Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Aug 29; 10(A):1429-1435.
https://doi.org/10.3889/oamjms.2022.9743
eISSN: 1857-9655
Category: *A - Basic Sciences*
Section: *Pharmacology*

Green Honey Deli Water Apple (*Syzygium aqueum* (Burm. f.) Alston "Madu Deli Hijau"): Evaluation of Antioxidant Activities and Phytochemical Content

Rika Hartati, Yasmine Widodo*, Chrisanta Tarigan, Irda Fidrianny

Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Bandung, Indonesia

Edited by: Sinisa Stojanoski
Citation: Hartati R, Widodo Y, Tarigan C, Fidrianny I. Green Honey Deli Water Apple (*Syzygium aqueum* (Burm. f.) Alston "Madu Deli Hijau"): Evaluation of Antioxidant Activities and Phytochemical Content. Open-Access Maced J Med Sci. 2022 Aug 29; 10(A):1429-1435. https://doi.org/10.3889/oamjms.2022.2927
Keywords: Antioxidant; CUPRAC; DPPH; *Syzygium aqueum*
***Correspondence:** Yasmine Widodo, Department of Pharmaceutical Biology, School of Pharmacy, Bandung Institute of Technology, Bandung - 40132, Indonesia. E-mail: syhrzqtyasmine@gmail.com
Received: 11-Apr-2022
Revised: 25-Jun-2022
Accepted: 19-Aug-2022

Funding: This research received funding from the PPMI Grant from Institute for Research and Community Service - ITB
Competing Interest: The authors have declared that no competing interest exists

Abstract

BACKGROUND: Antioxidants are able to fight against free radicals which then prevent degenerative diseases. Antioxidants can be found in many plants such as water apples. Comparison of antioxidant activities from three different parts of green honey deli water apple with different solvent polarity levels had not been yet reported.

AIM: This research is aimed to determine the antioxidant activity of green honey deli water apple (Syzygium aqueum) leaves, branches, and fruits extracts, total phenolic content (TPC), total flavonoid content (TFC), correlation of TPC and TFC on antioxidant activity, correlation between DPPH and CUPRAC methods, and content of flavonoid compounds found in ethanol fruit extract of green honey deli water apple.

METHODOLOGY: Antioxidant activities were examined by determining ascorbic acid equivalent (AAE) through DPPH and CUPRAC methods. TPC and TFC were determined using UV–Vis spectrophotometry. Correlation of TPC and TFC on antioxidant activity and correlation between DPPH and CUPRAC results were analyzed by Pearson's method. Contents of flavonoid compounds were determined using HPLC.

RESULTS: Antioxidant activities of green honey deli water apple leaves, branches, and fruits extracts according to DPPH and CUPRAC methods were 3.97–354.96 mg AAE/g and 10.46–222.51 mg AAE/g, respectively. Ethanol leaves extract had the highest TPC (68.14 ± 1.69 g GAE/100 g) and ethyl acetate leaves extract showed the highest TFC (18.65 ± g QE/100 g). TPC and TFC were found to correlate with the antioxidant activities. DPPH and CUPRAC results also correlated significantly positive.

CONCLUSION: Ethanol leaves and branches extracts of green honey deli water apple showed the highest antioxidant activities. Therefore, the two extracts have the most potential for further research as of discovery and development of antioxidant.

Introduction

The human body naturally produces reactive oxygen species (ROS) due to cellular metabolism [1]. In low to medium concentrations, ROS have physiological functions. However, in high concentrations, it causes oxidant and antioxidant imbalance that causes changes in cell components such as lipid, protein, and DNA [2]. Oxidative stress can trigger diseases such as Alzheimer's disease [3], atherosclerosis [4], Parkinson's disease [5], asthma [6], and cancer [7]. These pathological conditions can be prevented by antioxidants.

Antioxidants can fight free radicals both those from the body's metabolism and the environment (tobacco smoke, air pollution, and radiation). Therefore, antioxidants can prevent the aging process and degenerative diseases. Based on the source, antioxidants can be classified into two, endogenous and exogenous antioxidants. Endogenous antioxidants are antioxidants sourced from the body, for example, enzymes with antioxidant properties. Meanwhile, exogenous antioxidants are antioxidants sourced from outside of the body, antioxidants consumed through supplements or food. Exogenous antioxidants can come from herbs, spices, vegetables, etc. [8].

Indonesia has many plants that contain these radical-fighting antioxidants. One of those plants commonly found in Indonesia is the water apple. There are many varieties of water apple, for example, the green honey deli water apple. The antioxidant activity of water apple had previously been studied [9], [10]. However, the comparison of antioxidant activities of three different parts of this variety of water apple with different levels of solvent polarity had not yet been reported. This research is aimed to determine the antioxidant activities of green honey deli water apple leaves, branches, and fruits extracts, total phenolic content (TPC), total flavonoid content (TFC), correlation of TPC and TFC on antioxidant activities, correlation between the two test methods' results, and content of several flavonoids in ethanol fruits extract.

Methods

Preparation of sample

Leaves, branches, and fruits of green honey deli water apple were obtained from Dusun Bulak Timur, Cipayung Ward, Depok City, West Java-Indonesia. The parts were sorted, cleaned, cut, dried in the oven, and milled into crude drug powder, then stored in dry containers.

Extraction

The powdered samples were extracted using reflux method. The extraction was done with three solvents with increasing polarity: n-hexane, ethyl acetate, and ethanol. Each extraction was carried out for 2 h after the solvent has boiled. The extraction was done 3 times for each solvent. The extracts were then concentrated with a rotary evaporator.

Antioxidant activity with DPPH method

Determination of antioxidant activity of the extracts was carried out with DPPH and CUPRAC method. The DPPH method used ascorbic acid as the standard, pro-analytical methanol as the blank, and 50 μg/mL DPPH solution as the control. The original absorbance of the DPPH solution and the blank was measured with UV–Vis spectrophotometry at a wavelength of 517 nm. A stock solution of ascorbic acid in pro-analytical methanol was prepared at a 200 μg/mL concentration. As much as, 10, 12.5, 15, 20, 25, and 30 μL of the solution were taken, added pro-analytical methanol until 125 μL and the DPPH solution 750 μL, and incubated for 30 min in a dark place. Absorbances of each standard concentration were then measured 3 times. A calibration curve was obtained from the inhibition percentage. Extracts were dissolved in pro-analytical methanol and treated the same way as the standard. The absorbances of extracts were measured 6 times. Antioxidant activities of the extracts were obtained through the regression equation of the calibration curve and are expressed in mg of ascorbic acid equivalent (AAE) per g extract (mg AAE/g) [11].

Antioxidant activity with CUPRAC method

The method used ascorbic acid as the standard, ammonium acetate buffer as the blank, 100 μg/mL CUPRAC solution as the control. The original absorbance of the CUPRAC solution and the blank was measured with UV–Vis spectrophotometry at a wavelength of 450 nm. A stock solution of ascorbic acid in pro-analytical methanol was prepared at a 200 μg/mL concentration. As much as, 15, 17.5, 20, 22.5, 25, and 27.5 μL of the solution were taken, added ammonium acetate buffer until 250 μL and 750 μL of the CUPRAC solution, and incubated for 30 min. Absorbances of each standard concentration were then measured three times. A calibration curve is obtained from the increased capacity percentage. Extract solutions were prepared with pro-analytical methanol. Into 12.5 μL of the extracts were added ammonium acetate buffer until 250 μL and the CUPRAC solution 750 μL, then incubated for 30 min. The absorbances were measured 6 times. Antioxidant activities of the extracts were obtained through the regression equation of the calibration curve and were expressed in mg of AAE per g extract (mg AAE/g) [12].

Correlation between DPPH and CUPRAC methods

The correlation between the antioxidant activities based on DPPH and CUPRAC was analyzed statistically with Minitab Statistical Software 20. The analysis was done with Pearson's method.

TPC

Gallic acid was used as the standard and pro-analytical methanol was used as the blank. Gallic acid solutions were prepared in a series of concentrations from 60 to 130 μg/mL. Each concentration was taken 50 μL and added 500 μL Folin–Ciocalteu 10% and 400 μL Na_2CO_3 1 M. The mixture was incubated for 15 min. The standard and the blank's absorbance were measured with UV–Vis spectrophotometry at a wavelength of 765 nm. The absorbance of the standard was measured 3 times. A calibration curve was obtained from the measurements. Extracts dissolved in pro-analytical methanol were treated the same way as the standard. Absorbance measurements of each extract were done 6 times. TPC of the extracts was obtained through the regression equation of the gallic acid calibration curve and is expressed in g of gallic acid equivalent per 100 g extract (g GAE/100 g) [13].

TFC

Quercetin was used as the standard and pro-analytical methanol was used as the blank. Quercetin solutions were prepared in a series of concentrations from 40 to 110 μg/mL. Each concentration was taken 100 μL and added 300 μL pro-analytical methanol, 20 μL $AlCl_3$ 10%, 20 μL $NaCH_3COO$, and 560 μL distilled water. The mixture was incubated for 30 min. The standard and the blank's absorbance were measured with UV–Vis spectrophotometry at a wavelength of 415 nm. The absorbance of the standard was measured 3 times. A calibration curve was obtained from the measurements. Extracts were dissolved in pro-analytical methanol and treated the

same way as the standard. Absorbance measurements of each solution extract were done 6 times. TFC of the extracts was obtained through the regression equation of the quercetin calibration curve and was expressed in g of quercetin equivalent per 100 g extract (g QE/100 g) [14].

Correlation of TPC and TFC on antioxidant activity

The correlation of phenolic and flavonoid content on antioxidant activity was analyzed statistically with Minitab Statistical Software 20. The analysis was done with Pearson's method.

Content of several flavonoids

Identification and content determination of several flavonoids in ethanol fruit extract of green honey deli water apple were carried out with HPLC. The HPLC used was HPLC-20AD. The mobile phases used were water (eluent A) and methanol (eluent B). The separation system was linear gradient 40–60% eluent B for 5 min, gradient eluent B 70% until the 10th min, and gradient eluent B 40% until the 15th min. The stationary phase used was LiChrospher 100 RP-18 (5 μm). The flow rate was 1 mL/min, injection volume was 20 μL, and column temperature was 30°C. The retention time of compounds in ethanol green honey deli water apple fruit extract 500 μg/mL and a mixture of standards (luteolin-7-O-glucoside, rutin, quercetin, kaempferol, and apigenin) 2 μg/mL were compared. The contents of the compounds identified were then determined with one-point method.

Results

Antioxidant activity with DPPH method

Antioxidant activities of green honey deli leaves, branches, and fruits extracts were determined with DPPH method using DPPH inhibition percentage calibration curve regression equation y = 12.328x + 4.5328, R^2 = 0.997. Results are shown in Figure 1. In this experiment, ethanol leaves extract exposed the highest antioxidant activity (354.96 ± 13.21 g AAE/g).

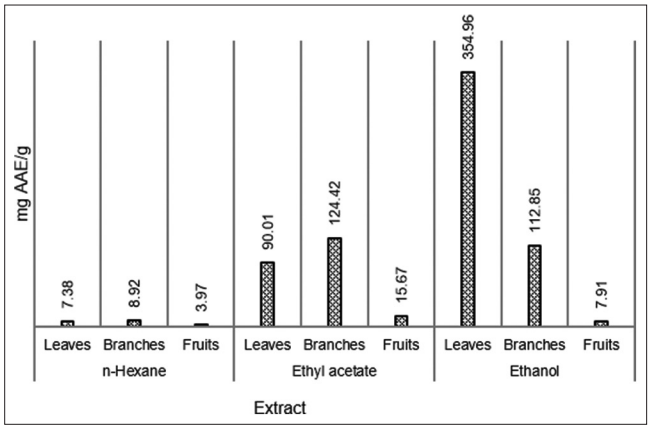

Figure 1: Antioxidant activities of green honey deli water apple leaves, branches, and fruits extracts according to DPPH

Antioxidant activity with CUPRAC method

Antioxidant activities of green honey deli leaves, branches, and fruits extracts were determined with CUPRAC method using increasing in capacity percentage calibration curve regression equation y = 8.3923x + 12.327, R^2 = 0.998. Results are shown in Figure 2. In this experiment, the highest antioxidant activity using CUPRAC method was found in both ethanol leaves extract (221.47 ± 9.18 g AAE/g) and ethanol branches extract (222.51 ± 10.44 g AAE/g).

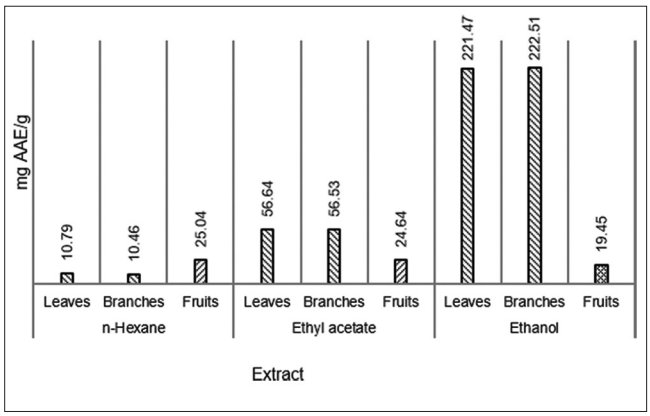

Figure 2: Antioxidant activities of green honey deli water apple leaves, branches, and fruits extracts according to CUPRAC

Table 1: Correlation between DPPH and CUPRAC methods

Antioxidant parameter	Pearson's correlation coefficient (r)								
	C1	C2	C3	C4	C5	C6	C7	C8	C9
D1	0.914**								
D2		0.908**							
D3			0.992**						
D4				0.944**					
D5					0.984**				
D6						0.889**			
D7							0.899**		
D8								0.668*	
D9									0.898**

**Significant at p < 0.01, *Significant at p < 0.05.

Correlation between DPPH and CUPRAC methods

The correlation between antioxidant activities based on DPPH and CUPRAC methods was analyzed. Results are shown in Table 1 with D = DPPH, C = CUPRAC, 1 = n-hexane leaves, 2 = n-hexane branches, 3 = n-hexane fruits, 4 = ethyl acetate leaves, 5 = ethyl acetate branches, 6 = ethyl acetate fruits, 7 = ethanol leaves, 8 = ethanol branches, and 9 = ethanol fruits.

Table 2: TPC in leaves, branches, and fruits extract of green honey deli water apple

Sample	TPC (g GAE/100 g)		
	n-hexane	Ethyl acetate	Ethanol
Leaves	1.14 ± 0.04^{ax}	13.96 ± 0.59^{ay}	68.14 ± 1.69^{az}
Branches	2.87 ± 0.12^{bx}	9.02 ± 0.69^{by}	14.40 ± 1.04^{bz}
Fruits	1.87 ± 0.09^{cx}	36.16 ± 0.65^{cy}	2.78 ± 0.10^{cz}

$^{a-c}$Different letters in a column show significant difference ($p < 0.05$), $^{x-z}$Different letters in a row show significant difference ($p < 0.05$). TPC: Total phenolic content.

TPC

TPC of green honey deli leaves, branches, and fruits extracts was determined using gallic acid calibration curve regression equation $y = 0.0057x - 0.0774$, $R^2 = 0.9916$. Results are shown in Table 2. In this experiment, ethanol leaves extract had the highest TPC (68.14 ± 1.69 g GAE/100 g).

Table 3: TFC in leaves, branches, and fruits extract of green honey deli water

Sample	TFC (g QE/100 g)		
	n-hexane	Ethyl acetate	Ethanol
Leaves	2.54 ± 0.14^{ax}	18.65 ± 1.04^{ay}	1.73 ± 0.27^{ax}
Branches	5.52 ± 0.73^{bx}	3.09 ± 0.13^{by}	0.39 ± 0.06^{bz}
Fruits	2.14 ± 0.26^{ax}	0.30 ± 0.03^{cy}	0.29 ± 0.02^{by}

$^{a-c}$Different letters in a column show significant difference ($p < 0.05$), $^{x-z}$Different letters in a row show significant difference ($p < 0.05$). TFC: Total flavonoid content.

TFC

TFC of green honey deli leaves, branches, and fruits extracts was determined using quercetin calibration curve regression equation $y = 0.0069x + 0.0167$, $R^2 = 0.9986$. Results are shown in Table 3. In this experiment, ethyl acetate leaves extract had the highest TFC (18.65 ± 1.04 g QE/100 g).

Table 4: Correlation of TPC and TFC on antioxidant activity

Antioxidant parameter	Pearson's correlation coefficient (r)	
	TPC	TFC
DPPH n-hexane leaves	0.927**	0.892**
DPPH n-hexane branches	0.953**	0.957**
DPPH n-hexane fruits	0.946**	0.847**
DPPH ethyl acetate leaves	0.952**	0.991**
DPPH ethyl acetate branches	0.924**	0.990**
DPPH ethyl acetate fruits	0.919**	0.823**
DPPH ethanol leaves	0.959**	0.855**
DPPH ethanol branches	0.802**	0.879**
DPPH ethanol fruits	0.976**	0.899**
CUPRAC n-hexane leaves	0.946**	0.855**
CUPRAC n-hexane branches	0.894**	0.965**
CUPRAC n-hexane fruits	0.898**	0.841**
CUPRAC ethyl acetate leaves	0.851**	0.940**
CUPRAC ethyl acetate branches	0.925**	0.978**
CUPRAC ethyl acetate fruits	0.972**	0.933**
CUPRAC ethanol leaves	0.873**	0.839**
CUPRAC ethanol branches	0.963**	0.880**
CUPRAC ethanol fruits	0.917**	0.901**

**Significant at $p < 0.01$, TPC: Total phenolic content, TFC: Total flavonoid content.

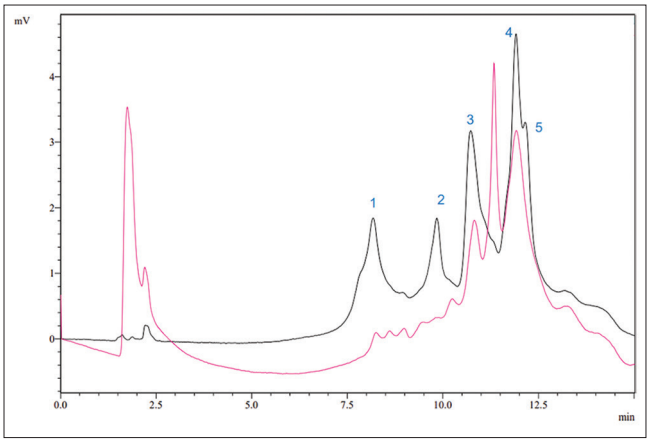

Figure 3: HPLC chromatogram of five flavonoids standard and ethanol fruit extract. 1: Luteolin-7-O-glucoside, 2: Rutin, 3: Quercetin, 4: Kaempferol, and 5: Apigenin, black line chromatogram = standards, pink line chromatogram = ethanol fruits extract

Correlation of TPC and TFC on antioxidant activity

The correlation of TPC and TFC on antioxidant activity was analyzed. Results are shown in Table 4.

Content of several flavonoids

Retention times of five flavonoids standard (luteolin-7-O-glucoside, rutin, quercetin, kaempferol, and apigenin) and ethanol fruit extract were used to identify the flavonoids present in the extract. Results are shown in Figure 3 and Table 5.

Table 5: Retention time and AUC of several flavonoids

Flavonoid	Retention time (min)		AUC	
	S	Std	S	Std
Luteolin-7-O-glucoside	-	8.17	-	59803
Rutin	-	9.84	-	30176
Quercetin	10.82	10.73	26330	66711
Kaempferol	11.92	11,91	95556	97657
Apigenin	-	12.15	-	23931

S = Sample (ethanol fruit extract), Std = Standard mixture.

The AUC of the flavonoids standard and compounds in sample was then used to determine the content of the compounds in the sample. Results are shown in Table 6.

Table 6: Content of several flavonoids in ethanol fruit extract

Flavonoid	Content (%)
Quercetin	0.16
Kaempferol	0.39

Discussion

ROS can come from the body's cellular metabolism or external factors such as tobacco smoke, pollution, and heavy metal. ROS can be classified into free radical and non-radical. A free radical is a molecule that has one or more unpaired electrons causing it to be highly reactive [1]. At low to middle

concentrations, ROS have physiological functions, but at high concentrations, it can cause changes to the cell's components [2]. This change within the body is called oxidative stress. Oxidative stress can cause pathological conditions. Aerobic living organisms can fight against ROS' effects with the antioxidant system they have, but in certain cases, this system can become overwhelmed [1]. Therefore, the body might need antioxidants from external sources.

Antioxidants are molecules that inhibit the oxidation of other molecules. Oxidation happens when a molecule loses its electrons and increases its oxidative state. This condition results in the formation of free radicals. Antioxidants can act by scavenging free radicals, decomposing peroxides, or binding with pro-oxidant metal ions [8].

It is known that phenolic and flavonoid compounds may contribute to antioxidant activities [15]. In the previous research, results showed that methanol leaves extract of water apple has TPC of 45.3 g GAE/100 g [9]. Another research's results expressed that leaves extracts of red water apple with methanol, ethyl acetate, and n-hexane as the solvent had TPC of 95.6 g GAE/100 g, 79.1 GAE/100 g, and 39.8 g GAE/100 g extract, respectively. Meanwhile, branches extract of red water apple with methanol, ethyl acetate, and n-hexane as the solvent had TPC of 33.8 g GAE/100 g, 20.1 g GAE/100 g, and 21.4 g GAE/100 g extract, respectively [10].

The TPC of the previous research revealed different results from the present research. In the present research, the TPC of green honey deli water apple leaves extracts ranges from 1.14 to 68.14 g GAE/100 g, branches extracts range from 2.87 to 14.40 g GAE/100 g, and fruits extracts range from 1.87 to 36.16 g GAE/100 g. Ethanol leaves extract figured the highest TPC of 68.14 ± 1.69 g GAE/100 g. The difference in results may come from the different solvents used to extract, different growth locations, and different varieties of the plant. However, the second previous research and the present research have a similarity that leaves extracts have higher TPC compared to branches extracts. Based on the one-way ANOVA method, all the nine extracts showed significant differences at $p < 0.05$.

In the previous research, results demonstrated that methanol fruits extract of water apple has TFC of 22.87 ± 8.59 mg catechin equivalent (mg CE)/100 g [16]. Another research's results presented that ultrasonic-assisted aqueous extract of java plum (*Syzygium cumini*) seed kernel powder had TFC of 10.11 mg CE/g extract [17]. The TFC of the previous research also exposed different results from the present research. In the present research, the TFC of green honey deli water apple leaves extracts ranges from 1.73 to 18.65 g QE/100 g, branches extracts range from 0.39 to 5.52 g QE/100 g, and fruits extracts range from 0.29 to 2.14 g QE/100 g. Ethyl acetate leaves extract had the highest TFC of 18.65 ± 1.04 g QE/100 g. In the previous research, TFC was expressed in catechin equivalent, whereas in the present research, TFC was expressed in quercetin equivalent/100 g extract. The difference in results may come from the different solvents used to extract, different parts used, different growth locations, and different varieties of the plant. Based on the one-way ANOVA method, n-hexane leaves and branches extracts have no significant difference. Ethanol branches and fruits extract also have no significant difference. N-hexane and ethanol leaves extracts' have no significant difference. Ethyl acetate and ethanol fruits extracts also have no significant difference. Meanwhile, the other extracts stated significant differences at $p < 0.05$.

When determining antioxidant activities, it is suggested to carry out the experiment in more than 1 method. Based on the chemical reaction involved, methods to determine antioxidant activities can be classified into two: Hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms. One method based on the SET mechanism is CUPRAC method and one method based on a combination of SET and HAT methods is DPPH method [18]. DPPH is a radical that then will be neutralized by antioxidants through electron transfer. This reaction causes a change of color which the change of absorbance can be measured at a wavelength of 517 *nm* [19].

In the previous research, results showed that methanol leaves extract of water apple has EC_{50} of 6.80 ± 0.15 μg/mL according to DPPH method [9]. Another research's results revealed that leaves extracts of red water apple with methanol, ethyl acetate, and n-hexane as the solvent had IC_{50} of 14.47 μg/mL, 35.72 μg/mL, and 748.30 μg/mL, respectively. Meanwhile, branches extract of red water apple with methanol, ethyl acetate, and n-hexane as the solvent gave IC_{50} of 9.71 μg/mL, 12.09 μg/mL, and 689.23 μg/mL, respectively, according to DPPH method [10].

The previous and the present research exposed different results. In the present research, antioxidant activities of green honey deli water apple leaves range from 7.38 to 354.96 mg AAE/g, branches range from 8.92 to 124.42 mg AAE/g, and fruits range from 3.97 to 15.67 mg AAE/g, according to DPPH method. Ethanol leaves extract had the highest activity of 354.96 ± 13.21 mg AAE/g extract. In one of the previous researches, results showed that branches extracts had higher antioxidant activity than leaves extract. In comparison, the present research showed that leaves extracts had higher activity than branches extracts. The difference in results may come from the difference of growth location as well as the different varieties of the plant. Based on the one-way ANOVA method, all nine extracts had significant differences at $p < 0.05$.

CUPRAC method measures antioxidant activities based on the reduction of Cu^{2+} into Cu^{+} by antioxidants with the presence of neocuproine which then will form a colored

complex with Cu^{+}. This complex has an absorbance that can be measured at a wavelength of 450 nm [20].

In the previous research, results showed that hot water branches extract of java apple (*Syzygium samarangense*) had an absorbance of 4.8 at 1000 μg/mL and hot water leaves extract of java apple has an absorbance of 4.1 at 1000 μg/mL, lower compared to L-ascorbic acid that had an absorbance of 4.2 at 100 μg/mL, according to CUPRAC method [21]. In the present research, antioxidant activities of green honey deli water apple leaves range from 10.79 to 221.47 mg AAE/g, branches range from 10.46 to 222.51 mg AAE/g, and fruits range from 19.45 to 25.04 mg AAE/g, according to CUPRAC method. Ethanol branches extract had the highest activity of 222.51 ± 10.44 mg AAE/g extract. Although, based on the one-way ANOVA method, branches and leaves extracts in all three solvents have no significant difference. N-hexane and ethyl acetate fruits extracts also have no significant difference.

Flavonoid and phenolic compounds may contribute to antioxidant activities. Flavonoid is a part of the phenolic group. Polyphenols can act as antioxidants depending on their chemical structure and ability to donate or accept electrons, thus delocalizing the unpaired electron within the aromatic structure [22]. As shown in Table 4, TPC and TFC correlated significantly positive at $p < 0.01$ with antioxidant activities according to Pearson's coefficient values. Therefore, it can be stated that phenolic and flavonoid compounds contribute to antioxidant activities whether by DPPH or CUPRAC method.

As shown in Table 1, the antioxidant activities by CUPRAC and DPPH method correlated positive and significant at $p < 0.01$ and $p < 0.05$. It showed that the two methods have linear results.

In general, *Syzygium* plants contain various secondary metabolites, namely, flavonoids [23]. According to the previous research, leaves of several *Syzygium* plants contained luteolin-7-O-glucoside, quercetin, kaempferol, and apigenin [24] and several fruits of Myrtaceae plants contained rutin [25]. In the present study, determination of flavonoid compounds in ethanol fruits extract of green honey deli water apple (Figure 3) demonstrated that the extract has two peaks aligned with the peaks from the standard mixture, which were quercetin and kaempferol. This concluded that quercetin and kaempferol were found within the extract. The calculation stated that the content of quercetin was 0.16% and kaempferol 0.39%.

Conclusion

Extracts of leaves, branches, and fruits of green honey deli water apple contain antioxidant activities, according to DPPH and CUPRAC method. According to DPPH method, the antioxidant activities' values range from 3.97 to 354.96 mg AAE/g extract, meanwhile, according to CUPRAC method range from 10.46 to 222.51 mg AAE/g extract. Based on the results, ethanol extracts of green honey deli water apple leaves and branches have the most potential for further research as of discovery and development of antioxidant. The highest TPC was found in ethanol leaves extract (68.14 ± 1.69 g GAE/100 g extract) and the highest TFC was found in ethyl acetate leaves extract (18.65 ± 1.04 QE/100 g extract). Phenol and flavonoid compounds gave great contribution to antioxidant activities. The results between DPPH and CUPRAC methods showed significant and positive correlation, therefore, the two methods gave linear results. Quercetin and kaempferol were found within ethanol fruits extract, with quercetin content 0.16% and kaempferol 0.39%.

Acknowledgments

The authors are grateful for receiving funding from the PPMI Grant 2022 No. 16A/IT1.C10/SK-KP/2022 from Institute for Research and Community Service – Bandung Institute of Technology and to the authorities of School of Pharmacy – Bandung Institute of Technology, for providing the necessary facilities to perform this research.

References

1. Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O. Oxidative stress and antioxidant defense. World Allergy Organ J. 2012;5(1):9-19. https://doi.org/10.1097/WOX.0b013e3182439613
PMid23268465
2. Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chem Biol Interact. 2006;160(1):1-40. https://doi.org/10.1016/j.cbi.2005.12.009
PMid16430879
3. Lyras L, Cairns NJ, Jenner A, Jenner P, Halliwell B. An assessment of oxidative damage to proteins, lipids, and DNA in brain from patients with Alzheimer's disease. J Neurochem. 1997;68:2061-9. https://doi.org/10.1046/j.1471-4159.1997.68052061.x
PMid9109533
4. Rahman K. Historical perspective on garlic and cardiovascular disease. J Nutr. 2001;131(1):977-9. https://doi.org/10.1093/jn/131.3.977S
PMid11238800
5. Blesa J, Trigo-Damas I, Quiroga-Varela A, Jackson-Lewis VR. Oxidative stress and Parkinson's disease. Front Neuroanat. 2015;9:91. https://doi.org/10.3389/fnana.2015.00091
PMid26217195
6. Andreadis AA, Hazen SL, Comhair SA, Erzurum SC. Oxidative and nitrosative events in asthma. Free Radic Biol Med. 2003;35(3):213-25. https://doi.org/10.1016/S0891-5849(03)00278-8

PMid12885584

7. Wang M, Dhingra K, Hittelman W, Liehr J, De Andrade M, Li D. Lipid peroxidation-induced putative malondialdehyde-DNA adducts in human breast tissues. Cancer Epidemiol Biomarkers Prev. 1996;5(9):705-10.
   PMid 8877062
8. Mehta SK, Gowder SJ. Members of antioxidant machinery and their functions. In: Gowder SJ, editor. Basic Principles and Clinical Significance of Oxidative Stress. London: InTechOpen; 2015.
9. Sobeh M, Mahmoud MF, Petruk G, Rezq S, Ashour ML, Youssef FS, *et al*. Syzygium aqueum: A polyphenol-rich leaf extract exhibits antioxidant, hepatoprotective, pain-killing and anti-inflammatory activities in animal models. Front Pharmacol. 2018;9:566. https://doi.org/10.3389/fphar.2018.00566
   PMid29922158
10. Itam A, Wati MS, Agustin V, Sabri N, Jumanah RA, Efdi M. Comparative study of phytochemical, antioxidant, and cytotoxic activities and phenolic content of Syzygium aqueum (Burm. f. Alston f) extracts growing in West Sumatera Indonesia. Sci World J. 2021;2021:5537597. https://doi.org/10.1155/2021/5537597
    PMid34234626
11. Celep E, Charehsaz M, Akyüz S, Acar ET, Yesilada E. Effect of in vitro gastrointestinal digestion on the bioavailability of phenolic components and the antioxidant potentials of some Turkish fruit wines. Int Food Res J. 2015;78:209-15. https://doi.org/10.1016/j.foodres.2015.10.009
    PMid28433284
12. Özyürek M, Bektaşoğlu B, Güçlü K, Güngör N, Apak R. Simultaneous total antioxidant capacity assay of lipophilic and hydrophilic antioxidants in the same acetone-water solution containing 2% methyl-β-cyclodextrin using the cupric reducing antioxidant capacity (CUPRAC) method. Anal Chim Acta. 2008;630(1):28-39. https://doi.org/10.1016/j.aca.2008.09.057
    PMid19068323
13. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol. 2006;5(11):1142-5.
14. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178-82. https://doi.org/10.38212/2224-6614.2748
15. Chaudari GM, Mahajan RT. Comparative antioxidant activity of twenty traditional Indian medicinal plants and its correlation with total flavonoid and phenolic content. Int J Pharm Sci Rev Res. 2015;30(1):105-11.
16. Tehrani M, Chandran S, Hossain AB, Nasrulhaq-Boyce A. Postharvest physico-chemical and mechanical changes in jambu air (Syzygium aqueum Alston) fruits. Aust J Crop Sci. 2011;5(1):32-8.
17. Mahindrakar KV, Rathod VK. Ultrasonic assisted aqueous extraction of catechin and gallic acid from Syzygium cumini seed kernel and evaluation of total phenolic, flavonoid contents and antioxidant activity. Chem Eng Process. 2020;149:10784. https://doi.org/10.1016/j.cep.2020.107841
18. Prior RL, Wu X, Schaich K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005;53(10):4290-302. https://doi.org/10.1021/jf0502698
    PMid15884874
19. Foti MC. Use and abuse of the DPPH (.) radical. J Agric Food Chem. 2015;63(40):8765-76. https://doi.org/10.1021/acs.jafc.5b03839
    PMid26390267
20. Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: A review. Int J Mol Sci. 2021;22(7):3380. https://doi.org/10.3390/ijms22073380
    PMid33806141
21. Hyun SB, Bae S, Hyun CG. Antioxidant activities of jeju wax apple (Syzygium samarangense) and safety of human keratinocytes and primary skin irritation test. Cosmetics. 2020;7(2):39. https://doi.org/10.3390/COSMETICS7020039
22. Lin KH, Yang YY, Yang CM, Huang MY, Lo HF, Liu KC, *et al*. Antioxidant activity of herbaceous plant extracts protect against hydrogen peroxide-induced DNA damage in human lymphocytes. BMC Res Notes. 2013;6:490. https://doi.org/10.1186/1756-0500-6-490
    PMid24279749
23. Aung EE, Kristanti AN, Aminah NS, Takaya Y, Ramadhan R. Plant description, phytochemical constituents and bioactivities of Syzygium genus: A review. Open Chem J. 2020;18(1):1256-81. https://doi.org/10.1515/chem-2020-0175
24. Rocchetti G, Lucini L, Ahmed SR, Saber FR. In vitro cytotoxic activity of six Syzygium leaf extracts as related to their phenolic profiles: An untargeted UHPLC-QTOF-MS approach. Food Res. 2019;126:108715. https://doi.org/10.1016/j.foodres.2019.108715
    PMid31732075
25. Reynertson KA, Yang H, Jiang B, Basile MJ, Kennelly EJ. Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits. Food Chem. 2008;109(4):883-90. https://doi.org/10.1016/j.foodchem.2008.01.021
    PMid21340048