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Research Article

EVALUATION OF ANTIOXIDANT ACTIVITIES OF FRUIT EXTRACTS OF CHAYOTE (*SECHIUM EDULE* [JACQ.] SWARTZ) GROWN IN DIFFERENT SITES IN JAVA - INDONESIA

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ABSTRACT

Objectives: The aim of this research were to determine antioxidant activity from various fruit extracts of chayote from three different sites using two antioxidant methods which were 2,2-diphenyl-1-picrylhydrazyl (DPPH) and phosphomolybdenum methods, correlation of total phenolic, flavonoid, and carotenoid content in various extracts of chayote with their IC_{50} of DPPH antioxidant activities and EC_{50} of phosphomolybdenum capacity.

Methods: An extraction was carried out by reflux using various polarity solvents. The extracts were evaporated using rotary evaporator. Antioxidant activities using DPPH and phosphomolybdenum assays, determination of total phenolic, flavonoid, and carotenoid content were conducted by ultraviolet-visible spectrophotometry and its correlation with IC_{50} of DPPH and EC_{50} of phosphomolybdenum were analyzed by Pearson's method.

Results: The lowest IC_{50} of DPPH scavenging activity was given by n-hexane fruit extract of chayote from Lembang (9.32 µg/ml), while the lowest EC_{50} of phosphomolybdenum capacity was given by ethyl acetate fruit extract of chayote from Semarang (209.87 µg/ml). Ethyl acetate chayote fruit extract from Malang gave the highest phenolic content and its n-hexane extract had the highest total flavonoid. There were negative and significant correlation between total flavonoid content in all of the chayote fruit extracts from three different sites with their IC_{50} of DPPH and EC_{50} of phosphomolybdenum.

Conclusions: N-hexane chayote fruit extract from Lembang and Semarang and ethyl acetate chayote fruit extract from Semarang were categorized as a very strong antioxidant by DPPH method. Flavonoid compounds in all of the chayote fruit extracts from three different locations were the major contributor in their antioxidant activities by DPPH and phosphomolybdenum methods. All of the chayote fruit extracts from Lembang, Semarang, and Malang had a linear result in DPPH and phosphomolybdenum assays.

Keywords: Antioxidant, 2,2-diphenyl-1-picrylhydrazyl, Phosphomolybdenum, Chayote, Fruit, Different sites.

INTRODUCTION

The phenolic compound can be found in plants, and reported to have multiple biological effects including antioxidant and antibacterial activity [1-4]. Previous research reported that phenolic and flavonoid content could be correlated to their antioxidant activities [5-7]. Consumption of antioxidant can reduce the excessive of oxidative stress which can cause many diseases.

2,2-diphenyl-1-picrylhydrazyl (DPPH), cupric reducing antioxidant capacity (CUPRAC), ferric reducing antioxidant power (FRAP), and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS) can be used to determine antioxidant activity of fruits, vegetables, and food [1,2,8]. The previous studies [9-12] expressed that ABTS, DPPH, FRAP, CUPRAC, and phosphomolybdenum methods could be used to determine antioxidant activity in many plants extracts. The plants included chayote contained phenolic and flavonoid compounds which were phenolic compounds that can act as antioxidant and determination its antioxidant activities had been performed by DPPH, FRAP, and CUPRAC assays [11-13].

The objective of this research was to determine antioxidant activities of various polarities extracts (n-hexane, ethyl acetate, and ethanol) of fruit of chayote from three different sites in Indonesia using DPPH and phosphomolybdenum assays, and correlations of total phenolic, flavonoid and carotenoid content with their antioxidant capacities.

METHODS

Materials

DPPH, sodium phosphate, ammonium molybdate, gallic acid, quercetin, and beta-carotene were purchased from Sigma-Aldrich (MO, USA), chayote from three different sites, ethanol. All other reagents were analytical grades.

Preparation of sample

Fruit of chayote were collected from three different sites: Lembang - West Java namely as LEM, Semarang - Central Java as SEM, and Malang - East Java as MAL were thoroughly washed with tap water, wet sortation, cut, dried, and grinded into powder.

Extraction

About 300 g 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 using ethyl acetate. Therefore totally, the remaining residue was extracted three times using ethanol. So totally, there were nine extracts: Three n-hexane extracts (namely - LEM1, SEM1, and MAL1), three ethyl acetate extracts (LEM2, SEM2, and MAL2), and three ethanolic extracts (LEM3, SEM3, and MAL3).

Total phenolic content (TPC)

TPC determination was using the modified Folin-Ciolcalteu method from Pourmorad *et al.* [7]. The absorbance was measured at wavelength 765 nm. The analysis was performed in triplicate for each extract. Standard solution of gallic acid (105-200 µg/ml) was used to obtain a calibration curve. TPC was expressed as a percentage of total gallic acid equivalent per 100 g extract (g GAE/100 g).

Total flavonoid content (TFC)

Determination of TFC was adapted from Chang *et al.* [14]. The absorbance was read at wavelength 415 nm. The analysis was performed in triplicate for each extract. Standard solution of quercetin

(36-100 µg/ml) was used to obtain a calibration curve. The TFC was stated as percentage of total quercetin equivalent per 100 g extract (g QE/100 g).

Total carotenoid content (TCC)

TCC was done using modification method from Thaipong *et al.* [10]. Each extract was diluted in n-hexane. The absorbance was measured at wavelength 470 nm. The analysis was done in triplicate for each extract. Standard solution of beta-carotene (30-100 µg/ml) was used to obtain a calibration curve. The TCC was demonstrated as percentage of total beta-carotene equivalent per 100 g extract (g BE/100 g).

DPPH scavenging activity

The preparation of DPPH solution and determination of DPPH scavenging activity was performed by using modification method from Blois [15]. Various concentrations of each extract were pipetted into DPPH solution 50 µg/ml (volume 1:1) to initiate the reaction for creating a calibration curve. The absorbance was measured after 30 minutes incubation at wavelength 515 nm using ultraviolet (UV)-visible spectrophotometer Beckman Coulter DU 720. Methanol was used as a blank. DPPH solution 50 µg/ml was used as a control. Ascorbic acid was used as a standard. The analysis was conducted in triplicate for each extract and standard. Antioxidant activity of each extract by DPPH method was measured by determining 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.

Phosphomolybdenum capacity

Determination of phosphomolybdenum capacity was conducted by adapting method from Prieto *et al.* [9]. Various concentrations of each extract were pipetted into phosphomolybdenum reagent with of 50 µg/ml (volume 1:1) to initiate the reaction for figuring a calibration curve. Incubation was performed at 95°C for 90 minutes and cooled 20 minutes, and then, the absorbance was read at wavelength 695 nm using UV-visible spectrophotometer Beckman Coulter DU 720. Aquadest was used as a blank. Phosphomolybdenum solution 50 µg/ml which was incubated at 95°C for 90 minutes and cooled 20 minutes was used as control, while alpha-tocopherol incubated at 37°C for 90 minutes and cooled 20 minutes was used as a standard. The analysis was conducted in triplicate for each extract and standard. Antioxidant capacity of each extract was determined based on increasing in phosphomolybdenum complex absorbance by calculating percentage of antioxidant capacity. EC_{50} of phosphomolybdenum capacity of each extract can be calculated using its calibration curve.

Statistical analysis

Each sample analysis was conducted in triplicate. All of the presented results are means±standard deviation of at least three independent experiments. A statistical analysis using ANOVA with a statistical significance level set at $p<0.05$ and *post-hoc* Tukey procedure was conducted 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

TPC in various extracts of chayote

TPC among the various fruit extracts was demonstrated in term of GAE using the standard curve equation $y=0.005x-0.198$, $R^2=0.9971$. The TPC in various fruit extracts from three different sites of chayote exposed result in the range of 1.02-6.18 g GAE/100 g. The highest phenolic content (6.18 g GAE/100 g) was given by ethyl acetate chayote extract from Malang (MAL2) and the lowest for n-hexane chayote fruit extract from Semarang (SEM1) 1.02 g GAE/100 g (Fig. 1).

TFC in various extracts of chayote

TFC among the various extracts was exposed in term of QE using the standard curve equation $y=0.007x+0.001$, $R^2=0.9991$. The TFC in various fruit extracts from three different sites of chayote had different result ranged from 0.15 to 6.01g QE/100 g (Fig. 2). N-hexane chayote fruit extract from Malang (MAL1) had the highest total flavonoid content (6.01 g QE/100 g), and the lowest was given by ethanol chayote extract from Semarang (SEM3).

TCC in various extracts of chayote

TCC among the various extracts was revealed in term of beta-carotene equivalent using the standard curve equation $y=0.007x-0.002$, $R^2=0.9979$. The TCC in various fruit extracts from three different sites of chayote gave result in the range of 0.59-4.11 g BE/100 g (Fig. 3). N-hexane chayote fruit extract from Malang (MAL1) gave the highest

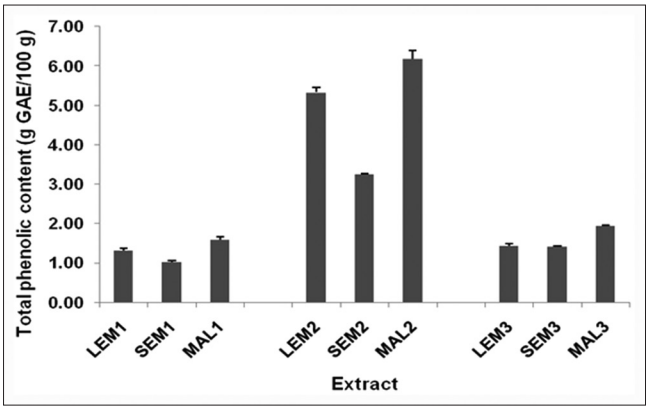

Fig. 1: Total phenolic content in fruit extracts from different sites of chayote, n=3

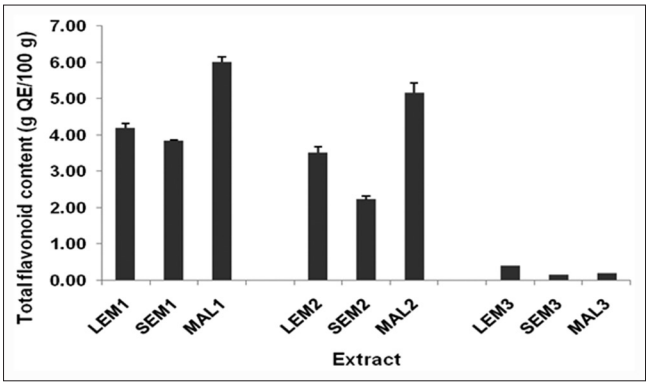

Fig. 2: Total flavonoid content in fruit extracts from different sites of chayote, n=3

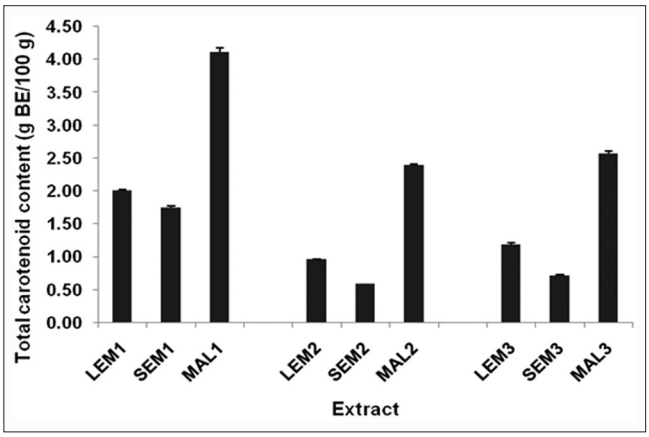

Fig. 3: Total carotenoid content in fruit extracts from different sites of chayote, n=3

carotenoid content (4.11 g BE/100 g), while the lowest carotenoid (0.59 g BE/100 g) for ethyl acetate extract of chayote from Semarang.

DPPH scavenging activity and phosphomolybdenum capacity

The IC_{50} of DPPH scavenging activities and EC_{50} of phosphomolybdenum capacity in various fruit extracts from three different sites of chayote by DPPH and phosphomolybdenum assays were shown in Figs. 4 and 5. IC_{50} of DPPH scavenging activities of each extract were compared to IC_{50} ascorbic acid and EC_{50} of phosphomolybdenum capacity compared to EC_{50} of alpha-tocopherol as standard. The lowest value of IC_{50} or EC_{50} of means had the highest antioxidant activity.

Correlations between total phenolic, flavonoid, carotenoid content in various extracts of chayote and IC_{50} of DPPH scavenging activities, EC_{50} of phosphomolybdenum capacity

Pearson's correlation coefficient between TFC in various extracts of chayote and their antioxidant activities exposed that TFC in all of extracts (LEM, SEM, and MAL) had negatively high correlation with their IC_{50} of DPPH scavenging activities ($r=-0.789$, $p<0.05$; $r=-0.904$; $r=-0.947$, $p<0.01$, respectively), and their EC_{50} of phosphomolybdenum capacity ($r=-0.988$; $r=-0.893$; $r=-0.989$, $p<0.01$). Only TCC in chayote extract from Lembang which had negative and high correlation with their IC_{50} DPPH scavenging activities ($r=-0.895$, $p<0.01$) (Table 1).

DISCUSSION

The previous research [13,17] reported that chayote had antioxidant capacity. There was no research regarding the antioxidant activity of various extracts (which were n-hexane, ethyl acetate, and ethanol) of chayote from three different sites using DPPH and phosphomolybdenum assays.

DPPH free radicals dissolve in methanol give absorption at wavelength 516 nm, while phosphomolybdenum complex dissolve in methanol has characteristic absorption at at wavelength 695 nm. The color of DPPH would be changed from purple to yellow color when the free radicals were scavenged by antioxidant [18]. Phosphomolybdenum reagent is sodium phosphate which was combined with ammonium molybdate in sulfuric acid solution. Color in phosphomolybdenum method would be changed from colorless to green-blue color. Intensity of green-blue color depends on amount of Mo (VI) that is reduced to Mo (V). Complex Mo (V) - sodium phosphate gives green-blue color and show characteristic absorption at wavelength 695 nm. The sample can act as antioxidant if it can reduce Mo (VI) to Mo (V), at the same time it will be oxidized. The reduction potential of Mo (VI)/Mo (V) is 0.43 V. Sample will act as antioxidant in phosphomolybdenum assay if sample had reduction potential lower than 0.43 V.

The phenolic content included phenolic acid can be correlated with antioxidant capacity [2,7,13,19]. In this study exposed that TPC in ethanolic fruit extract of chayote (*Sechium edule*) from different locations (Lembang, Semarang, and Malang) were 1.43, 1.41, 1.93 g GAE/100 g, respectively. It was similar to with the previous research [20] which showed that TPC in n-hexane, ethyl acetate and ethanolic fruit extracts of chayote from boyolali were 1.03, 3.21 and 0.88 g GAE/100 g, respectively, while ethanolic leaves extract of *S. edule* had higher TPC 3.05 g GAE/100 g compared to fruit 0.88 g GAE/100 g and pedicel 1.29 g GAE/100 g. Study by Ordonez *et al*., [13] regarding different extraction methods for leaves of chayote reported that ethanolic leaves extract of chayote by maceration gave higher TPC 1.16 mg/ml than decoction 0.91 mg/ml, while TPC in ethanolic stem extract of *S. edule* by maceration 0.25 mg/ml was similar with decoction 0.23 mg/ml. Polyphenolic content in acidified methanol leaves extract of the green one of the chayote 0.262 g GAE/100 g was higher than yellow one 0.063 g GAE/100 g [19]. The previous research [17] which studied regarding leaves extract of five species of Cucurbitaceae expressed that TPC in ethanolic leaves extract of *Momordica charantia* (0.36 g GAE/100 g) lower than TPC in ethanolic extract of *Cucumis sativus*,

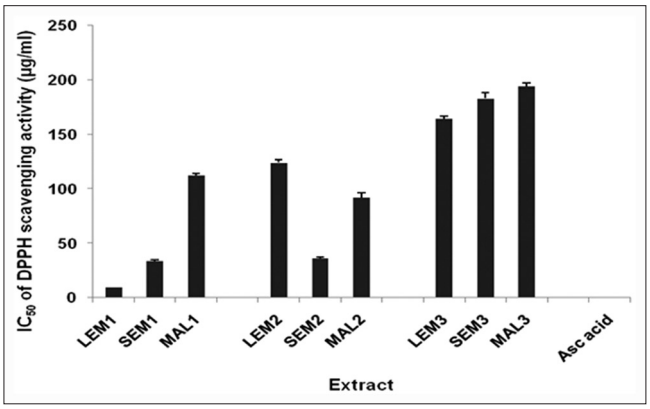

Fig. 4: IC_{50} of 2,2-diphenyl-1-picrylhydrazyl scavenging activities in various extracts of chayote, n=3

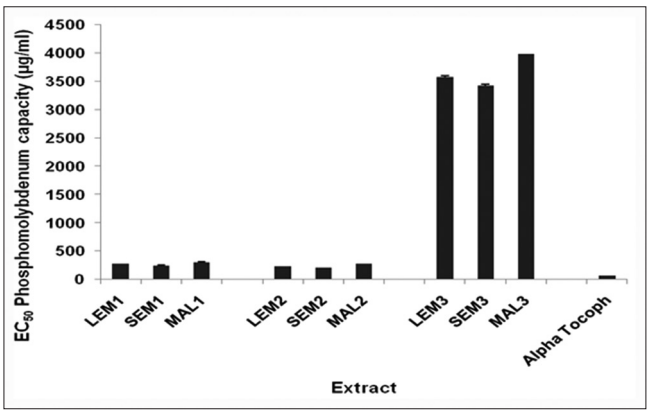

Fig. 5: EC_{50} of phosphomolybdenum capacity in various extracts of chayote, n=3

Table 1: Pearson's correlation coefficient of total phenolic, flavonoid, carotenoid content in various extracts of chayote with their IC_{50} of DPPH scavenging activities and EC_{50} of phosphomolybdenum capacity

Antioxidant activity	Pearson's correlation coefficient (r)					
	TPC	TFC	TCC	IC_{50} DPPH LEM	IC_{50} DPPH SEM	IC_{50} DPPH MAL
IC_{50} DPPH LEM	0.291 NS	−0.789 *	−0.895**			
IC_{50} DPPH SEM	−0.339 NS	−0.904 **	−0.442 NS			
IC_{50} DPPH MAL	−0.595 NS	−0.947 **	−0.246 NS			
EC_{50} phophomolybd LEM	−0.486 NS	−0.988**	−0.303 NS	0.694*		
EC_{50} phophomolybd SEM	−0.361 NS	−0.893**	−0.400 NS		0.999**	
EC_{50} phophomolybd MAL	−0.445 NS	−0.989**	−0.415 NS			0.982**

IC_{50} DPPH: IC_{50} DPPH scavenging activity, EC_{50} phosphomolybd: EC_{50} phosphomolybdenum capacity, LEM: Chayote from Lembang, SEM: Chayote from Semarang, MAL: Chayote from Malang, NS: Not significant, *: Significant at $p<0.05$, **: Significant at $p<0.01$, DPPH: 2,2-diphenyl-1-picrylhydrazyl, TPC: Total phenolic content, TFC: Total flavonoid content, TCC: Total carotenoid content

S. edule, Luffa acutangula, and *Cucurbita moschata* (2.47, 1.79, 2,88, and 1.43 g GAE/100 g, respectively).

In the previous research [20] exposed that ethanolic leaves extract of *S. edule* gave higher TFC (3.26 g QE/100 g) compared to its fruit extract and pedicel extract (0.35 and 0.31 g QE/100 g). It was similar to the present study which demonstrated that TFC in fruit extract of *S. edule* from three different locations (Lembang, Semarang, and Malang) were 0.39, 0.15 and 0.18 g QE/100 g, respectively. Ordonez *et al*., [13] stated that TFC in ethanolic leaves extract of *S. edule* which was extracted by maceration method (0.65 mg QE/ml) was higher than decoction method (0.20 mg QE/ml), while its stem extract had TFC 18 mg QE/ml and 0.07 mg QE/ml, respectively. The ethanolic leaves extract of *C. sativus*, *S. edule, L. acutangula, C. moschata* and *M. charantia* showed TFC 1.71, 5.47, 2.30, 1.59 and 0.77 g QE/100 g, respectively [17]. In the previous study by Chao *et al.* [19] expressed that the green one of *S. edule* gave TFC in acidified methanol leaves extract 0.42 g QE/100 g which was higher than in yellow one 0.18 g QE/100 g.

In the present research revealed that n-hexane fruit extracts of three different sites of *S. edule* had TCC 4.19, 3.85 and 6.01 g BE/100 g for Lembang, Semarang and Malang, respectively. It was contrast with the previous study which showed that TCC in n-hexane leaves extract (11.07 g BE/100 g) was higher than its fruit extract (0.44 g BE/100 g) and pedicel extract (2.45 g BE/100 g) [20]. Previous research [17] figured that TCC in n-hexane leaves extract of *C. sativus*, *S. edule, L. acutangula, C. moschata* and *M. charantia* were 4.56, 15.16, 13.19, 14.60 and 19.54 g BE/100 g, respectively, while its ethanolic extract gave TCC 0.04, 0.60, 0.09, 0.07 and 0.11 g BE/100 g, respectively.

The present study exhibited that IC_{50} of DPPH scavenging activities of various fruit extracts of *S. edule* from different locations in the range of 9.32-194.26 µg/ml. N-hexane extract and ethyl acetate fruit extract of *S. edule* from Semarang (SEM1 and SEM2) had IC_{50} of DPPH scavenging activity 33.61 and 35.99 µg/ml, which <50 µg/ml, so they could be categorized as a very strong antioxidant. Ascorbic acid standard had IC_{50} of DPPH scavenging activity 0.73 µg/ml. In the previous research [20] stated that IC_{50} of DPPH of ethyl acetate and ethanolic extract of leaves, fruit and pedicel were 5,1, 6,1, 1,3 µg/ml, respectively for ethyl acetate extract, while for ethanolic extract were 3.8, 34.7 and 45.7 µg/ml. Chao *et al*. [19] exposed that IC_{50} of DPPH of acidified methanol leaves extract of yellow one of *S. edule* 1503 µg/ml was lower than the green one 1801 µg/ml. Fidrianny *et al*. [17] reported that ethanolic leaves extract of *S. edule* from Garut and *L. acutangula* had IC_{50} of DPPH scavenging activity 94 and 73 µg/ml, which were classified as strong antioxidant. It was different to the previous study [20] which demonstrated that IC_{50} of DPPH of ethanolic leaves extract of *S. edule* from Boyolali was 3.8 µg/ml, which was categorized as a very strong antioxidant. Study by Ordonez *et al*. [13] expressed that ethanolic leaves and stem extract of *S. edule* which were extracted by maceration showed a higher percentage of DPPH scavenging activities (85% and 65%, respectively) than decoction method (80% and 30%, respectively). The ethyl acetate leaves extract of *S. edule* had the lowest EC_{50} of FRAP capacity 759 µg/ml compared to extracts of *C. sativus*, *L. acutangula, C. moschata,* and *M. charantia* [17].

This study revealed that the lowest EC_{50} of phosphomolybdenum capacity (209 µg/ml) among various fruit extracts of chayote (*S. edule*) from three different sites was given by ethyl acetate fruit extract of chayote from Semarang, while in the previous research [20] stated that ethyl acetate fruit extract of *S. edule* gave the lowest EC_{50} of CUPRAC capacity 147 µg/ml compared to ethyl acetate leaves extract (326 µg/ml) and ethyl acetate pedicel extract (227 µg/ml).

Phosphomolybdenum method which was used in the present study included reagent of sodium phosphate, ammonium molybdate, and sulfuric acid. In the first step of reaction will be produced 12-molybdophosphoric acid (Mo VI) [21]. Then, 12-molybdophosphoric acid (Mo VI) will be reduced to phosphomolydenum (Mo V) if there were antioxidant which can reduce Mo (VI) to Mo (V). The reduction potential of Mo VI/Mo V is 0.43 V, so antioxidant that can reduce Mo VI to Mo V should have reduction potential lower than 0.43 V. The absorbance of phosphomolybdenum will be measured at wavelength 695 nm. Increasing in amount of phosphomolybdenum which was produced will increase the intensity of green-blue color and also increase the absorbance.

In this study, the reagent of antioxidant and sample were prepared in the same volume 1:1. The reagent of DPPH and reagent of 12-molybdophosphoric acid were prepared in concentration of 50 µg/ml. In the first method (DPPH method) showed that 50 µg/ml free radical of DPPH was enough for serving complete reaction and sample could give lower IC_{50} of DPPH in the range of 1.3-157.3 µg/ml. However, in the second method (phosphomolybdenum method), sample had a high value of EC_{50} of phosphomolybdenum capacity ranged from 147 to 953 µg/ml. Based on the value of EC_{50} of phosphomolybdenum capacity, it can be supposed that the reagent of 12-molybdophosphoric acid 50 µg/ml was not enough to react with all of antioxidant in sample. Only a little antioxidant in sample reacted with the reagent and there were still most of antioxidant in sample which will oxidize again Mo V to Mo VI. This reaction will be repeated, between reduction from Mo VI to Mo V and then excessive of antioxidant in sample will oxidize again Mo V to Mo VI. Based on the explanation above it can be concluded that the high value of EC_{50} of phosphomolybdenum capacity because there were not enough amount of Mo VI (12-molybdophosphoric acid) and it means for completing reaction in phosphomolybdenum method it needed more than 50 µg/ml of 12-molybdophosphoric acid.

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 scavenging activity and EC_{50} of phosphomolybdenum capacity gave the highest antioxidant activity. Hence, negatively and high correlation will be given in good correlation between TPC, TFC and TCC with IC_{50} of DPPH or EC_{50} of phosphomolybdenum. It means increasing in TFC, TPC and TCC would increase antioxidant activities, which was figured by lower IC_{50} of DPPH scavenging activity and or EC_{50} of phosphomolybdenum capacity.

The IC_{50} of DPPH scavenging activities and EC_{50} of phosphomolybdenum capacity in various chayote fruit extracts from three different sites were shown in Figs. 1 and 2. The IC_{50} of DPPH scavenging activities and EC_{50} of phosphomolybdenum capacity in various fruit extracts were compared to IC_{50} of ascorbic acid standard. The lowest value of IC_{50} means had the highest antioxidant activity. 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 mg/ml was a medium antioxidant, while a weak antioxidant with IC_{50} or EC_{50} >150 µg/ml [15].

In Table 1, it can be seen that significantly negative correlation between TFC in all of fruits extracts of chayote (*S. edule*) from three different location with their IC_{50} of DPPH scavenging capacity ($r=-0.789$, $p<0.05$; $r=-0.904$; $r=-0.947$, $p<0.01$) and their EC_{50} of phosphomolybdenum capacity ($r=-0.988$; $r=-0.893$; $r=-0.989$, $p<0.01$). Based on the result above it can be predicted that antioxidant activity of fruit extract of chayote from Lembang, Semarang and Malang by DPPH and phosphomolydenum methods might be supposed by measuring its TFC. The previous study [20] demonstrated that there were a significant and negative correlation between TPC in leaves extract of *S. edule* with its IC_{50} of DPPH scavenging capacity ($r=-0.966$, $p<0.01$) and TPC in pedicel extract with its EC_{50} of CUPRAC capacity ($r=-0.831$, $p<0.01$). It could be supposed that antioxidant capacities in leaves extracts of *S. edule* with DPPH method and its pedicel extracts with CUPRAC method might be estimated indirectly by determining their TPC. The TPC of fruit and pedicel extracts of *S. edule* had high and significant correlation with their EC_{50} of CUPRAC capacity ($r=-0.705$, $p<0.05$, $r=-0.831$, $p<0.01$, respectively), also their TFC ($r=-0.994$, $r=-0.997$, $p<0.01$, respectively) and their TCC ($r=-0.997$, $r=-0.931$, $p<0.01$, respectively). It means that increasing in TPC and or TFC and or TCC in fruit and pedicel extracts

of *S. edule* would increase antioxidant activity by CUPRAC method. Based on this data, it can be reported that antioxidant capacity of leaves extract of *S. edule* by DPPH can be predicted indirectly by their TPC and antioxidant activity of fruit and pedicel extracts of *S. edule* by CUPRAC assay might be predicted indirectly by measuring their TFC and or TCC. Research by Fidrianny *et al*. [17] stated that TPC in leaves extract of *S. edule* had positively high correlation with their percentage of DPPH scavenging capacities (r=0.875, p<0.01).

Phenolic groups in plant included phenolic acid, flavonoid, tannins, qoumarine, and quinone. The previous study by Ragasa *et al*. [22] revealed that dichloromethane leaves extract of *S. edule* contained trans-cinnamic acid, phenylacetic acid, 3-octadecanoic acid, trinolenin and alpha-linolenic acid. Heim *et al*. [23] exposed that cinnamic acid had higher antioxidant activity compared to benzoic acid and phenylacetic acid. Trans-cinnamic acid, phenylacetic acid, 3-octadecanoic acid, trinolenin and alpha-linolenic acid soluble in n-hexane and ethyl acetate. In Fig. 1, it could be seen TPC in n-hexane fruit extract of chayote from Lembang (LEM1) 1.31 g GAE/100 g was similar to TPC in n-hexane fruit extract of chayote from Malang (MAL1) 1.58 g GAE/100 g, but IC_{50} of DPPH scavenging activity of LEM1 9.32 mg/ml, which was categorized as very strong antioxidant and IC_{50} of DPPH of MAL1 112.13 mg/ml as medium antioxidant. It can be predicted that LEM1 contained many cinnamic acid which had high antioxidant, while MAL1 contained many phenylacetic acid which had lower antioxidant than cinnamic acid. TPC in LEM1 (1.31 g GAE/100 g) was smaller than TPC in MAL2 (6.18 g GAE/100 g), but EC_{50} of phosphomolybdenum capacity of LEM1 (280.22 μg/ml) was similar to EC_{50} of phosphomolybdenum of MAL2 (285.45 μg/ml). It figured that many phenolic compounds in LEM1 have reduction potential lower than 0.43 V reduction potential of Mo VI/Mo V hence it can reduce Mo VI to Mo V, while in MAL2 only little phenolic compounds which have reduction potential lower than 0.43 V.

Flavonoid compound, which has OH in A ring and or B ring, will be included in phenolic groups. Flavonoid had higher antioxidant activity than phenolic acid [23]. Flavonoid which had ortho di OH at C-3', C-4', OH at C-3', oxo function at C-4', double bond at C-2', and C-3' have high antioxidant activity. The di OH with ortho position at C-3'-C-4' had the highest influence to antioxidant activity of flavonoid. The flavonoid glycosides would give lower antioxidant activity than flavonoid aglycones [23]. The previous study [24] stated that different organ of *S. edule* (leaves, roots and stems) contained apigenin and luteolin. Different organ of one plant might be contained the similar component, hence the fruit of *S. edule* which was used in the present study also contained the same component. It could be seen in Fig. 2 that TFC ethyl acetate fruit extract of chayote from Lembang LEM2 (3.52 g QE/100 g) was higher than TFC in ethyl acetate fruit extract of chayote from Semarang SEM2 (2.22 g QE/100 g), but IC_{50} of DPPH scavenging activity of SEM2 (35.99 μg/ml) which was classified as very strong antioxidant and IC_{50} of DPPH of LEM 2 (124.04 μg/ml) as medium antioxidant. Luteolin has di ortho OH at C-3'-C4', double bond at C-2' and C-3', oxo function at C4, OH at C-5' and C-7', while apigenin has the same OH and function group with luteolin, except OH at C-4'. Based on the explanation regarding flavonoid above, it can be seen that luteolin has higher antioxidant activity than apigenin. Luteolin has ortho di OH at C-3'-C4' which will give the highest antioxidant activity, so it can be predicted that SEM2 contained many luteolin, while LEM2 contained many apigenin. Increasing in antioxidant activity which be shown by lower IC_{50} of DPPH scavenging activity will be given by increasing in lipophilicity of carotenoid [25]. Foote [26] stated that carotenoid has antioxidant capacity by scavenging free radical. Beta-carotene was used as standard because of it has conjugation double bonds which have ability to scavenge free radicals [27]. More double bonds in carotenoid will give higher scavenging free radical activity. Previous research by Beutner *et al*. [28] revealed that carotenoid would give higher scavenging radical activity if contain greater than 7 double bonds. The highest TCC was given by n-hexane chayote fruit extract from Malang (MAL1) 4.11 g BE/100 g, while ethyl acetate chayote fruit extract of from Semarang (SEM2) gave the lowest TCC (0.59 g BE/100 g), but IC_{50} of DPPH scavenging activities of SEM2 (35.99 μg/ml) which was categorized as very strong antioxidant, was smaller than IC_{50} of DPPH of MAL1 (112.13 μg/ml) as medium antioxidant, it means antioxidant activity of SEM2 was greater than MAL1. It might be supposed that SEM2 consisted of many carotenoid compounds with more than seven double bonds which had high antioxidant activities, whereas MAL1 contained many carotenoid compounds with maximum 7 double bonds.

Correlation between two antioxidant methods which were used in the present study was evaluated by Pearson's correlation. The good correlation between two methods if there was a positive and significant correlation between one method to the other method. There were positively and significant correlation between IC_{50} of DPPH and EC_{50} of phosphomolybdenum in all of the chayote fruit extracts sample (Lembang r=0.694, p<0.05; Semarang r=0.999 and Malang r=0.982, p<0.01). It could be seen that IC_{50} of DPPH scavenging activities in chayote fruit extracts from three different sites were linear with their EC_{50} of phosphomolybdenum capacity.

CONCLUSION

Antioxidant activity of sample should be determined by different methods in parallel because various methods could give different results. N-hexane fruit extract from Lembang (LEM1) and Semarang (SEM1), ethyl acetate fruit extract from Semarang (SEM2) were categorized as very strong antioxidant by DPPH method. TFC in all of the chayote fruit extracts from three different sites had a negative and significant correlation with their IC_{50} of DPPH scavenging activities and EC_{50} of phosphomolybdenum capacity. Antioxidant activity by DPPH and phosphomolybdenum methods of chayote fruit extract from Lembang, Semarang and Malang indirectly can be estimated using its TFC data. Flavonoid compounds in fruit extracts from three different sites were the major contributor in their IC_{50} of DPPH and EC_{50} of phosphomolybdenum capacity. DPPH and phosphomolybdenum assays showed linear result for chayote fruit extracts from Lembang, Semarang, and Malang. Fruit of chayote may be exploited as sources of natural antioxidant.

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