

ANTIOXIDANT ACTIVITY OF WATER APPLE (*SYZYGIUM AQUEUM*) FRUIT AND FRAGRANT MANGO (*MANGIFERA ODORATA*) FRUIT

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ABSTRACT

Objective: The aim of this study was to compare the antioxidant activity of fruit of water apple and fragrant mango against 1,1-diphenyl-2-picrylhydrazyl as a free radical.

Methods: This study was conducted by visible spectrophotometry.

Results: The water apple and fragrant mango contain reducing compounds including vitamin C. The vitamin C content of water apple and fragrant mango fruit was 0.087% (w/w) and 0.050% (w/w), respectively. The inhibitory concentration 50% value of fresh fruit of water apple and fragrant mango was 4.857 µg/ml and 4.379 µg/ml, respectively.

Conclusion: Antioxidant activity of water apple fruit was higher than fragrant mango fruit.

Keywords: 1,1-diphenyl-2-picrylhydrazyl, Free radical, Visible spectrophotometry, Vitamin C.

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INTRODUCTION

Free radicals are responsible for the damage of lipids, proteins, and nucleic acid in cells [1] which lead to cardiovascular diseases, cancers, and other age-related degenerative diseases [2]. Fruits and vegetables can reduce the risk of stroke and cancer because of the antioxidant contained in the plant parts. Different fruits have different capacities due to different dietary antioxidants such as vitamin C, flavonoids, and other phenolic compounds [3].

Fruit of water apple (*Syzygium aqueum* (Burm.f.) Alston) and fragrant mango (*Mangifera odorata* Griffith) contains vitamin C. All physiological and biochemical actions of vitamin C are due to its action as an electron donor from a double bond. Vitamin C is an antioxidant, because of donating its electrons, which prevents other compounds from being oxidized. When vitamin C donates one electron, they are formed a free radical, semi-dehydroascorbic acid, or ascorbyl radical, which relatively stable (10^{-5} seconds of half-life) and fairly unreactive [4]. The aim of this study was to compare the antioxidant activity of fruit of water apple and fragrant mango against 1,1-diphenyl-2-picrylhydrazyl (DPPH) as a free radical.

MATERIALS AND METHODS

Materials

Fruit of water apple and fragrant mango was collected from Subang subdistrict, Indonesia, in July 2016. Fruits were identified by Laboratory of Plants Taxonomy, Department of Biology, Universitas Padjadjaran, Indonesia, with No. 450/HB/08/2016. All chemical reagents are analytical grade (Merck) including DPPH (Sigma-Aldrich).

Methods

Extraction

A total of 10 g of fruit and 15 ml of citrate buffer pH 4.2 were mashed and filtered into a 25 ml volumetric flask. The extract was centrifuged at 5000 rpm for 15 minutes to obtain supernatant. Citrate buffer pH 4.2 was added to the supernatant (1.0, 1.5, 2.0, 2.5, and 3 ml) in a 10 ml volumetric flask for antioxidant activity determination.

The moisture content determination

A total of 5 g of fruit was dried on 105°C at atmospheric pressure for 5 hrs, then weighed. Drying and weighing continued, every 1 hr, until a constant weight [5].

Qualitative analysis of reducing compounds

A total of 0.2 ml of 1% iodine solution was added to 2 ml of supernatant. The discoloration of the brown color was indicated the positive reaction.

Quantitative analysis of vitamin C

A total of 2 ml of 25 µg/ml methylene blue and 2 ml of distilled water incubated for 10 minutes. The absorbance was measured at 400-900 nm with spectrophotometer against distilled water as blank. A total of 2 ml of 25 µg/ml methylene blue was added to 2 ml of standard vitamin C (2, 4, 6, 8, and 10 µg/ml) or supernatant in 10 ml volumetric flask, accomplished with citrate buffer pH 4.2. The mixture was incubated for 10 minutes, then the absorbance was measured at maximum wavelength [6].

Antioxidant activity determination

The modified method of Molyneux (2004) was conducted [7]. A total of 1.5 ml of 40 µg/ml DPPH was added to various concentrations of 1.5 ml of vitamin C or supernatant. The mixtures were incubated in a dark chamber for 30 minutes, then absorbance was measured at 517 nm using spectrophotometer (Ray Leigh). The blank was 96% ethanol. Percentage of antioxidant activity was calculated using this formula:

$$\% \text{ of DPPH inhibition} = \frac{(Ab - Aa)}{(Ab)} \times 100$$

Aa and Ab are the absorbance values of the sample and the blank, respectively. A percent inhibition versus concentration was plotted, and the concentration of sample required for 50% inhibition was determined and expressed as inhibitory concentration 50% (IC₅₀) value.

Statistical analysis

The results were presented as the mean ± standard error of the mean. Data comparisons between groups were done by one-way ANOVA

followed by t-test. Values were considered statistically significant at $p < 0.05$.

RESULTS

A total of 10.127 ± 0.005 g of water apple fruit was produced the turbid pink extract, whereas 10.036 ± 0.232 g of fragrant mango was produced the turbid yellow extract. The turbid extract was centrifuged to obtain a clear supernatant, pink for water apple and yellow for fragrant mango. The moisture content for water apple and fragrant mango was $92.847 \pm 0.059\%$ and $84.927 \pm 0.099\%$, respectively. Water apple and fragrant mango supernatant were given the positive results.

There were statistically significant differences between groups on vitamin C content ($p = 3.65 \times 10^{-4}$) and IC_{50} value ($p = 2.19 \times 10^{-6}$).

DISCUSSION

Citrate buffer pH 4.2 was used to extract the reducing compounds, especially vitamin C, which is present in the fruit. Citrate buffer pH 4.2 was selected because of vitamin C is stable at acidic pH. Aqueous solutions are most stable at pH 5-6 and very unstable at alkaline pH [8]. The water apple and fragrant mango extracts were turbid, so centrifugation was conducted to separate the supernatant which contains the secondary metabolites from the residues. The moisture content for both fruits was high (more than 80%), which shown the content of water and volatile compounds in fruits. These values were determined to calculate the vitamin C content in dried plant materials because the used samples were fresh fruits.

Qualitative analysis of reducing compounds, including vitamin C in the supernatant, was showed the positive results. There was the oxidation-reduction reaction, the vitamin C is oxidized by the iodine into dehydroascorbic acid, and the iodine is reduced [9]. These results indicate the presence of a reducing compound in the supernatant which can react with DPPH as free radical oxidants.

Table 1: Vitamin C content in fruit

Sample	Absorbance	Vitamin C content ($\mu\text{g/ml}$)	Vitamin C content (% w/w)
Water apple	0.886 ± 0.001	4.047 ± 0.115	0.087 ± 0.002
Fragrant mango	0.894 ± 0.001	2.313 ± 0.115	0.050 ± 0.002

Values are mean \pm SD (n=3), SD: Standard deviation

Quantitative analysis of vitamin C was based on oxidation-reduction reactions which observed from discoloration of methylene blue to leucomethylene blue, from blue to colorless [6]. The methylene blue solution was provided the maximum wavelength at 664.4 nm. This wavelength was consistent with the literature [6]. The instrument response was proportional to the vitamin C content, due to the correlation coefficient was 0.9993 (Fig. 1) which meets the criteria by ICH [10].

The vitamin C content in both fruits was small (Table 1) because of the used sample was fresh fruit with high moisture content. If converted to a dried plant material, the vitamin C content for water apple and fragrant mango was $1.216 \pm 0.001\%$ and $0.332 \pm 0.001\%$ (w/w), respectively. High vitamin C content in water apple due to the time harvest was the harvest season for water apple in July to August in Indonesia [11]. Low vitamin C content in fragrant mango due to the time harvest was not the harvest season, i.e., from September to December in Indonesia [12]. This study was conducted in July, so we suggested that the secondary metabolites content in fragrant mango were not maximally produced.

The chemical composition of horticultural crops was influenced by climatic conditions including light and average temperature [13]. The intensity and amount of light during the growing season influence the vitamin C content because of vitamin C was synthesized from sugars through photosynthesis. The outside fruit which exposed to maximum sunlight contains a higher amount of vitamin C than inside and shaded fruit on the same plant [14]. Climatic conditions and cultural practices also influence the preharvest [8,15]. The plant maturity when harvested, harvesting method, postharvest handling conditions [16],

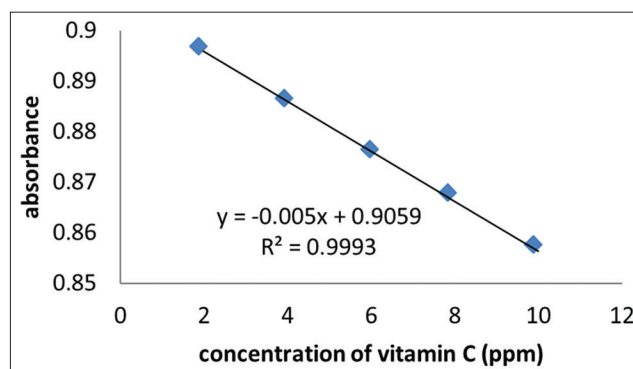


Fig. 1: Calibration curve of standard vitamin C (n=3)

Table 2: The antioxidant activity

Sample	Concentration ($\mu\text{g/ml}$)	Absorbance	% inhibition	Linear regression equation	IC_{50} ($\mu\text{g/ml}$)
Vitamin C standard	2	0.432 ± 0.003	32.448 ± 0.477	$y = 7.7318x + 17.755$ $R^2 = 0.998$	4.170
	4	0.325 ± 0.011	49.167 ± 1.685		
	6	0.221 ± 0.005	65.521 ± 0.786		
	8	0.138 ± 0.003	78.490 ± 0.502		
	10	0.031 ± 0.005	95.104 ± 0.705		
Water apple fruit	4	0.571 ± 0.002	45.587 ± 0.220	$y = 6.0857x + 20.444$ $R^2 = 0.995$	4.857
	5	0.523 ± 0.002	50.159 ± 0.198		
	6	0.461 ± 0.002	56.127 ± 0.198		
	7	0.383 ± 0.003	63.556 ± 0.240		
Fragrant mango fruit	8	0.318 ± 0.004	69.714 ± 0.343	$y = 9.2476x + 9.8794$ $R^2 = 0.990$	4.379
	2	0.766 ± 0.014	16.571 ± 1.746		
	3	0.645 ± 0.001	27.016 ± 1.361		
	4	0.537 ± 0.001	38.540 ± 0.110		
	5	0.475 ± 0.001	48.889 ± 0.110		
	6	0.366 ± 0.004	54.762 ± 0.095		

Values are mean \pm SD (n=3), SD: Standard deviation

storage duration and conditions, processing, and physical damage [17] also affect the vitamin C content.

The antioxidant activity with the DPPH method was characterized by the color alteration, from purple to yellow after incubation for 20 minutes. It is because of the free radical DPPH was reduced to DPPH [18]. Table 2 showed that IC_{50} value for water apple and fragrant mango was similar as standard vitamin C. Both fruits were classified as a very strong antioxidant because the IC_{50} value is less than 50 $\mu\text{g/ml}$ [18] due to high vitamin C content (Table 1). The reactive free radical (DPPH) was reduced by vitamin C (ascorbic acid), and the ascorbyl radical formed in its place is less reactive [19]. Then, the ascorbyl radical loss of a second electron to form the dehydroascorbic acid, which its stability (only a few minutes) depends on factors such as pH and temperature [20]. The vitamin C content and the IC_{50} value for both fruits were statistically significant, due to harvest time which also influenced by climate.

CONCLUSION

The vitamin C content and antioxidant activity of water apple fruit were higher than fragrant mango fruit.

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