



# Phytochemical and Antioxidant Profile: Cucumber Pulp and Leaves Extracts

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## Abstract

**BACKGROUND:** Many vegetables and fruits have been shown to be sources of antioxidant such as lemons, apples, cabbage, mangoes, beets, and guavas.

**AIM:** This research aimed to determine the antioxidant activity of *Cucumis sativus* L. (cucumber) pulp and leaves extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and cupric reducing antioxidant capacity (CUPRAC) methods, total phenolic content (TPC), total flavonoid content (TFC), correlation of TPC and TFC on antioxidant activity, correlation between the two methods, identification of marker, and total marker content.

**METHODS:** Antioxidant activity was examined by determining IC<sub>50</sub> and AAI of DPPH and EC<sub>50</sub> and AAI of CUPRAC. TFC and TPC were measured using UV-visible spectrophotometer. Correlation of TPC and TFC on antioxidant activity was analyzed by Pearson's method.

**RESULTS:** The AAI of DPPH cucumber pulp and leaves extracts were in the range of 0.22 - 2.18, while AAI of CUPRAC were 0.07 - 0.95. All extracts showed antioxidant activity. Ethyl acetate cucumber pulp extract had highest antioxidant by DPPH assay, whereas n-hexane cucumber leaves extract had highest antioxidant activity by CUPRAC assay. Ethyl acetate cucumber leaves extract had highest TFC value (21.47 g QE/100 g) and TPC value (2.34 g GAE/100 g). Flavonoids in cucumber pulp extract contributed to antioxidant activity of CUPRAC method and phenolic compounds in cucumber pulp extract gave a contribution to antioxidant activity of DPPH method. Quercetin content as marker in ethanol cucumber pulp extract was 0.00114%. AAI CUPRAC and DPPH of cucumber leaves extract showed positive correlation but not significant.

**CONCLUSION:** Antioxidant activity between CUPRAC and DPPH methods on cucumber extracts was not linear.

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## Introduction

Accumulation of free radicals causes a condition called oxidative stress [1]. Oxidative stress is an unbalance number between free radicals in the body and the ability of biological systems to neutralize them. High level of free radicals causes chain reaction which can cause cell damage [2]. Cell damage triggers the development of cancer, autoimmune, and cardiovascular disease [1]. The body has mechanisms to fight oxidative stress by producing antioxidant. The antioxidant naturally produced in the body is called endogenous antioxidants. Antioxidant also supplied externally through food [1]. Endogenous antioxidants produced on the body are limited, so antioxidants from external are needed.

Many vegetables and fruits have been shown to be sources of antioxidant such as lemons, apples, cabbage, green chilies, mangoes, beets, bell peppers, and guavas [3]. One of the plants that are thought to have the potential to be a source of antioxidant is *Cucumis sativus* L. (cucumber). Cucumber fruits are often consumed by Indonesian people. Based on previous research, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to measure the antioxidant

activity of cucumber. The results stated that 500 µg/mL cucumber fruit extract has DPPH scavenging activity with 56.15 ± 2.32% inhibition [4]. If a part of the plant has an antioxidant effect, then other parts of the plant can be suspected of having the potential to have an antioxidant effect as well because there is a possibility that it has the same chemical content. Therefore, in this research, the antioxidant activity was tested on the leaves and pulp of cucumber. The test was carried out using DPPH and cupric reducing antioxidant capacity (CUPRAC) method accompanied by the determination of total phenolic content (TPC) and total flavonoid content (TFC), correlation of TPC and TFC on antioxidant activity, the correlation between the two test methods, identification of marker compound, and determination of total marker content.

## Materials and Methods

### Preparation of sample

Leaves and pulp of *C. sativus* were collected from EcoCamp Garden Ciburial, Cimenyan District,

Bandung City, West Java, Indonesia. Before being made into powder, the ingredients were sorted first. The making of crude drug powder begins with cutting the material so that it becomes smaller then dried in the oven. After that, the materials were grinded into powder. The powder obtained was stored in dry containers [5].

### **Extraction**

Powdered sample was extracted using reflux method. Reflux was done using three solvents with various polarities, namely, n-hexane, ethyl acetate, and ethanol. The extraction of powdered sample was carried out for 2 h after the solvent boiled. Extraction was done three times for each solvent. The extract obtained was stored then concentrated by a rotary evaporator [5].

### **IC<sub>50</sub> and AAI of DPPH scavenging activity**

The determination of IC<sub>50</sub> DPPH was done using a standard ascorbic acid solution, 50 µg/mL DPPH control solution, and pro-analytical methanol as a blank. Ascorbic acid solution in pro-analytical methanol was prepared in various concentrations. A total of 1 mL of ascorbic acid was added with 1 mL of DPPH 50 µg/mL and then incubated for 30 min in a brown vial. Its absorbance was measured by UV–visible spectrophotometer at a wavelength of 517 nm. The extract was given the same treatment as the ascorbic acid. Each measurement was carried out 3 times. The reduction of DPPH absorbance was calculated as a percent decrease in DPPH absorbance after addition of extract [6]. Through the calibration curve of each sample, the regression equation was determined. IC<sub>50</sub> value was calculated using the regression equation. The determination of AAI value was done by dividing the final concentration of DPPH by the calculated IC<sub>50</sub> value.

### **EC<sub>50</sub> and AAI of CUPRAC**

The determination of EC<sub>50</sub> CUPRAC was done using ascorbic acid standard solution, CUPRAC control solution, and ammonium acetate buffer as a blank. The CUPRAC control solution was diluted to 100 µg/mL using ammonium acetate buffer with a pH of 7. Ascorbic acid in pro-analytical methanol was prepared in various concentrations. One milliliter of ascorbic acid was added with 1 mL of CUPRAC solution, then 30 min incubation was performed. Its absorbance was measured using a UV–visible spectrophotometer at a wavelength of 450 nm. The extract was given the same treatment as the ascorbic acid. Each measurement was carried out 3 times. CUPRAC capacity was calculated as a percent increase in CUPRAC absorption after addition of extract [7]. Through the calibration curve of

each sample, the regression equation was determined. The EC<sub>50</sub> value was calculated using the regression equation. The determination of AAI value was done by dividing the final concentration of the CUPRAC solution by the calculated EC<sub>50</sub> value [6].

### **TFC**

The standard solution used in the determination of TFC is quercetin solution. Quercetin solutions were prepared in the concentration range of 30–120 µg/mL to obtain a standard calibration curve. A half milliliter of quercetin solution was added with 1.5 mL of methanol, 0.1 mL of 1 M sodium acetate, and 0.1 mL of 10% aluminum (III) chloride, then 2.8 mL of distilled water was added, then incubation 30 min was conducted. The extract in pro-analytical methanol and pro-analytical methanol as a blank was given the same treatment as quercetin. The absorbance of these solutions was measured at a wavelength of 415 nm using UV–visible spectrophotometer. Extract absorbance measurements were carried out 3 times for each extract. The obtained quercetin calibration curve was used to determine the regression equation. TFC was calculated using the regression equation and expressed in grams of quercetin equivalent per 100 grams extract (g QE/100 g) [8].

### **TPC**

The standard solution used in the determination of TPC is gallic acid solution. Gallic acid solution was prepared in the concentration range of 40–160 µg/mL to obtain a standard calibration curve. A half milliliter of gallic acid which had been dissolved in pro-analytical methanol was added to 5 mL of 10% Folin–Ciocalteu reagent which had been mixed with 4 mL of 1 M Na<sub>2</sub>CO<sub>3</sub>. This mixture was incubated for 15 min. The extract in the pro-analytical methanol and pro-analytical methanol as a blank was given the same treatment as gallic acid. The absorbance of these solutions was determined using UV–visible spectrophotometer at a wavelength of 765 nm. Absorbance measurements were carried out three times for each extract. The obtained gallic acid calibration curve was used to determine the regression equation. TPC in the extract was calculated using the regression equation and expressed in grams of gallic acid equivalent per 100 grams extract (g GAE/100 g) [9].

### **Correlation between various extracts of cucumber pulp and leaves**

Correlations between various extracts of cucumber pulp and leaves were measured using IBM SPSS Statistics 35. This analysis used one-way ANOVA method. The statistical level was set to p<0.05 and using the *post hoc* Tukey procedure [5].

### Correlation of TPC and TFC on antioxidant activity

The correlation of phenolic and flavonoid content to antioxidant activity was measured statistically using IBM SPSS Statistics 25. The analysis of the results was carried out using bivariate method. Correlation analysis between antioxidant activity assays was performed applying the Pearson's method [5].

### Identification marker of the ethanol extract of cucumber pulp

Identification marker of the ethanol extract of cucumber pulp was carried out using the HPLC method. The HPLC used was HPLC-20AD with 0.01% H<sub>3</sub>PO<sub>4</sub> (eluent A) and methanol (eluent B) as mobile phases. This HPLC separation system was linear gradient 40%–60% eluent B for 5 min, then gradient eluent B 70% for 5 min, and gradient eluent B 40% until 15 min. The stationary phase used was LiChrospher® 100 RP-C18 5 µm (length 100 mm, diameter 4 mm, 20 mm per column [Merck]). This HPLC flow rate was 1 mL/min using a CTO-20A pump, Shimadzu, Japan. Injection volume in this HPLC system was 20 µL. The quercetin used was 1 µg/mL. Ethanol cucumber pulp extract was prepared at 10,000 µg/mL. HPLC column temperature was 30°C. To identify marker compound in cucumber pulp, retention time of five standard compounds (luteolin 7-O-glucoside, rutin, quercetin, kaempferol, and apigenin) were determined. After that, ethanol cucumber pulp extract was injected into the HPLC system; then, the retention time and AUC were observed. The retention time of cucumber pulp extract was compared with the retention time of five standards to find out the marker compound.

### Total marker content

Total marker content of ethanol cucumber pulp extract was measured using HPLC. The AUC value of ethanol cucumber pulp extract was compared with the AUC value of standard compound. Total marker content in ethanol cucumber pulp extract was calculated by the following equation:

$$\frac{AUC_{\text{extract}}}{AUC_{\text{control}}} \times \frac{\text{Final Concentration}_{\text{control}}}{\text{Final Concentration}_{\text{extract}}} \times 100\%$$

## Results

IC<sub>50</sub> of DPPH of n-hexane cucumber pulp extract (NH1), n-hexane extract cucumber leaves (NH2), ethyl acetate cucumber pulp extract (EA1), ethyl acetate cucumber leaves extract (EA2), ethanol cucumber pulp extract (ET1), and ethanol cucumber

leaves extract (ET2) was compared to the standard, IC<sub>50</sub> of ascorbic acid. IC<sub>50</sub> of DPPH scavenging activity data in various extracts of cucumber pulp and leaves is exposed in Figure 1. The extract with a lower IC<sub>50</sub> value has a higher antioxidant activity. In this experiment, ethyl acetate cucumber pulp extract had the highest antioxidant activity with IC<sub>50</sub> value 11.52 µg/mL. AAI of DPPH in various extracts of cucumber pulp and leaves is given in Figure 2. EA1 had the highest antioxidant activity with AAI value 2.18.

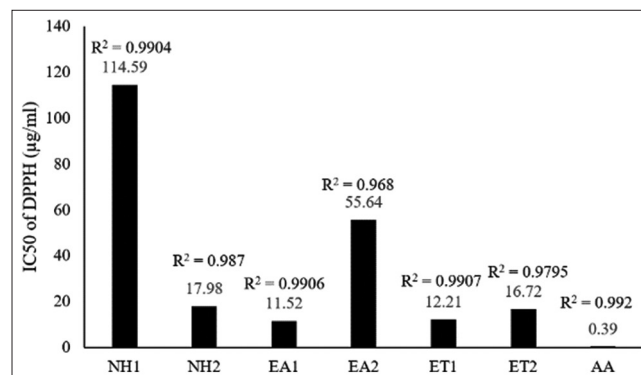


Figure 1: IC<sub>50</sub> DPPH of cucumber pulp and leaves extracts

EC<sub>50</sub> of CUPRAC capacity measured from NH1, NH2, EA1, EA2, ET1, and ET2 was compared to EC<sub>50</sub> of ascorbic acid as a standard. EC<sub>50</sub> of CUPRAC capacities in various extracts of cucumber pulp and leaves is shown in Figure 3. The extract with a lower EC<sub>50</sub> value had a higher antioxidant activity. In this experiment, n-hexane cucumber leaves extract had the highest antioxidant activity with EC<sub>50</sub> value 52.91 µg mL. AAI of CUPRAC in various extracts of cucumber pulp and leaves is expressed in Figure 4. NH1 had the highest antioxidant activity with AAI value 0.95.

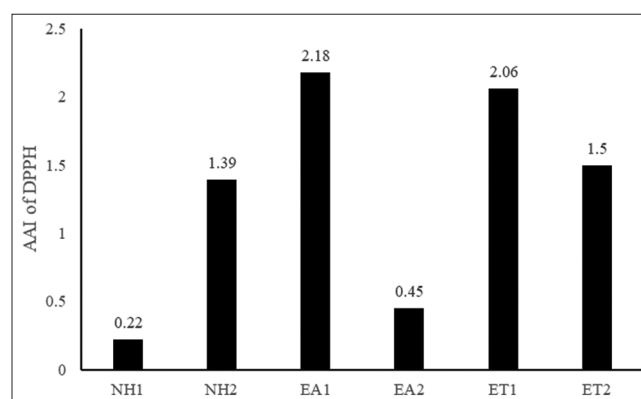


Figure 2: AAI DPPH of cucumber pulp and leaves extracts antioxidant activity with AAI value 0.95.

TFC from NH1, NH2, EA1, EA2, ET1, and ET2 was measured using quercetin as a standard with calibration curve regression equation  $y = 0.00655x + 0.0232$ ,  $R^2 = 0.9961$  and expressed in gram quercetin equivalent per 100 g extract. TFC in various extracts of cucumber pulp and leaves is exposed in Table 1. In this experiment, ethyl acetate cucumber leaves extract showed the highest TFC (21.47 g QE/100 g extract).

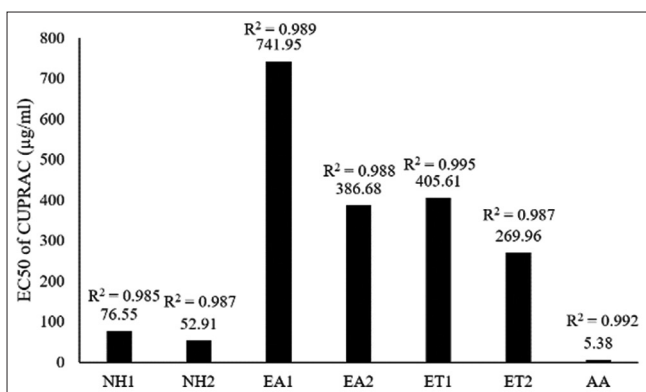


Figure 3: EC<sub>50</sub> CUPRAC of cucumber pulp and leaves extracts

TPC from NH1, NH2, EA1, EA2, ET1, and ET2 was measured using gallic acid as a standard with calibration curve regression equation  $y = 0.0053x + 0.0368$ ,  $R^2 = 0.9989$  and expressed in gram gallic acid equivalent per 100 g extract.

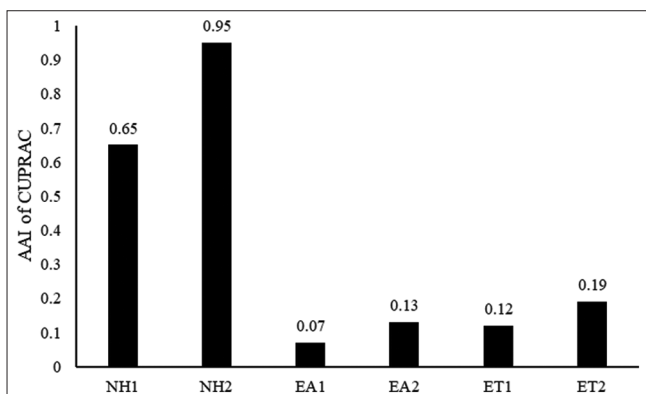


Figure 4: AAI CUPRAC of cucumber pulp and leaves extracts

TPC in various extracts of cucumber pulp and leaves is presented in Table 2. In this experiment, ethyl acetate cucumber leaves extract showed the highest TPC (2.34 g GAE/100 g extract).

Table 1: TFC in various extracts of cucumber pulp and leaves

Extract	TFC (g QE/100 g extract)	
	Pulp	Leaves
n-Hexane	7.41 ± 0.07 <sup>a</sup>	9.04 ± 0.60 <sup>a</sup>
Ethyl acetate	3.11 ± 0.24 <sup>b</sup>	21.47 ± 1.41 <sup>b</sup>
Ethanol	0.77 ± 0.78 <sup>c</sup>	1.46 ± 0.05 <sup>c</sup>

Correlation between TFC and TPC with AAI of DPPH and AAI CUPRAC was determined statistically using a software called IBM SPSS Statistics 25. The results are expressed in Table 3.

Table 2: TPC in various extracts of cucumber pulp and leaves

Extract	TPC (g QE/100 g extract)	
	Pulp	Leaves
n-Hexane	0.64 ± 0.05 <sup>a</sup>	0.33 ± 0.04 <sup>a</sup>
Ethyl acetate	2.08 ± 0.12 <sup>b</sup>	2.34 ± 0.11 <sup>b</sup>
Ethanol	1.75 ± 0.33 <sup>b</sup>	1.67 ± 0.07 <sup>c</sup>

Correlation between AAI of DPPH and AAI CUPRAC was also determined statistically using a software called IBM SPSS Statistics 25. The results are represented in Table 4.

Retention time of five standards (luteolin 7-O-glucoside, rutin, quercetin, kaempferol, and

Table 3: Correlation between TFC and TPC with AAI of DPPH and AAI CUPRAC

Parameters	Pearson's correlation coefficient (r)	
	TFC	TPC
AAI DPPH pulp	-0.904**	0.972**
AAI DPPH leaves	-0.945**	-0.682*
AAI CUPRAC pulp	0.904**	-0.389 <sup>ns</sup>
AAI CUPRAC leaves	-0.197 <sup>ns</sup>	-0.959**

ns: Not significant, \*significant at the 0.05 level, \*\*significant at the 0.01 level

apigenin) for identification and total marker content of the ethanol extract of cucumber pulp determination were exposed on the chromatogram, as shown in Figure 5 and Table 5.

Quercetin content in ethanol cucumber pulp extract was calculated by the following calculation:

$$(8.199/71.801) \times (1 \mu\text{g/mL}/10000 \mu\text{g/mL}) \times 100\% = 0.00114\%$$

Table 4: Correlation between AAI of DPPH and AAI CUPRAC

Parameters	Pearson's correlation coefficient (r)	
	AAI DPPH pulp	AAI DPPH leaves
AAI CUPRAC pulp	-0.991**	
AAI CUPRAC leaves		0.470 <sup>ns</sup>

ns: Not significant, \*significant at the 0.05 level, \*\*significant at the 0.01 level

## Discussion

Free radicals are chemically reactive species that contains one or more unpaired electrons [10]. Free radicals are unstable and short-lived [11]. Unpaired electrons in free radicals can be formed from non-radical compound that loses one electron or gain one electron [12]. Free radicals in the body come from metabolism and from external influences such as pollution, radiation, certain drugs, and cigarette smoke. Free radicals are useful for the body's defense system, but high level of free radicals in the body can be dangerous [12]. If the number of free radicals in the body is excessive, the body is unable to neutralize them. Excessive amounts of free radicals in the body can cause oxidative stress condition [1]. Unpaired electrons in free radicals pass through the cell structure, oxidation will occur in cell components and molecules related to protein, lipid, and DNA so that it can cause cell

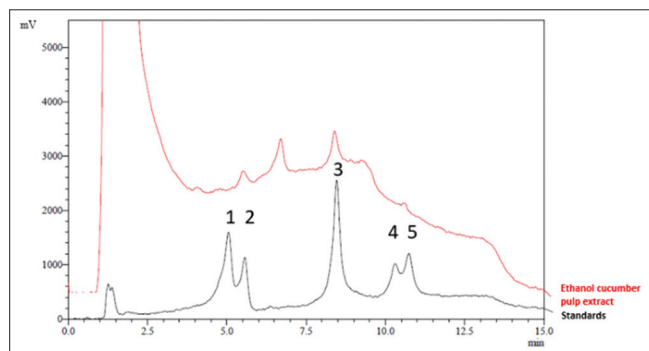


Figure 5: HPLC chromatogram for marker identification. 1: Luteolin-7-O-glucoside, 2: Rutin, 3: Quercetin, 4: Kaempferol, and 5: Apigenin

damage [13]. This cell damage triggers aging and the development of several diseases such as autoimmune, cardiovascular disease, and cancer [1].

Antioxidants are compounds that can inhibit free radicals in the body [12]. Inhibition of free radicals can prevent cell damage that triggers aging and the development of disease. Antioxidants can be produced naturally as a result of body metabolism (endogenous antioxidants) or externally (exogenous antioxidants). Endogenous antioxidants produced on the body are limited, so antioxidants from external are needed.

**Table 5: Retention time and AUC data for marker identification**

Standard (1 µg/mL)	Control peak		Sample peak	
	Retention time (min)	AUC	Retention time (min)	AUC
Luteolin 7-O-glucoside	4.958	26419	-	-
Rutin	5.466	22.291	5.511	4651
Quercetin	8.345	71801	8.399	8199
Kaempferol	10.109	13555	-	-
Apigenin	10.564	15124	-	-

DPPH assay is commonly practiced as the antioxidant activity assessment [14]. DPPH is a free radical that has good stability because it undergoes delocalization of electrons throughout the molecule so that the molecule does not dimerize like other free radicals. Electron delocalization on DPPH showed a dark purple adsorption in ethanol at a wavelength of 517 nm. When the DPPH solution is mixed with a substrate (antioxidant) that can donate a hydrogen atom, a reduced form will be produced which is indicated by the loss of the purple color. IC<sub>50</sub> value is evaluated applying calibration curve regression equation. The inhibitory activity can be calculated using the following equation: % inhibitory =  $([A_0 - A_1]/A_0) \times 100\%$ . A<sub>0</sub> is the absorbance of control solution and A<sub>1</sub> is the absorbance of sample. The inhibitory concentration of the sample in scavenging 50% free radicals in this DPPH method is called IC<sub>50</sub>. The IC<sub>50</sub> value is inversely proportional to the antioxidant activity. If the IC<sub>50</sub> value is getting lower, it means the antioxidant activity higher. AAI was investigated by dividing the final concentration of DPPH with calculated IC<sub>50</sub> value. Antioxidant activity can be categorized very strong AAI > 2, strong 1 ≤ AAI ≤ 2, medium 0.5 ≤ AAI < 1, and weak AAI < 0.5 [15].

CUPRAC assay is a method to determine antioxidant capacity of sample. In CUPRAC method, bis(neocuproine)copper (II) chloride [Cu (II)-Nc] as the chromogenic oxidant reacts with polyphenols [Ar(OH)<sub>n</sub>]. Free protons are supported with ammonium acetate buffer solution. The Ar-H groups from the polyphenols are oxidized to quinones and Cu (II)-Nc is reduced to a colored Cu(I)-Nc chelate, showing a maximum absorption at λ 450 nm in this reaction [16]. Concentration of sample or standard which can exhibit 50% of CUPRAC capacity is called exhibitory concentration 50% (EC<sub>50</sub>). The lower EC<sub>50</sub> value means a higher antioxidant activity. AAI value was calculated by dividing the final concentration of CUPRAC with EC<sub>50</sub> value. According to the classification [15], antioxidant activity was considered as a very strong antioxidant if AAI > 2.

In the present research, IC<sub>50</sub> of DPPH measured from different extract from cucumber pulp and leaves was ranged from 11.52 to 114.59 µg/mL, while standard solution ascorbic acid IC<sub>50</sub> value was 0.39 µg/mL. NH1 had the highest IC<sub>50</sub> value, which means that NH1 had the lowest antioxidant activity, whereas EA1 showed the lowest IC<sub>50</sub> value, which expresses that EA1 had the strongest antioxidant activity among the other extracts. AAI value is inversely proportional to the IC<sub>50</sub> value. If AAI value is getting higher, it means the antioxidant activity higher. AAI of DPPH calculated from IC<sub>50</sub> of different extracts from cucumber pulp and leaves were ranged from 0.22 to 2.18. EA1 had the highest AAI value among the other extracts, it means that EA1 also gave the highest antioxidant activity among the other extracts.

In the previous research [17], cucumber pulp water extract was tested by DPPH assay and used butylated hydroxytoluene as the standard for its antioxidant activity. This research found that cucumber fruit had a high antioxidant activity with IC<sub>50</sub> value 14.73 ± 1.42 µg/mL. The result was similar with this research, cucumber pulp extract was considered as a very strong antioxidant. Cucumber leaves also were previously examined by DPPH assay for their antioxidant activity. The result showed that cucumber leaves have a high antioxidant activity with IC<sub>50</sub> value 13.06 µg/mL [18]. The result was different from the present research, cucumber leaves extract was only considered as a strong antioxidant.

EC<sub>50</sub> of CUPRAC measured from different extract from cucumber pulp and leaves was ranged from 52.91 to 741.95 µg/mL, while standard solution ascorbic acid EC<sub>50</sub> value was 5.38 µg/mL. NH2 had the lowest EC<sub>50</sub> value, which means that NH2 showed the highest antioxidant activity among the other extracts. EC<sub>50</sub> of NH2 was among 50–100 µg/mL, so NH2 was considered as strong antioxidant. AAI CUPRAC of cucumber pulp and leaves extracts was ranged from 0.07 to 0.95. NH<sub>2</sub> exposed the highest AAI value among the other extracts, it means based on this assay, NH<sub>2</sub> had the highest antioxidant activity among the other extracts.

The previous research [19] measured the antioxidant activity using FRAP method and reported that percentage of FRAP capacity from methanolic cucumber leaves extract was 1.63%. On the other hand, other plants from the Cucumis genus *Cucumis melo* (cantaloupe) also showed good antioxidant activity. Other research [20] reported that IC<sub>50</sub> of DPPH from methanolic cantaloupe flesh extract was 11.9 ± 1.00 µg/mL and methanolic cantaloupe leaves extract was 1.52 ± 0.01 µg/mL.

There might be correlation between antioxidant activity with TPC and TFC [21]. In the previous research, measurements of TPC and TFC of aqueous cucumber pulp extract have also been examined. The results showed that the TFC of aqueous cucumber pulp

extract was  $9.33 \pm 0.33$  mg QE/g extract and the TPC of aqueous cucumber pulp extract  $40.68 \pm 2.9$  mg GAE/g extract [22]. The other research [23] reported that TFC value in methanolic cucumber pulp extract was  $12 \pm 1.41$  mg QE/g extract and in petroleum ether cucumber pulp extract  $62.5 \pm 0.71$  mg QE/g extract, whereas the TPC value in methanolic cucumber pulp extract was  $23.75 \pm 6.19$  mg GAE/g extract and in petroleum ether cucumber pulp extract  $16.25 \pm 0.88$  mg GAE/g extract. It has also been found that the TFC value of ethanolic cucumber peel extract was  $14.02$  mg QE/g extract and the TPC value of ethanolic cucumber peel extract was  $23.08$  mg GAE/g extract [24].

The TFC of the previous results was different with the result of the present research. In present research, we reported that the TFC of cucumber pulp and leaves extracts were varied from  $0.77$  to  $21.47$  g QE/100 g. The result in the present research was higher than the previous study. EA2 had the highest TFC value ( $21.47$  g QE/100 g). Using the one-way ANOVA method, TFC value of n-hexane extract, ethyl acetate extract, and ethanol extract of cucumber pulp showed significant differences at  $p < 0.05$ . TFC value of n-hexane extract, ethyl acetate extract, and ethanol extract of cucumber leaves also showed significant differences ( $p < 0.05$ ). TFC value of cucumber pulp and leaves extracts is given in Table 1.

The TPC of the previous results was also different with the present research. In the present research, we reported that the TPC of cucumber pulp and leaves extracts were in the range of  $0.33 - 2.34$  g GAE/100 g. The result in the present research was higher than the previous research. EA2 had the highest TPC value ( $2.34$  g GAE/100 g). There was no significant different between TPC value of ethanol extract and ethyl acetate extract of cucumber pulp. But both extracts showed significant difference with n-hexane extract ( $p < 0.05$ ) using one way ANOVA method. TPC value of n-hexane, ethyl acetate, and ethanol cucumber leaves extract had significant difference ( $p < 0.05$ ). TPC value in cucumber pulp and leaves extracts is exhibited in Table 2.

Antioxidant activity can be proportional to the concentration of phenolic compounds in the sample. Flavonoids are phenolic compound because flavonoids have phenolic group in their chemical structure. Phenolic compounds have an ability to donate electrons and delocalizing unpaired electrons within aromatic structure [21].

TPC and TFC affect the antioxidant activity if Pearson's correlation coefficient value is positive and significant at  $p < 0.01$  and  $p < 0.05$  [25]. According to Table 3, TFC has positive Pearson's correlation coefficient and significant at  $p < 0.01$  with AAI CUPRAC of cucumber pulp extract. TPC also had positive Pearson's correlation coefficient and significant at  $p < 0.01$  with AAI DPPH of cucumber pulp extract. Therefore, it can be stated that flavonoids in cucumber pulp extract

contributed to antioxidant activity of CUPRAC method and phenolic compounds in cucumber pulp extract contributed to the antioxidant activity of DPPH method.

According to Table 4, AAI between CUPRAC and DPPH methods for cucumber leaves extract showed positive but not significant correlation. Therefore, the results between CUPRAC and DPPH methods were not linear.

In this study, marker identification was also examined on the ethanol cucumber pulp extract. The HPLC chromatogram results of ethanol cucumber pulp extract showed peaks at retention times of  $5.511$  and  $8.399$  min, which means that there were rutin and quercetin contained in the extract. The quercetin peak exposed higher AUC value than the other peaks, so it can be stated that quercetin is a marker compound of ethanol cucumber pulp extract.

Marker content in ethanol cucumber pulp extract was calculated using the AUC value quercetin in extract and AUC value quercetin standard in chromatogram. According to the calculation in results section, quercetin content in ethanol cucumber pulp extract was  $0.00114\%$ .

## Conclusion

AAI of DPPH of different extracts from cucumber pulp and leaves was ranged from  $0.22$  to  $2.18$ , whereas AAI of CUPRAC in the range of  $0.07-0.95$ . Ethyl acetate cucumber leaves extract had the highest TFC value ( $21.47$  g QE/100 g) and TPC value ( $2.34$  g GAE/100 g). Flavonoids in cucumber pulp extract gave a contribution to antioxidant activity by CUPRAC method and phenolic compounds in cucumber pulp extract gave a contribution to antioxidant activity by DPPH method. AAI between CUPRAC and DPPH methods for cucumber leaves extract showed positive correlation but not significant. Therefore, the results between CUPRAC and DPPH methods were not linear. Quercetin was a marker compound of ethanolic cucumber pulp extract. Quercetin content in ethanol cucumber pulp extract was  $0.00114\%$ . Based on this study, cucumber pulp and leaves are recommended to be sources of natural antioxidants for the food or nutrition industry.

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