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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2024.69.79

Research Article
Antioxidant Activities of Stem, Leaves and Fruits Extracts of Pepino (*Solanum muricatum* Aiton)

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Abstract

Background and Objective: Pepino (*Solanum muricatum* Aiton), rich with vitamin C and flavonoids, constitutes an abundant source of potent antioxidants. This research was conducted to determine antioxidant activity from three different parts of pepino based on equivalence with ascorbic acid, to analyze the relationship between total phenolic content (TPC) and total flavonoid content (TFC) on antioxidant activities and to determine flavonoid compounds. **Materials and Methods:** Antioxidant activities were determined using 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and Cupric Ion Reducing Antioxidant Capacity (CUPRAC) methods. The TPC and TFC were determined by UV-visible spectrophotometry. The correlation between TPC, TFC and antioxidant activity was analyzed using Pearson's method. Flavonoid compound content was performed by HPLC. **Results:** The ethyl acetate pepino fruit extract expressed the highest antioxidant activity by DPPH and CUPRAC assays. The highest TPC was obtained from the ethyl acetate extract of pepino stem (18.493 g GAE/(100 g)), while the highest TFC was obtained from the hexane extract of pepino leaves (9.541 g QE/(100 g)). **Conclusion:** The DPPH and CUPRAC assays demonstrated that pepino exhibits potential as a source of natural antioxidants, especially in its fruit part.

Key words: Antioxidant, Cupric Ion Reducing Antioxidant Capacity (CUPRAC), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), *Solanum muricatum*, pepino

Citation: Hartati, R., N.A. Febiana, H. Pramastya and I. Fidrianny, 2024. Antioxidant activities of stem, leaves and fruits extracts of pepino (*Solanum muricatum* Aiton). Pak. J. Biol. Sci., 27: 69-79.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Free radicals are compounds that have one or more unpaired electrons and are highly reactive, capable of oxidizing surrounding molecules[1,2]. Due to their unpaired electrons, free radicals exhibit instability and a high reactivity level. Consequently, free radicals seek to attain electron stability by attracting electrons from other compounds. Molecules attacked by free radicals will lose electrons and become free radicals, triggering a chain of reactions that ultimately damage living cells[3]. Free radicals that are toxic to body cells are oxygen free radicals or superdioxide and its derivatives. Excessive free radical species in the body can cause cellular damage to DNA, protein and lipids[4]. Continuous cellular damage will lead to chronic diseases and other degenerative diseases[5]. Additional research indicated that free radicals have a substantial role in the pathogenesis of cancer[6] and cardiovascular disease[7].

The effect of free radicals in the body can be prevented by antioxidant compounds. Antioxidants are stable compounds that can donate electrons, making them easily oxidized[4]. Antioxidants can protect body cells from free radical species by donating electrons, which can bind to free radical compounds[6]. Therefore, free radicals will oxidize antioxidants first and other molecules in the cell can be protected from damage due to free radical oxidation.

Antioxidants can be produced naturally or synthetically. There are two sources of natural antioxidants, namely endogenous (from inside the body) and exogenous (from outside the body). Endogenous antioxidants can neutralize the impact of free radicals in normal amounts. However, when the number of free radicals in the body is excessive, the body needs exogenous antioxidants to help neutralize the impact of free radicals. Exogenous antioxidants are widely obtained from nature, such as from plants or minerals[8]. One example of a plant rich in antioxidants is pepino.

Solanum muricatum or pepino is a species of the *Solanum* genus and Solanaceae family, originating from the Andes Mountains and widely distributed in tropical and subtropical regions, including Indonesia. Pepino has a high content of vitamin C, carotenoids, flavonoids, alkaloids and tannins[9]. The content of pepino can be utilized as an antioxidant, antidiabetic, antitumor and antiscorbutic[10]. Although pepino has many beneficial contents, it is generally only used as food, an ornamental plant and an indoor mood enhancer[11]. The usefulness of pepino can be optimized by further research on the activity of pepino content.

Dominant contents of pepino are vitamin C and flavonoids, which play an important role in antioxidant activity. Therefore, this study was conducted to determine antioxidant activity from three different parts of pepino based on equivalence with ascorbic acid using DPPH and CUPRAC methods, to analyze the relationship between total phenolic content (TPC) and total flavonoid content (TFC) on antioxidant activities and to determine flavonoid compounds in the selected extract.

MATERIALS AND METHODS

Study area: The study was conducted at the Pharmaceutical Biology Laboratories of Bandung Institute of Technology, with a duration of approximately 7 months, from October, 2022 until April, 2023.

Chemicals: The gallic acid, quercetin, ascorbic acid, ammonium acetate, 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), neocuproine were purchased from Sigma Aldrich (USA). Analytical grade compounds were also used.

Sample collection: Three parts of pepino (*Solanum muricatum* Aiton) were stem (ST), leaves (LV) and fruits (FR). The parts were collected and identified in the School of Life Sciences and Technology, Bandung Institute of Technology, West Java, Indonesia. The sample was sorted, washed, dried, milled into powder and stored in a dry and closed container.

Extract preparation: The samples (300 g) were weighed and refluxed with n-hexane, ethyl acetate and ethanol solvents sequentially. The extraction process was performed 3 times for each extraction solvent to obtain optimum yields. The extract was concentrated until thick extract was obtained. There were nine different extracts. There were three n-hexane extracts (ST1, LV1 and FR1), three ethyl acetate extracts (ST2, LV2 and FR2) and three ethanol extracts (ST3, LV3 and FR3).

***In vitro* antioxidant activities by DPPH assay:** *In vitro* antioxidant activity by DPPH method was carried out using the method of Celep *et al.*[12] with modifications. The reference standard used was ascorbic acid, the control used was DPPH 50 μg mL^{-1} and the blank used was pro-analysis grade methanol. Samples were prepared at a variety of concentrations. Sample 12.5 μL was sequentially mixed with 112.5 μL of methanol pro-analysis, followed by 750 μL of DPPH

50 g mL^{-1} solution. The mixture was placed in a sealed and dark environment and incubated for 30 min. Subsequently, the absorbance of each mixture was measured at a wavelength of 517 nm using a UV-visible spectrophotometer (Thermo Scientific Orion Aquamate 8100, Singapore). The measurements were conducted in six repetitions for each extract. The obtained absorbance readings represent the percentage of sample inhibition. Antioxidant activity was determined by substituting sample inhibition percentage into the ascorbic acid calibration curve. The results reported as (AEAC)/g or ascorbic acid equivalent antioxidant capacity per gram of extract.

***In vitro* antioxidant activities by CUPRAC assay:** *In vitro* antioxidant activity by CUPRAC method was carried out using the method of Özyürek *et al.*[13] with modifications. Ascorbic acid was used as the standard, CUPRAC 100 µg mL^{-1} was used as the control and ammonium acetate buffer was used as the blank. The CUPRAC stock solution was obtained by mixing $CuCl_2 \cdot H_2O$ solution with neocuproine solution. The CUPRAC stock solution was diluted with ammonium acetate buffer pH 7.0 to the concentration of 100 µg mL^{-1}. Samples were prepared at a variety of concentrations. Add 12.5 µL of sample along with ammonium acetate until 250 µL, then add 750 µL of CUPRAC solution. The mixture was placed in a sealed and dark environment and incubated for 30 min. Subsequently, the absorbance of each mixture was measured at a wavelength of 450 nm using a UV-visible spectrophotometer. The measurements were conducted in six repetitions for each extract. The obtained absorbance readings represent the percentage increase in the sample content. The antioxidant activity was determined by substituting the percentage increase in a sample into an ascorbic acid calibration curve. The results reported as (AEAC)/g or ascorbic acid equivalent antioxidant capacity per gram of extract.

Total phenolic content (TPC): Determination of TPC was performed using gallic acid as the standard and Folin-Ciocalteu reagent. Gallic acid was prepared in pro-analytical grade methanol with concentrations ranging from 60 to 130 µg mL^{-1}. Each concentration of the gallic acid standard 50 µL was added by 500 µL of 10% Folin-Ciocalteu reagent and 400 µL of 1 M sodium carbonate. The mixture was incubated for 30 min at room temperature and the absorbance was read at wavelength of 765 nm. The calibration curve was prepared based on the absorbance measurement results of the gallic acid standard solution.

The determination of TPC in each sample was performed with the same procedure. Measurements were performed in six replicates for each extract. The TPC was determined using gallic acid calibration curve and reported as g GAE 100 g^{-1} (g gallic acid equivalents per 100 g of extract)[14].

Total flavonoid content (TFC): Determination of total flavonoid content was performed using quercetin as the standard. Quercetin was prepared in pro-analytical grade methanol with concentrations ranging from 45 to 100 µg mL^{-1}. Each concentration of the quercetin standard 100 µL was added sequentially by 300 µL of pro-analytical grade methanol, 20 µL of 10% aluminum (III) chloride, 20 µL 1 M sodium acetate and 560 µL distilled water. The mixture was incubated for 30 min at room temperature and the absorbance was investigated at wavelength of 415 nm. The calibration curve was prepared based on the absorbance measurement results of the quercetin standard solution.

The determination of TFC in each sample was performed with the same procedure. The measurements were conducted in six repetitions for each extract. The TFC was determined using quercetin calibration curve and reported as g quercetin acid equivalents per 100 g of extract (g QE 100 g^{-1})[15].

Statistical analysis: The results were averaged and reported with its standard deviation. Statistical analysis was performed with a one-way ANOVA-Tukey's method and an independent t-test using Minitab 2.0 with a significance level of 0.05 or 5%. The correlation test between TPC and antioxidant activity, the correlation between TFC and antioxidant activity and the correlation between antioxidant activity using the DPPH and CUPRAC methods were carried out using Pearson's method.

Identification and determination of the flavonoid compounds in the selected extract: Identification and determination of the flavonoid compounds in the selected extract were conducted using High-Performance Liquid Chromatography (HPLC). Luteolin-7-O-glucoside, rutin, quercetin, kaempferol and apigenin were used as reference standard compounds, dissolved in methanol at a concentration of 500 µg mL^{-1}, except for kaempferol (50 µg mL^{-1}). The selected extract used in this study was FR2, which was dissolved in methanol to produce a concentration of 50,000 µg mL^{-1}. Identification and determination of flavonoid compounds were performed using LC-20AD liquid chromatography system with a Shimadzu UV-vis SPD-20A detector at λ 360 nm. The mobile phase used in the HPLC

system consisted of water and methanol with a linear gradient of 40-60% methanol for 5 min, followed by a gradient of 70% methanol until 10 min and a gradient of 40% methanol until 15 min. The stationary phase used was Li-Chrospher® 100 RP-C18 5 µm (100 mm in length, 4 mm in diameter). The flow rate was set at 1 mL min^{-1} (CTO-20A pump, Shimadzu, Japan) and the injection volume was 20 µL with a column temperature of 30°C (CTO-20A oven, Shimadzu, Japan). The presence of the flavonoid compound was determined by the retention time, which was the same as that of the reference compound. The concentration of flavonoid compound was determined using a one-point method by comparing the area under the curve (AUC).

RESULTS

The extraction of the crude drug was carried out by the reflux method. The crude drug was extracted using three solvents with ascending polarity. The use of solvents with increasing polarity aims to obtain optimum non-polar, semi-polar and polar compounds from each part of the plant. The extraction process started by using n-hexane, a non-polar solvent followed by using ethyl acetate, a semi-polar solvent and ended by using ethanol, a polar solvent. The n-hexane solvent selectively extracted non-polar compounds. Ethyl acetate extracted most semi-polar compounds and a small fraction of non-polar compounds. Ethanol extracted most polar compounds and a small fraction of non-polar and semi-polar compounds. The extraction process ended with the separation of the extract from the crude drug residue by decantation. The liquid extract was concentrated using a rotary evaporator.

The assessment of activity and levels of phytochemical compounds involves density as a crucial indicator. Higher density indicates stronger activity and/or higher phytochemical content. Density determination of concentrated extract was conducted using 1% extract solution in pycnometer. The density obtained from the extracts of pepino stems, leaves and fruits indicated that the density among the extracts was similar in the range of 0.740-1.045 g mL^{-1}.

Antioxidant activities of pepino's stem, leaves and fruit extracts were analyzed with DPPH and CUPRAC methods. The antioxidant activities of both samples were expressed as the equivalence of ascorbic acid antioxidant capacity per gram of extract (mg AEAC g^{-1}). The greater the value of ascorbic acid equivalence, the stronger the antioxidant activity of the extract.

The antioxidant activity of pepino's stem, leaves and fruit extracts in the DPPH method was determined by a linear regression equation obtained from the ascorbic acid calibration curve. The equation obtained from the linear regression of the ascorbic acid calibration curve with the DPPH method was $y = 11.289x+15.05$, with an R^2 value of 0.992. The values for DPPH scavenging activity of different extracts from various parts of pepino ranged from 1.625 to 39.269 mg AEAC g^{-1} sample shown in Table 1. The highest antioxidant activity was shown in FR2, which was 39.269±1.558 mg AEAC g^{-1} sample.

The values of antioxidant activity by the DPPH method of different extracts from various parts of pepino were statistically processed by one-way ANOVA-Tukey's method to determine significant differences between extracts at $p<0.05$. Based on the plant parts, the results showed significant differences among the antioxidant activity values of n-hexane, ethyl acetate and ethanol extracts of pepino leaves. In contrast, the antioxidant activity value of ST2 did not significantly differ from ST3, but both were significantly different from ST1 ($p<0.05$). Additionally, the antioxidant activity value of FR1 did not significantly differ from FR3, but both were significantly different from FR2 ($p<0.05$).

Based on the extracting solvents, the results showed significant differences among the antioxidant activity values of ST2, LV2 and FR2 ($p<0.05$). Moreover, significant differences were also found among the antioxidant activity values of ST3, LV3 and FR3 ($p<0.05$). In contrast, the antioxidant activity value of LV1 did not significantly differ from FR1, but both were significantly different from ST1 ($p<0.05$).

Table 1: Antioxidant activities of pepino extracts using DPPH method

	Antioxidant activity (mg AEAC g^{-1} extract)		
Sample	n-hexane extract	Ethyl acetate extract	Ethanol extract
Stem	1.625 ± 0.100^{ax}	2.184 ± 0.058^{ay}	2.245 ± 0.167^{ay}
Leaves	5.289 ± 0.222^{bx}	4.460 ± 0.318^{by}	6.783 ± 0.438^{bz}
Fruits	1.787 ± 0.106^{ax}	39.269 ± 1.558^{cy}	3.034 ± 0.187^{cx}

$^{a-c}$Distinct letter in the same column indicates significant difference ($p<0.05$), $^{x-z}$Distinct letter in the same row indicates significant difference ($p<0.05$) and AEAC: Ascorbic acid equivalent antioxidant capacity

Table 2: Antioxidant activities of pepino by CUPRAC method

	Antioxidant activity (mg AEAC g^{-1} extract)		
Sample	n-hexane extract	Ethyl acetate extract	Ethanol extract
Stem	5.273 ± 0.331^{ax}	7.834 ± 0.546^{ay}	3.079 ± 0.274^{az}
Leaves	ND	10.400 ± 0.558^{ax}	12.042 ± 1.152^{by}
Fruits	2.336 ± 0.725^{bx}	54.488 ± 3.738^{by}	5.645 ± 0.341^{cx}

[a-c]Distinct letter in the same column indicates significant difference ($p<0.05$), [x-z]Distinct letter in the same row indicates significant difference ($p<0.05$), ND: Not detected and AEAC: Ascorbic acid equivalent antioxidant capacity

The antioxidant activity of pepino's stem, leaves and fruit extracts in the CUPRAC method was determined by a linear regression equation obtained from the ascorbic acid calibration curve. The equation obtained from linear regression of the ascorbic acid calibration curve with the CUPRAC method was $y = 3.8789x+48.253$, with an R^2 value of 0.9904. The values for CUPRAC antioxidant activity of different extracts from various parts of pepino ranged from 2.336 to 54.488 mg AEAC g^{-1} sample shown in Table 2. The highest antioxidant activity was shown in FR2, which was 54.488 ± 3.738 mg AEAC g^{-1} sample.

The values of antioxidant activity by the CUPRAC method of different extracts from various parts of pepino were also statistically processed by the one-way ANOVA-Tukey's method and independent t-test method to determine significant differences between extracts at $p<0.05$. To determine significant differences among three data variables, one-way ANOVA-Tukey's was used, whereas an independent t-test was used for two data variables. The CUPRAC method did not detect antioxidant activity in the n-hexane extract of pepino leaves. Therefore, a significant difference in antioxidant activity involving data from ethyl acetate and ethanol extracts of pepino leaves could only be determined using an independent t-test method.

Based on plant parts, the results showed significant differences among the antioxidant activity values of n-hexane, ethyl acetate and ethanol extracts from pepino stem ($p<0.05$). A significant difference was also found between the antioxidant activity values of ethyl acetate and ethanol extracts from pepino leaves ($p<0.05$). Conversely, the antioxidant activity value of FR1 was not significantly different from the antioxidant activity value of FR3, but both were significantly different from the antioxidant activity value of FR2 ($p<0.05$).

Based on the extracting solvents the results showed significant differences among the antioxidant activity values of ethanol extracts from pepino stems, leaves and fruits ($p<0.05$). In addition, a significant difference was found between the antioxidant activity values of ST1 and FR1 ($p<0.05$). The antioxidant activity value of ST2 was not significantly different from the antioxidant activity value of LV2, but both were significantly different from the antioxidant activity value of FR2 ($p<0.05$).

The total phenol was shown as equivalence to gallic acid, or g gallic acid equivalent (GAE) 100 g^{-1}. The regression equation obtained for the gallic acid calibration curve is $y = 0.0046x+0.0025$, with an R^2 value of 0.9927. The highest TPC was obtained from the ST2, which was 18.493 ± 1.080 g GAE 100 g^{-1}.

The total flavonoid was shown as equivalence to quercetin or g quercetin equivalent (QE) 100 g^{-1}. The regression equation obtained for quercetin calibration curve is $y = 0.0055x+0.0004$, with an R^2 value of 0.9997. The highest TFC was obtained from LV1, which was 9.451 ± 0.636 g QE 100 g^{-1}. The results of TPC and TFC from n-hexane, ethyl acetate and ethanol extracts of pepino's stem, leaves and fruits can be seen in Fig. 1 and 2.

A quantitative correlation analysis between TPC and TFC of stem, leaves and fruit extracts of pepino was performed with Minitab Statistical Software to determine their correlation with DPPH and CUPRAC antioxidant activity, using the Pearson correlation test. Pearson correlation test results between the TPC-TFC and antioxidant activity can be seen in Table 3. Pearson correlation test results obtained in Table 3 indicated a positive and strong correlation between TPC, DPPH and CUPRAC antioxidant activities, except for the correlation result between TPC of LV1 and CUPRAC of LV1. The Pearson correlation test also showed a positive and strong correlation between TFC, DPPH and CUPRAC antioxidant activities, except for the correlation between TFC of LV1 and CUPRAC antioxidant activity. The correlation test results of TPC and TFC with CUPRAC antioxidant activity in LV1 were undetectable. This was because the CUPRAC antioxidant activity value of the n-hexane extract of pepino leaves was not detected, so the correlation between TPC and TFC with CUPRAC antioxidant activity could not be performed.

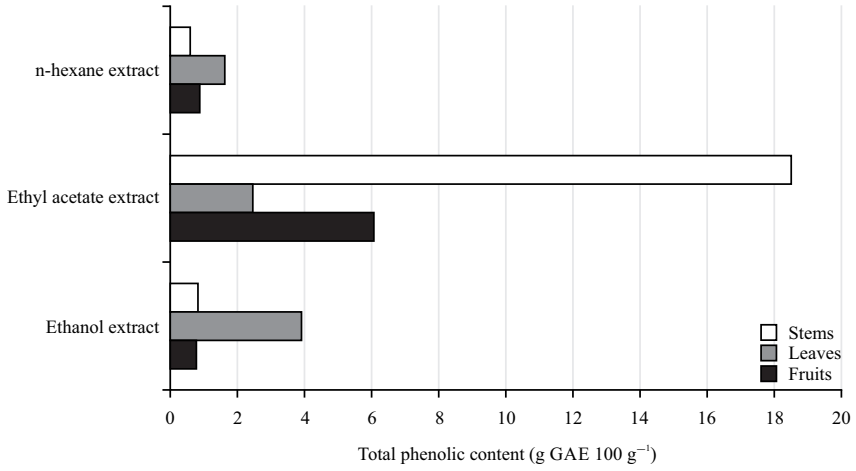

Fig. 1: Total phenolic content in different parts of pepino

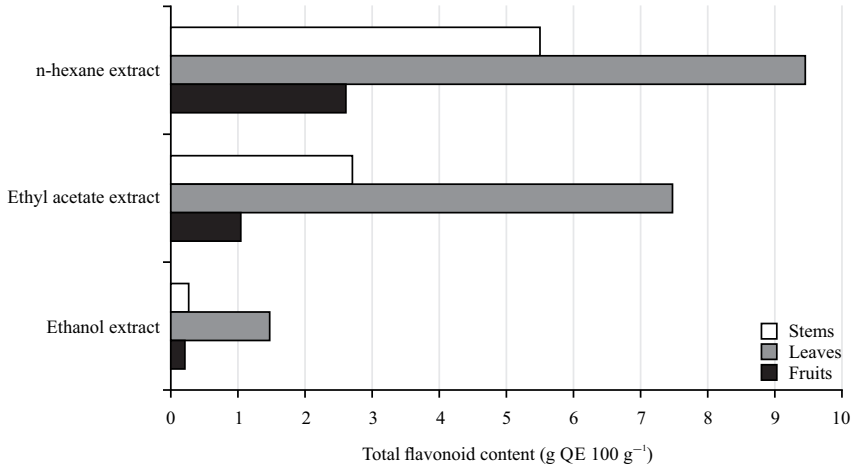

Fig. 2: Total flavonoid content in different parts of pepino

Table 3: Correlation of TPC and TFC with antioxidant activities

	Pearson's correlation coefficient (r)	
Antioxidant parameter	TPC	TFC
DPPH ST1	0.949****	0.956****
DPPH LV1	0.913****	0.874***
DPPH FR1	0.831***	0.969****
DPPH ST2	0.984****	0.989****
DPPH LV2	0.772***	0.968****
DPPH FR2	0.947****	0.925****
DPPH ST3	0.817***	0.974****
DPPH LV3	0.641**	0.823***
DPPH FR3	0.906****	0.842***
CUPRAC ST1	0.867***	0.961****
CUPRAC LV1	ND	ND
CUPRAC FR1	0.731***	0.953****
CUPRAC ST2	0.814***	0.926****
CUPRAC LV2	0.907****	0.942****
CUPRAC FR2	0.799***	0.990****
CUPRAC ST3	0.728***	0.971****
CUPRAC LV3	0.988****	0.976****
CUPRAC FR3	0.897****	0.809***

TPC: Total phenolic content, TFC: Total flavonoid content, **Moderate correlation, ***Strong correlation, ****Very strong correlation and ND: Not detected

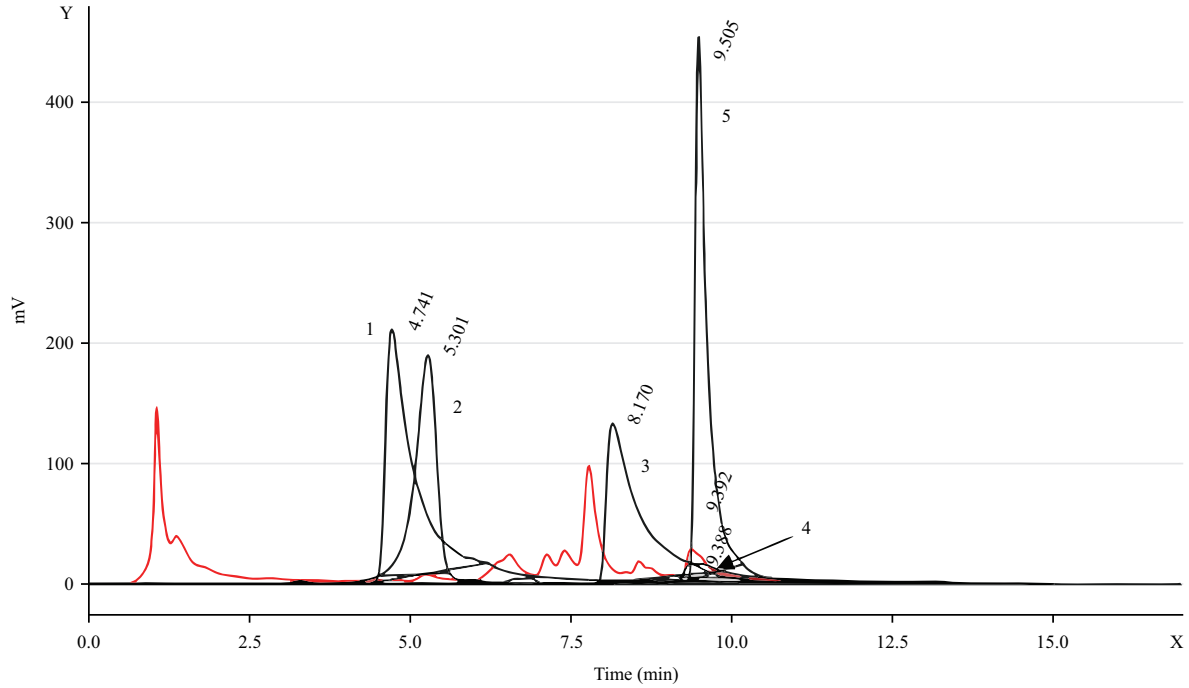

Fig. 3: HPLC chromatogram pattern of 5 flavonoids standard and ethyl acetate extract of pepino fruits
1: Luteolin 7-O-glucoside, 2: Rutin, 3: Quercetin, 4: Kaempferol, 5: Apigenin, Red line chromatogram: Sample and Black line chromatogram: Standard

Table 4: Correlation of DPPH and CUPRAC methods of n-hexane, ethyl acetate and ethanol extract

	Pearson's correlation coefficient (r)								
Antioxidant parameter	CUPRAC ST1	CUPRAC LV1	CUPRAC FR1	CUPRAC ST2	CUPRAC LV2	CUPRAC FR2	CUPRAC ST3	CUPRAC LV3	CUPRAC FR3
DPPH ST1	0.957****								
DPPH LV1		ND							
DPPH FR1			0.853***						
DPPH ST2				0.901****					
DPPH LV2					0.860***				
DPPH FR2						0.870***			
DPPH ST3							0.973****		
DPPH LV3								0.738***	
DPPH FR3									0.954****

***Strong correlation, ****Very strong correlation and ND: Not detected

A quantitative correlation analysis between antioxidant activity by DPPH and CUPRAC methods of stem, leaves and fruit extracts of pepino was performed with Minitab Statistical Software to determine their correlation, using the Pearson correlation test. The Pearson test results between the DPPH and CUPRAC antioxidant activity methods can be seen in Table 4. In general, DPPH and CUPRAC antioxidant activity gave a strong to very strong correlation for each extract, except for LV1.

Identification of flavonoid compounds was performed by High Performance Liquid Chromatography (HPLC), using a liquid chromatography system with UV-vis detector at λ 360 nm. Retention times of five standard flavonoids (luteolin 7-O-glucoside, rutin, quercetin, kaempferol, apigenin) was compared to retention time of FR2. The chromatogram results, retention times and area under curve (AUC) can be seen in Fig. 3 and Table 5.

Based on the chromatogram results obtained from Fig. 3, there was only one peak on the extract chromatogram that overlapped with the peak on the reference chromatogram. The extract chromatogram at 9.392 min was similar to the reference compound, kaempferol (number 4), with a retention time of 9.388 min. The determination of the identified compound, kaempferol, in the extract, was performed using the one-point method and the kaempferol content in FR2 was found to be 0.112% (Table 5).

Table 5: Flavonoids content in ethyl acetate extract of pepino fruits (FR2)

	Retention time (min)		AUC		
Flavonoid	Sample	Standard	Sample	Standard	Content (%)
Luteolin 7-O-glucoside	-	4.741	-	6080442	-
Rutin	-	5.301	-	4132432	-
Quercetin	-	8.170	-	4539154	-
Kaempferol	9.392	9.388	487489	435126	0.112
Apigenin	-	9.505	-	5917291	-

AUC: Area under curve

DISCUSSION

The highest antioxidant activity with DPPH and CUPRAC method was shown by ethyl acetate fruit extract (FR2), which was 39.269±1.558 mg AEAC g^{-1} sample for the DPPH method and 54.488±3.738 mg AEAC g^{-1} sample for CUPRAC method. Antioxidant activity in LV1 was not detected by the CUPRAC method. This is due to the different mechanisms of antioxidant activity exhibited by the n-hexane extract of pepino leaves. The antioxidant compounds in the n-hexane extract of pepino leaves cannot reduce Cu^{2+} ions to Cu^{+} ions in the CUPRAC method. However, LV1 showed antioxidant activity results using the DPPH method. Therefore, it was proven that the n-hexane extract of pepino leaves had antioxidant activity through the mechanism of donating hydrogen atoms, as was the case in testing antioxidant activity using the DPPH method.

Pepino's antioxidants activity levels measured using DPPH and CUPRAC methods varied. Antioxidant activities measured with DPPH method generated a range of values from 1.625 to 39.269 mg AEAC g^{-1}, while the CUPRAC method generated a range of values from 2.336 to 54.488 mg AEAC g^{-1}. The difference in antioxidant activity values between the DPPH and CUPRAC methods was due to the difference in reaction mechanisms. The value of antioxidant activity in the DPPH method was determined based on its ability to donate a hydrogen atom, thereby reducing DPPH. On the other hand, the value of antioxidant activity in the CUPRAC method was determined based on its ability to reduce Cu^{2+} ions to Cu^{+} ions.

The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) acts as a stable free radical. This is due to DPPH's ability to delocalize excess electrons throughout the molecule, resulting in an inability to dimerize[16]. Antioxidant assay with DPPH method measures the ability of antioxidants to donate hydrogen atoms, thereby causing the reduction of DPPH. The DPPH receives electrons from the antioxidant, forming DPPH-H, which will cause decolorization[17]. This process changes the original purple color of DPPH to yellow and reduces its absorbance as measured by UV-visible spectrophotometry at a wavelength of 517 nm.

An antioxidant assay using the CUPRAC method measures the ability of antioxidants to reduce Cu^{2+} ions to Cu^{+} ions using neocuproine reagent. The bis(neocuproine) copper (II) chloride (Cu(II)-Nc) reagent reacts with electrons originating from the antioxidant, resulting in the reduction of Cu^{2+} to Cu^{+} ions, forming a chromophoric complex with neocuproine and the absorbance can be measured at a wavelength of 450 nm[13]. The formation of the Cu^{+} chromophoric complex with neocuproine is indicated by the appearance of a yellow color that has a proportional color change rate to the ability to reduce Cu^{2+} ions[16].

In this study, the antioxidant activity values were expressed as the equivalence of ascorbic acid antioxidant capacity per gram of extract (mg AEAC g^{-1} extract). The greater the equivalence value of ascorbic acid antioxidant capacity, the greater the antioxidant activity of a sample. In another study, the antioxidant activity was expressed as the EC_{50} value. The smaller the EC_{50} value, the greater the antioxidant activity of a sample. Sudha *et al.*[18] showed that, the ethyl acetate extract of pepino fruits had DPPH, ABTS and FRAP antioxidant activities with EC_{50} values of 0.44, 10.03 and 1.51 mg mL^{-1}, respectively. These findings indicated that the DPPH method showed higher antioxidant activity for the ethyl acetate extract of pepino fruits than the other methods. However, the present study showed that the ethyl acetate extract of pepino fruits had lower DPPH antioxidant activity (39.269±1.558 mg AEAC g^{-1} extract) than CUPRAC method (54.488±3.738 AEAC g^{-1} extract). In another study, Sudha *et al.*[19] reported that the ethanol extract of pepino fruits had DPPH, ABTS and FRAP antioxidant activities with EC_{50} values of 2.20, 34.06 and 8.56 mg mL^{-1}, respectively. Ahmad *et al.*[20] revealed that methanol and n-hexane extracts of the pepino leaves showed DPPH antioxidant activities with IC_{50} values of 67.55 and 139.58 mg mL^{-1}, respectively. The ethanol and water extracts of *Cyphomandra betacea* or tamarillo, a plant in the same family as pepino (Solanaceae), had DPPH antioxidant activities with EC_{50} values of 44.25±0.82 and 47.38±1.11 µm mL^{-1}, respectively[21]. The methanol extract of *Solanum anguivi* or African eggplant, another plant in the Solanaceae family, showed antioxidant activity with an IC_{50} value of 275.03±7.8 µg mL^{-1} using the DPPH method[22].

The highest TPC was obtained from the ethyl acetate extract of pepino stem, which was 18.493±1.080 g GAE 100 g^{-1}. The other research which was conducted by Sudha *et al.*[18] reported that the ethyl acetate extract of pepino fruits contained TPC of 24.68±0.71 mg GAE g^{-1}, while another study by Sudha *et al.*[19] found that the ethanol extract of pepino fruits contained TPC of 14.44±0.42 mg GAE g^{-1}. These findings were consistent with the results of this study, which demonstrated that the TPC of ethyl acetate extract from pepino fruits (6.068±0.380 g GAE 100 g^{-1}) was higher than ethanol extract of pepino fruits (0.790±0.059 g GAE 100 g^{-1}). The ethanol and water extracts of *Cyphomandra betacea* or tamarillo, a plant belonging to the same family as pepino, Solanaceae, contained a TPC of 7.63±0.37 and 1.83±0.50 mg GAE g^{-1}, respectively[21]. The methanol extract of *Solanum anguivi* or African eggplant, which also belongs to the Solanaceae family like pepino, contained a TPC of 17.13±0.73 mg GAE g^{-1} [22].

The quantification of total phenol based on a redox reaction between phenolic compounds and the Folin-Ciocalteu reagent in a basic environment. The basic environment is obtained by adding sodium carbonate so that the protons in the phenolic compound can dissociate into phenolic ions. The redox process occurs as a result of the oxidation of the phenolic compound by the Folin-Ciocalteu reagent. The oxidized phenolic compound will reduce the heteropoly acid in the Folin-Ciocalteu reagent, consisting of phosphotungstic and phosphomolybdic acid, into a blue-colored molybdenum-tungsten complex. The molybdenum-tungsten complex can be measured by a UV-visible spectrophotometer at a wavelength of 765 nm[23].

The highest TFC was obtained from the n-hexane extract of pepino leaves, namely 9.451±0.636 g QE 100 g^{-1}. In this study, TFC was shown as quercetin equivalent (QE). Meanwhile, in other studies, TFC was shown as rutin equivalent (RE). Based on previous research conducted by Sudha *et al.*[18], the ethyl acetate extract of pepino fruits contained a TFC of 53.60±1.50 mg RE g^{-1}. Meanwhile, another research that was also performed by Sudha *et al.*[19] reported that ethanol extract of pepino fruits had a TPC of 23.62±0.61 mg RE g^{-1}. The results from previous studies were aligned with the results of this study, which found that the TFC of the ethyl acetate extract of pepino fruits (1.044±0.045 g QE 100 g^{-1}) was greater than the ethanol extract of pepino fruits (0.214±0.016 g QE/100 g^{-1}). The ethanol and water extracts of *Cyphomandra betacea* or tamarillo, a plant belonging to the same family as pepino (Solanaceae), contained TFC of 6.44±0.16 and 2.22±0.31 mg CE (catechin equivalent) g^{-1}, respectively[21]. The methanol extract of *Solanum anguivi* or African eggplant, a plant also belonging to the Solanaceae family, contained a TFC of 9.53±0.49 mg QE g^{-1} [22].

The quantification of total flavonoid based on color formation reaction or colorimetry. The color formation reaction occurs due to aluminum chloride forming a complex with the carbonyl functional group at C-4 and the hydroxyl functional group at C-3, C-5, C-3' or C-4' atoms[15]. The formation of the complex will result in the formation of yellow color caused by a wavelength shift towards the visible spectrum, which can be observed at a wavelength of 415 nm[23]. The addition of sodium acetate in the determination of total flavonoid content reaction will provide a basic environment, which aims to create a stable complex bond.

The Pearson correlation coefficient (denoted as "r") represents the linear relationship between two continuous random variables[24]. The correlation between two related continuous variables that are associated with changes in the other can be mathematically described using the Pearson correlation coefficient. The interpretation of the Pearson correlation coefficient can be done using various approaches. The approach used to interpret the Pearson correlation coefficient is based on Schober *et al.*[24] interpretation of the coefficient. Pearson correlation (r) is categorized based on the obtained value. Correlation values between 0.00-0.10 are ignored, 0.10-0.39 weak correlation, 0.40-0.69 moderate correlation, 0.70-0.89 strong correlation and 0.90-1.00 very strong correlation.

The correlation describes the contribution of TPC and TFC to the antioxidant activity of the extract. A positive correlation and the level of correlation strength indicate that there is a contribution of TPC and TFC to the antioxidant activity of the extract. The correlation results of TPC generally showed a strong ($0.70 \leq r < 0.89$) and very strong ($0.90 \leq r < 1.00$) correlation with DPPH and CUPRAC antioxidant activities, except for LV3 which showed a moderate correlation with DPPH antioxidant activities ($0.40 \leq r < 0.69$). In addition, the correlation test results of TFC generally showed a strong ($0.70 \leq r < 0.89$) and very strong ($0.90 \leq r < 1.00$) correlation with all DPPH and CUPRAC antioxidant activities. In general, it can be concluded that phenolic and flavonoid compounds in different parts of pepino contributed significantly to DPPH and CUPRAC antioxidant activities.

Previous studies showed a positive and significant correlation between phenolic and flavonoid compounds and the antioxidant activity of DPPH and CUPRAC in ethanol extract of *Solanum melongena* L., a plant that belongs to the same family as pepino, Solanaceae[25].

The Pearson test results between the DPPH and CUPRAC antioxidant activity methods resulted in general, DPPH and CUPRAC antioxidant activity gave a strong to very strong correlation for each extract, except for LV1. Thus, it can be concluded that the antioxidant activity of different parts of pepino using DPPH and CUPRAC methods was generally linear.

Determination of flavonoid compound in ethyl acetate extract of pepino fruits was performed by HPLC, using a liquid chromatography system with a UV-vis detector at λ 360 nm. Retention times of five standard flavonoids (luteolin 7-O-glucoside, rutin, quercetin, kaempferol, apigenin) was compared to retention time of FR2. The selected extract was FR2, as it exhibited the highest antioxidant activity in both DPPH and CUPRAC methods. The standards were used based on previous research. It had been found that pepino contained flavonoid compounds including rutin and quercetin. Furthermore, *Solanum torvum* or "takokak", a plant that also belongs to the same family as pepino (Solanaceae), was known to contain kaempferol and apigenin[26]. The compound luteolin-7-O-glucoside was also found in *Physochlaina physaloides*, a plant that also belongs to the Solanaceae family[27]. Thus, the compounds used as standards to identify and quantify flavonoids in the ethyl acetate extract of pepino fruits were luteolin-7-O-glucoside (500 μg mL^{-1}), rutin (500 μg mL^{-1}), quercetin (500 μg mL^{-1}), kaempferol (50 μg mL^{-1}) and apigenin (500 μg mL^{-1}).

Determination of kaempferol content identified in FR2 was carried out using a one-point method, resulting in kaempferol content of 0.112%. Based on a study which was performed by Tian *et al.*[28], kaempferol is a natural flavonol compound known to have antioxidant activity. Therefore, it can be assumed that the kaempferol content in the ethyl acetate extract of pepino fruits (FR2) contributes to its antioxidant activity.

Further studies regarding the antioxidant activity of pepino can be carried out using methods other than the DPPH and CUPRAC methods, such as the ABTS (2,2-Azino-Bis(3-Ethylbenzothiazoline-6-Sulfonic Acid) method and the FRAP (ferric reducing antioxidant power) method. In addition, further studies regarding the antioxidant activity of pepino can be carried out by isolating, elucidating and determining the levels of specific compounds that have antioxidant effects.

CONCLUSION

The highest DPPH and CUPRAC results were obtained from the ethyl acetate extract of pepino fruits. These results indicated that pepino fruits, which are generally consumed as a food item, had potential as a source of antioxidants. In general, the phenolic and flavonoid compounds in the extracts of stem, leaves and fruits of pepino significantly contributed to the DPPH and CUPRAC antioxidant activities. The DPPH and CUPRAC methods yielded linear results. The ethyl acetate extract of pepino fruits contained 0.112% of kaempferol content.

SIGNIFICANCE STATEMENT

Pepino (*Solanum muricatum* Aiton) has many beneficial contents, such as vitamin C and flavonoids, which play an important role in antioxidant activity. However, it is generally only used as food, an ornamental plant and an indoor mood enhancer. The usefulness of pepino can be optimized by further research on the activity of pepino content. This research found that DPPH and CUPRAC assays demonstrated that pepino exhibits potential as a source of natural antioxidants, especially in its fruit part. The highest antioxidant activity obtained from pepino fruit ethyl acetate extract. The results show that the pepino fruit, a part that is generally only consumed as food, has the potential to be a source of antioxidants.

ACKNOWLEDGMENTS

This research was funded by PPMI Grant 2023 No 56/IT1.C10/SK-KU/2023 from the Institute for Research and Community Service, Bandung Institute of Technology. The authors are grateful to School of Pharmacy, Bandung Institute of Technology for the necessary facilities to conduct this research.

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