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Antioxidant and Tyrosinase Enzyme Inhibition Activity of Lime Peel and Seed Ethanol Extract

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Abstract

UV Light exposure and Oxidative stress can cause the accumulation of free radicals and increase melanogenesis. The various herbal plant enriches Indonesia. One of them is lime that content various phytochemicals. This study was aimed to explore antioxidants property and tyrosinase enzyme inhibition activity of lime peel and lime seed. This study used ethanol extract from lime peel and seed. The antioxidant activity was evaluated by the ABTS reduction assay, while tyrosinase enzyme inhibitor activity by tyrosinase enzyme from fungi. The results of this study showed that the IC50 Value of Lime Seed (14.09 ± 0.88 µg/mL) was lower than lime peel (20.16 ± 0.23 µg/mL). Meanwhile, the IC50 value against tyrosine enzyme of Lime Peel (34.23 ± 0.93 µg/mL) was lower than lime seed (67.88 ± 3.73µg/mL). Hence lime peels potentially become a tyrosinase enzyme inhibitor agent while the lime seed has better antioxidant potential than the peel.

Keywords: lime; peel; seed; ABTS; tyrosinase enzyme.

1. Introduction

Exposure of UV light supports the effect of free radical primarily Reactive Oxygen Species (ROS) and increases melanin synthesis (melanogenesis) that is catalyzed by tyrosinase enzyme. The melanin is responsible for skin color appearance and protection of the skin against UV light. There are two types of melanin include eumelanin and pheomelanin, that are conjugated by cysteine or glutathione.

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The initiation of melanogenesis is oxidation of L-tyrosinase to dopaquinone by the key enzyme (Tyrosinase enzyme), and due to some reaction, it will form L-DOPA that is also a substrate for tyrosinase enzyme. Some enzymes that take a role in melanogenesis are tyrosinase enzyme, tyrosinase-related protein-1, and tyrosinase-related protein-2 [1,2]. Tyrosinase enzyme is a critical enzyme in melanogenesis due to most of the reactions are catalyzed by this enzyme. Tyrosinase enzyme (polyphenol oxidase) is a copper-containing monooxygenase at melanosome that catalyzed two different reactions includes hydroxylation of tyrosine into dopaquinone and oxidation of dopachrome and L-DOPA into dopaquinone. Furthermore, dopaquinone will form eumelanin through some reaction [2,3]. Other than UV light exposure, the accumulation of free radicals can increase the melanogenesis process. Furthermore, excessive levels of free radicals can enhance several skin diseases by oxidative injury like fine wrinkles of the skin around the eyes, forehead, and around the mouth that need to treat. Meanwhile, the increase of melanogenesis can cause an increase of melanin in the skin and cause a severe esthetic problem [4,5]. Recently, the modality of treatment has been back to nature, the higher demand for the herbal product. Data from *Promoting the Role of Traditional Medicine in Health Systems: Strategy for the African Region* showed that around 80% of people in some countries group of WHO in Africa used traditional medicine for health. Indonesia is the second most extensive tropic country that rich by various herbal plants around 2,500 species plant [6,7]. Lime is one of the fruit as a herbal plant that rice by Vitamin C and antioxidants that have several health benefits. Meanwhile, the extract and essential oil of lime peels are used to be food and beverages additive. The lime peels have some phytochemicals like limonene, terpinene, and linalyl acetate [8]. Based on the facts above, this study was aimed to explore the antioxidant and tyrosinase enzyme inhibition activity from lime peel and seed.

2. Methods

2.1. Material

Lime, 70% Ethanol Solution, DMSO Solution, ABTS Solution, phosphate buffer solution, tyrosinase enzyme, and L-DOPA.

2.2. Preparation of sample

The lime as the sample was collected from a traditional market in Bandung town, West Java. The peel and seed of lime were sorted and dried by food dehydrator. Then it was mesh and form Simplicia powder. The simplicia powder was macerated by 70% ethanol for 24 hours, and it was filtrated. The residue was remacerated using the same solvent for the next two days. All filtrate from macerated and remacerated was collected and evaporated using a rotary evaporator at 50oC. After that, it formed a concentrated form. The concentrated form was dissolved by DMSO into several concentration include 50 µg/ml, 25 µg/ml, 12,50 µg/ml, 6.25 µg/ml, 3.13 µg/ml, and 1.56 µg/ml for ABTS reduction assay meanwhile other serial concentration include 100 µg/dl, 50 µg/dl, 25 µg/dl, 12.5 µg/dl, 6.25 µg/dl, 3.13 µg/dl, and 1.56 µg/dl for anti-tyrosinase enzyme assay [9–12].

2.3. ABTS Reduction assay

The amount of 2 µl sample filled into 96 well microplates, and it was mixed with 198 µl ABTS Solution, while

it also prepared some plate that was filled by 200 µl ABTS solution as a control. The absorbance of the sample was measured by spectrophotometry at 745 nm wavelength. The following formulation was used to determine the inhibition percentage of the sample against ABTS [9,10]:

Inhibition percentage = [(Abs Control- Abs Sample)/Abs Control] x 100%

2.4. Tyrosinase Enzyme Inhibition assay

The amount of 120 µl of the sample was mixed with 80 µl phosphate buffer solution (pH 6.8) and 40 µl tyrosinase enzyme, and it was incubated for 5 minutes at room temperature. After that, it was added 40 µl L-DOPA (Sigma), and it was incubated for 30 minutes at room temperature. The absorbance of the sample was measured by spectrophotometry at 510 nm wavelength. The following formulation was used to determine the inhibition percentage of the sample against the Tyrosinase [13,14].

Inhibition percentage = [(Abs Control- Abs Sample)/Abs Control] x 100%

2.5. Data Analysis

The inhibition percentage of the sample against ABTS and tyrosinase enzyme were analyzed using descriptive analysis (Mean and SD), One-Way ANOVA and followed by Post Hoc Test Tukey HSD at 95% confidence interval (α=0.05). Based on inhibition percentage, it was determined the Inhibition Concentration 50 (IC50) for each assay by linear regression.

3. Result and Discussion

3.1. Antioxidant Properties

The ABTS reduction assay determined the antioxidant activity. The following table shows the result of the ABTS assay.

Table 1: Inhibition Percentage of Lime Seed and Peel against ABTS as Antioxidant Properties (Post Hoc Test Tukey HSD)

Concentration (µg/ml)	Inhibition Percentage (%)	
	Seed	**Peel**
50	88.73 ± 0.60^{e}	77.69 ± 0.9^{e}
25	63.70 ± 1.16^{d}	54.24 ± 3.4^{d}
12.5	48.92 ± 2.52^{c}	44.26 ± 1.2^{c}
6.25	42.35 ± 2.18^{b}	38.16 ± 8.8^{b}
3.13	36.83 ± 2.49^{a}	33.63 ± 0.6^{a}
1.56	34.79 ± 1.75^{a}	30.37 ± 2.2^{a}

Data were expressed as Mean ± SD. The difference between small letters in the same column showed

significance at P-Value < 0.05.

Based on the table above, there are significant differences in each concentration of the sample, except at least two lower sample concentrations (3.13 µg/ml and 1.56 µg/ml), while the increasing percentage ABTS inhibition follows the higher concentration of the sample. Furthermore, the analysis is followed by linear regression for the determination of IC_{50} Value against ABTS. The following table shows the result of the analysis.

Table 2: IC50 Value against ABTS for Lime Seed and Peel (Linear Regression)

Sample	Equation	R^2	IC50 (µg/mL)	IC50 (µg/mL)
Seed (1st Repetition)	Y= 1.102x + 35.08	0.99	13.53	14.09 ± 0.88
Seed (2nd Repetition)	Y= 1.087x + 35.17	0.98	13.64	
Seed (3rd Repetition)	Y= 1.131x + 32.90	0.99	15.11	
Seed (Mean)	Y= 1.107x + 34.38	0.99	14.10	
Peel (1st Repetition)	Y= 0.984x + 30.01	0.99	20.30	20.16 ± 0.23
Peel (2nd Repetition)	Y= 0.936x + 31.32	0.99	19.93	
Peel (3rd Repetition)	Y= 0.905x + 31.46	0.98	20.48	
Peel (Mean)	Y= 0.942x + 30.93	0.99	20.24	

Based on the table above, the IC50 Value of Lime Seed (14.09 ± 0.88 µg/mL) is lower than lime peel (20.16 ± 0.23 µg/mL). So, it needed a lower concentration of Lime seed than lime for inhibition half of ABTS as free radical. Hence the lime seed is better than lime peel for antioxidant assay based on ABTS assay.

3.2. Tyrosinase Enzyme Inhibition Properties

The result of Tyrosinase enzyme inhibition assay was showed as the inhibition percentage. The following table shows the result of the analysis.

Table 3: Inhibition Percentage of Lime Seed and Peel against Tyrosinase Enzyme as Antioxidant Properties (Post Hoc Test Tukey HSD)

Concentration (ug/ml)	Inhibition Percentage (%)	
	Peel	Seed
100	84.14 ± 2.66^{e}	69.45 ± 4.58^{e}
50	60.02 ± 1.72^{d}	39.63 ± 6.48^{d}
25	47.51 ± 3.48^{c}	24.13 ± 3.49^{c}
12.5	40.24 ± 1.24^{b}	$16.75 \pm 0.63^{b,c}$
6.25	33.98 ± 0.48^{a}	$11.28 \pm 1.65^{a,b}$
3.13	32.50 ± 1.31^{a}	$9.94 \pm 0.78^{a,b}$
1.56	30.10 ± 1.41^{a}	6.05 ± 0.20^{a}

Data were expressed as Mean ± SD. The difference between small letters in the same columns showed significance at P-Value < 0.05.

Based on the table above, there is a significant difference that is observed mainly at a higher concentration than in lower concentration, while the increase of the percentage tyrosinase enzyme inhibition followed by the higher concentration of the sample. Furthermore, the linear regression was used to determination of IC50 value.

Table 4: IC50 Value against Tyrosinase Enzyme for Lime Seed and Peel (Linear Regression)

Sample	**Equation**	**R^2**	**IC50 (µg/mL)**	**IC50 (µg/mL)**
Peel (1st Repetition)	Y = 0.544x + 31.39	0.99	34.37	34.23 ± 0.93
Peel (2nd Repetition)	Y = 0.517x + 31.92	0.98	35.08	
Peel (3rd Repetition)	Y = 0.555x + 31.63	0.98	33.24	
Peel (Mean)	Y = 0.539x + 31.64	0.99	34.21	
Seed (1st Repetition)	Y = 0.584x + 7.917	0.99	72.00	67.88 ± 3.73
Seed (2nd Repetition)	Y = 0.657x + 6.011	0.98	66.93	
Seed (3rd Repetition)	Y = 0.636x + 8.778	0.98	64.72	
Seed (Mean)	Y = 0.626x + 7.569	0.99	67.76	

Based on the table above, the IC50 value against tyrosine enzyme of Lime Peel (34.23 ± 0.93 µg/mL) is lower than lime seed (67.88 ± 3.73µg/mL). So, it needed a lower concentration of Lime seed than lime for inhibition half of the tyrosinase enzyme. Hence the lime peel is better than lime seed as an inhibitor of tyrosinase enzyme [15].

The result of this study was similar to the report of Stevenie and his colleagues (2019) that antioxidant property by hydrogen peroxide assay of lime seed was better than lime peel, due to the IC50 value (194.82 ± 1.49 µg/mL) of lime seed that was lower than IC50 value of lime peel (246.84 ± 4.08 µg/mL). Other than antioxidant properties, Stevanie and his colleagues (2019) also explored the anti-aging effect of lime seed and peel by inhibition of elastase enzyme assay, which showed the inhibition of elastase enzyme was higher in the lime peel than the lime seed. It was shown by the IC50 value of Lime peels that were lower than the IC50 value of lime seed [15]. The higher inhibition activity against the tyrosinase enzyme of lime peel than lime seed. It due to the presence of inhibitor melanogenic phytochemical in the lime peel include flavonoid and triterpenoid. Other phytochemical compounds that have inhibitor melanogenic properties include isoflavone that is the derivate of flavonoid, chalcone, stilbene, and polyphenols [16]. Other than an antioxidant, elastase, and tyrosinase enzyme inhibition properties, the lime also has antibacterial property against several bacterial pathogens include *Staphylococcus aureus, Salmonella thypii, Bacillus subtilis,* and *Escherichia coli* [17] and mosquito (*Aedes aegypti L*) repellent properties [18].

4. Conclusion

The lime seed has a better antioxidant effect than lime peel. However, the lime peel has better tyrosinase

enzyme inhibition than lime seed.

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