidoside, luteolin 6-C-arabinosyl-2"-O-rhamnoside, and luteolin 2"-O-rhamnosyl-C-(6-deoxy-ribo-hexos-3-ulosyl) [12]. Fig. 1 revealed that TPC in ethanolic herbs extract of CW3 12.53 g GAE/100 g was higher than TPC in ethanolic extract of CC3 9.29 g GAE/100 g, but antioxidant activity of CC3 was higher than CW3 by DPPH method, as represented by IC_{50} of DPPH scavenging activity of CC3 2.75 µg/ml lower than CW3 5.32 µg/ml. Phenolic acid had lower antioxidant activity than flavonoid [24]. It can be estimated that many phenolic compounds in CC3 had higher antioxidant activity, such as luteolin 6-C-glucoside and luteolin 7-O-glucoside, which were extracted in ethanol solvent, than phenolic compounds in CW3 such as chlorogenic acid with lower antioxidant activity. It was similar to the second antioxidant method which was demonstrated that EC_{50} of FRAP capacity CC3 (12.22 µg/ml) was lower than EC_{50} of FRAP CW3 (17.23 µg/ml). It can be suggested that many phenolic compounds in CC3 have reduction potential lower than that of Fe (III)/Fe (II) 0.77 V, while phenolic compounds in CW3 have reduction potential higher than 0.77 V. Sample will be oxidized and at the same time Fe (III) reduce to Fe (II) and then the Fe (II) react with TPTZ, gave the blue color complex.

TFC in ethyl acetate herbs extract of CC2 12.71 g QE/100 g was higher than TFC in its ethanolic herbs extract (CC3) 2.87 g QE/100 g, but IC_{50} of DPPH of CC3 (2.75 µg/ml) was lower than IC_{50} of DPPH of CC2 (4.56 µg/ml). Aluminum (III) chloride was used as a reagent in determining TFC. The complex reaction between sample with aluminum (III) chloride would be happened if sample contained a flavonoid with ortho di- OH at C3'-C4' and or OH at C-3 and oxo function at C-4 and or OH at C-5 and oxo function at C-4. The weakness of TFC determination using aluminum (III) chloride reaction is complex reaction would not be carried out only with flavonoid compound but it could also react with any phenolic compounds, which has ortho di-OH. It was similar to a phenolic compound which has ortho di-OH-OCH_3. Based on the result, it can be predicted that many phenolic compounds but not flavonoid such as caffeic acid which was extracted in ethyl acetate solvent, reacted with aluminum (III) chloride, detected as TFC and gave higher TFC value, and they have lower antioxidant compared to flavonoid. In contrary with CC2, many flavonoid compounds in CC3 such as luteolin glycoside which was extracted in ethanol solvent have higher antioxidant activity compared to caffeic acid.

Carotenoid has antioxidant capacity by scavenging free radical [29] and carotenoid which contain more double bonds will give higher scavenging free radical activity. The previous research [30] expressed that a carotenoid would give higher scavenging radical activity if it contain >7 double bonds. Beta carotene was used as standard because it has conjugation double bonds which have ability to scavenge free radicals [31]. Increasing in lipophilicity of carotenoid would increase antioxidant activity which was revealed by lower IC_{50} of DPPH scavenging activity [32]. In Fig. 3, it could be showed that TCC in n-hexane herbs extract of CC1 10.19 g BE/100 g was lower than TCC in n-hexane herbs extract of CM1 10.91 g BE/100 g, but the higher TCC did not linear with its antioxidant activity, which stated that IC_{50} of DPPH of CC1 (11.27 µg/ml) was lower than IC_{50} of DPPH of CM1 (17.61 µg/ml). It can be predicted that many carotenoid compounds in CM1 contained maximum seven double bonds which have low antioxidant activity while in CC1 many carotenoid compounds had more than seven double bonds which have high antioxidant activity.

DPPH and FRAP assays have different principles. In this study showed that IC_{50} of DPPH scavenging activities of three kinds of lemon grass (CC, CW and CM) had positive and significant correlation with their EC_{50} of capacities (r=0.999; r=0.960, p<0.01, r=0.748, p<0.05, respectively). It could be seen that IC_{50} of DPPH scavenging activities of herbs extract of three kinds of lemon grass gave linear result with their EC_{50} of FRAP capacities.

CONCLUSION

Antioxidant activity of samples should be determined using different methods in parallel, because various methods could give different results. All herbs extracts of three kinds of lemon grass (CC, CW and CM) were a very strong antioxidant using DPPH assay. TPC in herbs extracts of CC had significantly negative correlation with their IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities. Phenolic compounds in herbs extracts of CC were the major contributor in their antioxidant activity by DPPH and FRAP methods. There was linear correlation between IC_{50} of DPPH scavenging activities and EC_{50} of FRAP capacities of all of the sample extracts. Herbs of CC, CW and CM have many benefits to prevent oxidative stress and may be exploited as sources of natural antioxidant.

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