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Correlation between Antioxidant Activity, Total Flavonoid and Green Color Index, Bitterness Value of *Carica papaya* Leaves

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Abstract:
Papaya leaves is a common vegetable consumed by the people of Indonesia, especially Java people. Carica papaya leaves is known to contain flavonoids as antioxidants in the body. The diversity of varieties of Carica papaya leaves in Indonesia raises questions from the public about how to pick a papaya leaves that has flavonoids and high antioxidant activity. Selection of papaya leaves is easy to do with the physical properties of papaya leaves is the color and the level of bitterness papaya. This study aims to determine the relationship between antioxidant activity and total flavonoids in green color index and the degree of bitterness papaya. Papaya leaves used in the study is papaya Grendel, Bangkok, Purple, California and Gold. Analysis of antioxidant activity using DPPH and FRAP method while total flavonoid testing using spectrophotometric method. Analysis of papaya leaves color index using chroma meter, while the bitterness value using Europian Pharmacopoeia method. The results showed antioxidant activity and total flavonoids five varieties of papaya leaves significantly different and the highest is papaya Grendel leaves. Green color index five varieties of papaya leaves significantly different, and the greenest is papaya Grendel leaves. The bitterness value of five varieties of papaya leaves significantly different and the lowest degree of bitterness is papaya Gold leaves and papaya Grendel leaves. Based on the results of this study can be concluded that there is correlation between antioxidant activity and total flavonoids with green color index. There is a correlation between antioxidant activities and bitterness value. It means high green color index of papaya leaves have high antioxidant activity and papaya leaves with low bitterness value have high antioxidant activity.

Keywords*: green color index, bitterness value, antioxidant, flavonoid, papaya*

1. Introduction

Papaya leaves is one vegetable that consume by Indonesian especially Javanese people. Papaya *(Carica papaya*) included in the family of *Caricaceae* and some types *Caricaceae* has been used for treatment against various diseases (Mello et al., 2008). *Carica papaya* come from southern Mexico and has spread beyond the tropics. All parts of the papaya plant can be used for medicinal include pulpy, seeds and flowers. Several studies have been conducted to test the biological activity as part of the papaya fruit, shoots, leaves, rinds, seeds, roots and latex (Maisarah et al., 2008).

Polyphenols, flavonoids, vitamin C and Vitamin E are phytochemicals in foods that have been known as a natural antioxidant that has antioxidant activity. Polyphenols and flavonoids are known to have chemopreventive activity as ROS scavenger (Lee and Lee, 2006; Moon et al., 2006). Reactive oxygen species (ROS) play a role in various pathological processes including cancer, aging, and atherosclerosis (Valko et al., 2004). Chronic disease can be reduced by ROS scavenging. Some studies suggest that increased intake of foods containing polyphenols and flavonoids can reduce oxidative stress, inflammation, tumor and coronary heart disease (Moon et al., 2006; Poulsen et al., 1998; Laranjiha et al., 1994). Antioxidant function in reducing DNA damage, lowering lipid peroxidation, maintaining the immune system and inhibit the malignant transformation of cells (Gropper et al., 2009).

Carica papaya leaves is known contain flavonoids as antioxidants in the body. The diversity of varieties of *Carica papaya* leaves in Indonesia raises questions from the public about how to pick a papaya leaves that has flavonoids and high antioxidant activity. Selection of papaya leaves is easy to do with the physical properties of papaya leaves is the color and the level of bitterness papaya. This study aims to determine the relationship between antioxidant activity and total flavonoids in green color index and the degree of bitterness papaya.

2. Materials and Methods

2.1. Materials

Carica papaya leaves are used in this study were obtained from local farmers in Yogyakarta with five varieties Gold, Purple, California, Bangkok and Grendel. Sampling of *Carica papaya* leaves in this study based on observation of the number of leaves in the tree then divided 3 parts. Number of papaya leaves in every tree ranged between 18-25 leaves. The calculation of the number of leaves starting from the leaves that had bloomed. *Carica papaya* leaves were taken from the seventh or eighth from above.

2.2. Determination of Bitterness Value

The degree of bitterness papaya extract is determined by sensory methods of the European Pharmacopoeia (2005). The determination threshold using the standard bitter quinine HCl in various concentrations i.e. 3.6 ml to 5.8 ml solution of quinine HCl 10 µg/ml of distilled water plus 6.4 ml to 4.2 ml. After determination of threshold bitter, dilute papaya extract by adding 100 ml of hot water into 1 gram of extract and heated in a water bath temperature of 50^0C then filtered. 2 ml of the initial filtrate discarded and the volume is rounded up to 100 ml. This solution became a stock solution for further dilution by taking a certain volume and is made in the range as in Table 1.

	Volume (ml)						
Sample	1,2	1,5	2	3	6	8	10
Water	8,8	8,5	8	7	4	2	0

Table 1: Volume of sample solution and water

The entire solution was tested at a minimum of six panelists, each panelist determined correction factors: k = n / 5, n is the number of ml solution in the beginning to be felt bitter by the panelists. The degree of bitterness is calculated using the formula: (Y x k) / (X x 0.1), Y: the dilution factor, X: ml were beginning to be felt bitter, k: correction factor

2.3. Determination of Green Color Index

Carica papaya leave green color index is determined by using Chroma meter. Chroma meter is a tool used to measure the color of the surface of an object. The basic principle of this tool is the interactions between light energy diffuse with atoms or molecules of the object being analyzed. Color analysis using Chroma meter based on three parameters: *L* value that indicates the brightness level of the sample. *L* grade scale ranging from 0 to 100 samples darkest to brightest sample. The second parameter is the value of *a* shows the chromatic color mixture of red and green. The positive value of *a* means that color samples tend to be colored red, while the value of *a* negative sample color tends to green. Green color index is determined by using the numbers listed on Chroma meter.

2.4. Determination of Antioxidant Activity

Determination antioxidant activity using DPPH assay and FRAP assay. Sample preparation was conducted by extraction 1 g powdered papaya leaves with water by microwave. DPPH assay was conducted by adding 1.5 ml of freshly DPPH solution (20 µg/ ml) to 0.75 ml of extract sample and vortex. Absorbance reading of sample and control was conducted after 5 min incubation in dark condition at 517 nm (Taie et al., 2008). Antioxidant activity = [(control absorbance - sample absorbance) / control absorbance] × 100%.

Determination of antioxidant activity using FRAP assay refers to the method Vichitphan et al. (2007) with FeSO4.7H2O as standard. Antioxidant capacity of the samples was determined by the ability of antioxidant compounds to reduce the sample ions Fe^{3+} to Fe^{2+} (Halvorsen et al., 2002). Preparation of FRAP reagent performed with mixing a solution of 0.1 M acetate buffer (pH 3.6), the solution 2,4,6-tripydyl-s-triazine (TPTZ) 10 mM in 40 mM HCl as many as 0.15 grams TPTZ 10 mM dissolved in 50 ml of HCl 40 mM and 20 mM $FeCl_3$.6H2Osolution with a volume ratio of 10: 1: 1. A total of 50 mL and 150 mL of distilled water sample is added to the tube which already contains 1.5 ml of reagent FRAP. Solution then was incubated for 8 minutes in a dark room and the room temperature. Absorbance of the sample was measured at a wave length of 594 nm and the results are calculated in equivalent of Fe^{2+} using a standard curve equation $FeSO_4.7H_2O$ with concentration range 4-24 mol/ml.

2.5. Determination of total flavonoid

Total flavonoid was analyzed using the method of Taie et al. (2008) 0.5 ml $AlCl_3$ 2% in ethanol solution was added to 0.5 ml of papaya leaves extract. Absorbance reading was conducted after 1 hours at room temperature at 420 nm. Total flavonoid was calculated using a standard curve equation of quercetin with a concentration range from 0.01 to 0.08 mg/ml.

2.6. Statistical Analysis

Results of tables are presented as mean ± standard deviation and the analyses were done in triplicate. Data obtained were tabulated and analyzed using variance analysis (ANOVA). The differences of the result would be tested using Post Hoc Duncan.

3. Results and Discussions

3.1 Green color Index

Table 2 shows the green color index of papaya leaves with different varieties. Grendel papaya leaves have a green color index higher than others. It means that the Grendel papaya leaves more green than others. The green color of the papaya leaves is affected by chlorophyll content. The chlorophyll content of a barley plant can be determined by analyzing the leaf image color or color index (Hu et al., 2010). Chlorophyll is the green photosynthetic pigments that determine the nutrient content in plant leaves. The content of chlorophyll has been used as an evaluation of nutritional status in some plant varieties (Argenta et al., 2001). Based on research results of Setiari and Nurchayati (2009) papaya leaves contain chlorophyll higher than spinach leaves, gotu kola leaves, cassava leaves, basil leaves and grass jelly. Chlorophyll content is influenced by several factors such as the age of the plant, age leaves, leaf morphology and genetic factors (Biber, 2007; Setiari and Nurchayati, 2009) The results of this study prove that genetic factors or varieties affect the content of chlorophyll in the leaves of papaya indicated by the green color index.

Papaya leaves have a bitter taste, which is why the papaya leaves less favored by Indonesian people. Phytochemical analysis results of papaya leaves showed that papaya leaf contains saponins, glycosides and alkaloids, but does not contain tannin (Ayoola and Adeyee, 2009). A bitter taste in papaya leaves suspected to be caused by the content of alkaloids. The bitter taste of papaya leaves can be removed by boiling the leaves of papaya with clay or cassava leaves or guava leaves. This study shows that variety of papaya leaves affect bitterness value of papaya leaves.

Variety	Green Color Index
California	2.01 ± 0.25^{a}
Gold	$2,31\pm0,17^{a}$
Bangkok	$4,09\pm0,27^{b}$
Grendel	$10,09\pm0,21^{a}$

Table 2: Green color index of Carica papaya leaves extract at different variety

3.2. Bitterness Value of Papaya Leaves

Table 3 show the bitterness value of Grendel papaya leaves and Gold papaya leaves lower than California, Bangkok and and Purple papaya leaves. Papaya leaves that have a low degree of bitterness has the potential to be developed into functional foods. Bitterness value of papaya leaves is lower than traditional tonic plants of southern Africa in the range 5.000-10.000 (Olivier and Wyk, 2013). This study shows that papaya leaves with low bitterness value has high antioxidant activity. The causes of why high antioxidant activity of papaya leaves have low bitterness value not yet known. Allegedly caused by the content of alkaloids in papaya leaves that can inhibit the antioxidant activity of papaya leaves active compound. Bitter taste may be masked by compounds producing tastes such as saltiness, sweetness, sourness and astringency by competing for receptor sites (Ley, 2008; Reinberger, 2006). Some compounds (such as phospholipids) are known to selectively inhibit bitterness without affecting others tastes (Katsuragi, 1997). Antibacterial, antifungal, antioxidant and hepatoprotective properties have also been observed for bitter substances, apart from digestive stimulation (Kusar et al., 2006; Kondo et al., 1994). The results of this study are very beneficial to humans because papaya is not bitter have high antioxidant activity.

Variety	Bitterness Value
California	$340,63\pm31,44^{bc}$
Gold	$241,88\pm22,66^{a}$
Bangkok	$381,50\pm67,91^{c}$
Purple	$384,56\pm11,84^{c}$
Grendel	$262,74\pm14,98^{ab}$

Table 3: Bitterness value of Carica papaya leaves extract at different variety

3.3. Antioxidant Activity and Total flavonoid

Table 4 shows that Grendel papaya leaves has a higher antioxidant activity than other varieties by DPPH and FRAP method. The total flavonoid varied significantly among samples ranging from 36.93 to 76.69 µg/g of dry papaya leaves. Grendel papaya leaves have total flavonoid highest.

Varieties	DPPH (%)	FRAP (mmol/mg)	Total Flavonoid (µg/g)
California	71.15±0.00^{ab}	16.64±0.71^{a}	46.02±3.38^{ab}
Gold	78.37±3.40^{c}	24.65±4.74^{ab}	36.93±2.72^{a}
Bangkok	67.31±0.00^{a}	19.25±1.16^{ab}	50,34±6,63^{b}
Purple	74.52±2.04^{bc}	30.68±4.99^{b}	60.97±0.31^{c}
Grendel	77.40±0.68^{c}	42.18±6.72^{c}	76.69±0.13^{d}

Table 4: Antioxidant activity and total flavonoid of Carica papaya leaves extract at different variety

3.4. Correlation between green color index, bitterness value and antioxidant activity and total flavonoid

Based on the results of correlation analysis known that there is a correlation between antioxidant activity by FRAP method and total flavonoids with green color index in papaya leaves. There is correlation between antioxidant activity by DPPH method and bitterness value in papaya leaves (Table 5).

This study shows that papaya leaves with high green color index has high antioxidant activity and high total flavonoid. Chlorophyll is one of the most valuable components of plants and foods high in their content can prevent a number of diseases (Rosu et al., 2012; Usatiuk et al., 2014). Different with the previous study shows that low chlorophyll leaf has higher antioxidant activity by DPPH than high chlorophyll leaf with percentage inhibition of 75.73±1.10% and 58.62±1.13% respectively in *Moringa oleifera* leaf(Abdulkadir et al., 2015). Pattanayak et al. (2012) reported that high antioxidant activity by DPPH associated with lower IC_{50} value. Sreelatha and Padma (2009) reported that high chlorophyll leaf or matured leaf has low IC_{50}. Oloyede et al (2013).Also, reported that high chlorophyll leaf or matured leaf of *Amaranthus cruentus* and *Celosia argentea* have high antioxidant activity. But the result of the flavonoids content same with this study, where high chlorophyll leaf or matured leaf have flavonoids content three times higher than low chlorophyll leaf (Abdulkadir et al., 2015). Different with study result by Simao et al. (2013) that the content of the antioxidant compounds has negative correlation with chlorophyll means that when the amount of antioxidants increased chlorophyll decreased in plants. Based on these results it can be concluded that the green colored leaves that contain high chlorophyll and chlorophyll is the active compound has antioxidant activity that have benefit for health.

	Antioxidant Activity by DPPH	Antioxidant Activity by FRAP	Total Flavonoid
Green color index	p=0.374 r=-0.316	p=0.001 r=0.872	p=0.000 r=0.938
Bitterness value	p=0.018 r=-0.723	p=0.341 r=0.316	p=0.896 r=-0.048

Table 5: Correlation between of green color index, bitterness value and antioxidant activity, total flavonoid of Carica papaya leaves extract at different variety

4. Conclusion

The green color index of papaya leaves with different varieties in a range 2-10 and Grendel papaya leaves have a green color index higher than others. The bitterness value of papaya leaves with different varieties in a range 200-400. Grendel and Gold papaya leaves have bitterness value lower than California, Bangkok and and Purple papaya leaves. There is correlation between antioxidant activity and total flavonoids with green color index of papaya leaves. There is a correlation between antioxidant activities and bitterness value of papaya leaves.

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