American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Comparison Activities of Peel and Extract of Lime (*Citrus amblycarpa*) as Antioxidant and Antielastase

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Abstract

Aging of the skin is a physiological process that occurs in all living things, along with increasing age, hormones, nutrition, and exposure to sunlight both directly and indirectly. This process will form Reactive Oxygen Species ROS, free radicals that interfere with one of the most important enzymes in the cell matrix, namely the elastase enzyme. Free radicals can be neutralized by the presence of antioxidants. Lime plants have natural phytochemical compounds that act as antioxidants and anti-aging. This study was to compare the antioxidant activity and anti-elastase from the peel and lime seed extract (*Citrus amblycarpa*) by conducting H2O2 trapping tests and inhibition of elastase. The test results of antioxidant activity and anti-aging trapping of H2O2 lime seed extract had an IC50 value lower than lime peel extract, with yields of 194.82 µg / ml better than those of lime skin extract of 246.84 µg / ml. The results of the elastase inhibition activity test showed that lime peel extract had a lower IC50 value of 36.94 µg / ml compared to lime seed extract with an IC50 value of 62.39 µg / ml. A lime seed extract has antioxidant activity through better H2O2 trapping compared to lime peel extract while lime skin extract has antiaging activity through inhibition of elastase which is better than lime seed extract.

Keywords: Lime extract; Antioxidant ; Antielastase.

1. Introduction

The aging process is a physiological process that will occur in all living things that are included in all organs including the skin. Every human being certainly looks young, but the aging process happens gradually and gradually goes on and the skin is one of the body's tissues that directly addresses the aging process. Even though aging is something that must happen, but efforts to prevent it never recede.

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Various ways can be done to keep skin healthy and young [1].The external factor of the aging process comes mostly from free radicals. These free radicals will induce the formation of ROS (Reactive Oxygen Species). Free radicals are an atomic molecule that has electrons that have no partners [2]. The formation of ROS if overproduced causes oxidative stress. Oxidative stress is an imbalance between the amount of ROS and antioxidant activity in the body [3].Antioxidants are substances that can neutralize free radicals so that unpaired atoms get an electron pair so they are no longer reactive. Aging of the skin itself can be caused by internal factors which are interpreted as natural aging and stimulated by changes in skin elasticity. Elastase is part of the chymotrypsin protease which functions to breakdown elastin which is an extracellular matrix protein. This protein can break down elastin, collagen, fibronectin and other proteins [4]. The elastin decomposition process is caused by activated elastase enzyme by UV or ROS so that it can inhibit elastase activity to avoid the aging process [5].Today, technological advances have developed rapidly, especially in the field of beauty. Many cosmetics industries use chemicals that are unsafe and have harmful side effects for the skin when used in the long term so that other alternative ingredients are safe to use. Lime is one source of antioxidants that are beneficial for health and are often used as drinks. Lime is widely found in lowland areas up to 300 mpdl highlands. The place to grow lime is a place that is heavily exposed to the sun [6].This is the background of the authors to examine ingredients that are safe, affordable and can be a natural treatment for the skin. In this case, this study aims to determine the antioxidant activity and antielastase activity in the peel and seeds of lime.

2. Material and Method

2.1 Materials

Micropippete 200 µl, 1000 µl (Eppendorf), Multichannel pippete 30-300 µl (Thermo Fisher Scientific), Multiskan GO Reader (Thermo Scientific 1510), Vortex (WiseMix VM-10), 96-well plate (TPP 92096), Falcon tube 15 ml (SPL 50015), Falcon tube 50 ml (SPL 50050), Tube Effendorf 1,5ml (SPL 60015-1), Tips kuning (NEPTUNE), Tips biru (NEPTUNE), pH meter (OHAUS Starter300 portable), Tabung Erlenmeyer, Spatula, Magnetic stirrer and hot plate (Thermo Fisher Scientific), Multiskan Go Reader (Thermo Fisher Scientific 1510), Incubator (ESCO IFA-32-8), Mikropipet (1-10 µl, 50- 200 µl, 100-1000 µl) (Eppendorf), 96well-plate (TPP 92096), Falcon tube 15 ml (SPL 50015), Falcon tube 50 ml (SPL 50050), Analytical Balance (AXIS), Vortex (WiseMix VM-10), Ferrous Ammonium Sulfate (Sigma 7783859), Hydrogen peroxide (Merck 1.08597.1000), Asam sulfat (Merck 109981), 1,10-phenanthroline (Sigma 131377), Dymethilsufoxide (DMSO) (Merck 1.02952.1000 lot K46505352), Akuades, Pemerangkapan H_2O_2, N-Sucanyl-Ala-Ala-Ala-P-nitroanilide, elastase substrate(Sigma 54760), Elastase from porcine pancrease (Sigma 45124), Tris (Pharmacia biotech 17-1321-01), Sodium chloride (Merck), ddH_2O, Dymethilsulfoxide (Merck), Hydrochloride acid solution (Merck).

2.2 Preparation of peel and seed of lime extract

lime seeds and peel are washed with running water and dried using a food dehydrator. The purpose of drying is to get simplicia not easily damaged in long storage. Dry simplicia is made powder by grinding, then soaked in 70% ethanol. Making lime skin and seed extract using maceration technique with 70% ethanol solvent. The simplicia was weighed approximately 150 g, added with a solution of liquid, which is 70% ethanol as much as

500 mL, then the filtrate was accommodated 24 hours until the ethanol filtrate was colorless, then the ethanol filtrate was evaporated.

2.3 Phytochemical screening of peel and seed of lime extract

Phytochemical screening of extract ethanol kemangi leaf by using modification of fransworth method consisted of identification of phenol, steroids/terpenoids, saponins, flavonoids, tannin and alkaloids [7-9].

2.4 Antioxidant activity test by using H_2O_2 trapping method

The trapping of the radical activity of H2O2 was measured by the method described [10] by with a slight modification. The solution mixture is made according to the table 1 below:

Table 1: Solution

Reagen	control	Sample test	Blank
Sample	-	60 µL	60 µL
Ferrous Ammonium Sulfate (1 mM, Sigma 7783859)	12 µL	12 µL	-
DMSO	63 µL	-	90 µL
H_2O_2 (5 mM, Merck 1.08597.1000)	-	3 µL	-
1,10-*phenanthroline* (1 Mm, Sigma 131377)	-	75 µL	75 µL

Then after adding H2O2 the mixture of the control solution, sample and blank which was inserted into the 96-well plate was incubated for 5 minutes in a dark room with room temperature. Then each mixture of sample and blank was added 1,10-phenanthroline as much as 75 µL, then incubated again for 10 minutes in a dark room with room temperature. Absorbance is measured using a wavelength of 510 nm.The trapping activity of hydrogen peroxide (H2O2) was measured by ferrous ammonium sulfate and phenanthroline reaction methods with little modification. If ferrous ammonium sulfate reacts with phenanthroline it will form Fe2 + -tri-phenanthroline complex which is orange, but if it is H2O2 in the reaction complex is not formed, so if there are antioxidants that trap H2O2, Fe2 + -tri-phenanthroline complex which is orange that is.

Percentage activity of trapping calculated by this formula

% Trapping = [(A absorbance of control – Absorbance of samplel)/ absorbance of sample x 100]

2.5 Inhibition of elastase enzyme activity test (In-Vitro)

Inhibition of elastase enzyme activity was measured based on the method described by Sigma Aldrich and Thring and his colleagues (2009) with a few modifications. A mixture of solutions consisting of 10 µL samples (0.78 - 50 µg / mL), 5 µL Elastase from porcine pancreas enzyme (0.01 mg / mL, Sigma 45124) and 125 µL tris buffer (100 mM, pH 8, Pharmacia Biotech 17-1321 -01) incubated at 25 ° C for 15 minutes. Also, it was also

prepared for controls containing only 5 µL enzymes and 135 µL tris buffers and blanks containing only 130 µL tris buffers and 10 µL samples. Next, a mixture of 10 µL of the SucAla3-pNA substrate was added and the incubation was returned at 25 ° C for 15 minutes. The absorbance is measured using a wavelength of 410 nm.

Formula of inhibition of elastase :

$$\textbf{\% anti-elastase} = \frac{C-S}{C} \textbf{ x 100}$$

C : absorbance enzyme without samples

S : absorbance enzyme with samples

2.6 Statistical analysis

Test analysis was carried out by using one-way analysis of variance (ANOVA) followed by Post Hoc Test using the Tukey HSD test. $P<0.05$ was considered as statistical significance.

3. Result and discussion

3.1 Analysis of antioxidant activities by using H_2O_2 trapping method

The antioxidant activity of H2O2 in the extract of peel and seeds lime was analyzed by the H2O2 trapping method. Data from the analysis of antioxidant activity were analyzed by the Post Hoc Test Turkey HSD test, as shown in the table below.

Table 2: Results of analysis of post hoc test of tukey HSD test on antioxidant activity data with trapping method on peel and seed extract of lime

Final concentration (ug/ml)	Mean activity of trapping H_2O_2 (%) by samples	
	Peel extract of lime	Seed extract of lime
500	75.23 ±0.43^{f}	88.47 ±1.12^{f}
250	50.77 ±1.26^{e}	61.31 ±0.50^{e}
125	40.92 ±0.33^{d}	44.44 ±0.54^{d}
62.5	32.44 ±0.85^{c}	36.85 ±0.09^{c}
31.25	26.77 ±0.72^{b}	24.24 ±0.16^{b}
15.63	22.11 ±0.20^{a}	19.52 ±0.07^{a}

*Data were presented as mean ±standart deviation. Different small letters in the same colomn are significant at $P<0.05$ (Tukey HSD post hoc test)

Based on the table above it can be seen that the antioxidant activity in lime peel extract at the highest concentration of 500 ug/ml showed an average of 75.23 ± 0.43, and at the lowest concentration of 15.63 ug/ml

showed an average activity of 19.52 ± 0.07. Whereas the lime seed extract at the highest concentration of 500 ug/ml showed an average of 88.47 ± 1.12, and at the lowest concentration of 15.63 ug/ml showed an average activity of 22.11 ± 0.20.

Table 3: IC50 value inhibition of H_2O_2 from peel and seed extract of lime

Samples	equation	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
Peel extract (repeted 1)	Y= 0.1041x+24.681	0.98	243.22	
Peel extract (repeted 2)	Y= 0.1036x+23.972	0.98	251.24	246.84±4.06
Peel extract (repeted 3)	Y= 0.105x+24.162	0.98	246.08	
(Average)	Y= 0.1042x+24.272	0.98	246.91	
Seed extract (repeted 1)	Y= 0.1364x+23.449	0.97	194.66	
Seed extract (repeted 2)	Y= 0.1382x+23.271	0.97	193.41	194.82±1.49
Seed extract (repeted 3)	Y= 0.1341x+23.665	0.96	196.38	
(Average)	Y= 0.1362x+23.462	0.97	194.85	

Based on the table above, it can be seen that the antioxidant and antiaging activity seen from the IC50 value of inhibition of H2O2 from lime peel extract was 246.84 ± 4.06, and IC50 inhibition value of H2O2 from lime seed extract was 194.82 ± 1.49. which means that lime seed extract (*Citrus amblycarpa*) has antioxidant activity through the trapping of H2O2 which is better than that of lime skin extract (*Citrus amblycarpa*).

3.1 Comparison of percentage activities antioxidant of peel and seed extract of lime

In this study, it can be seen in Figure 1 and Figure 2 below that the anti-elastase and antioxidant activity with the trapping of H2O2 from peel and seed extract of lime showed increased activity in line with the increase in concentration.

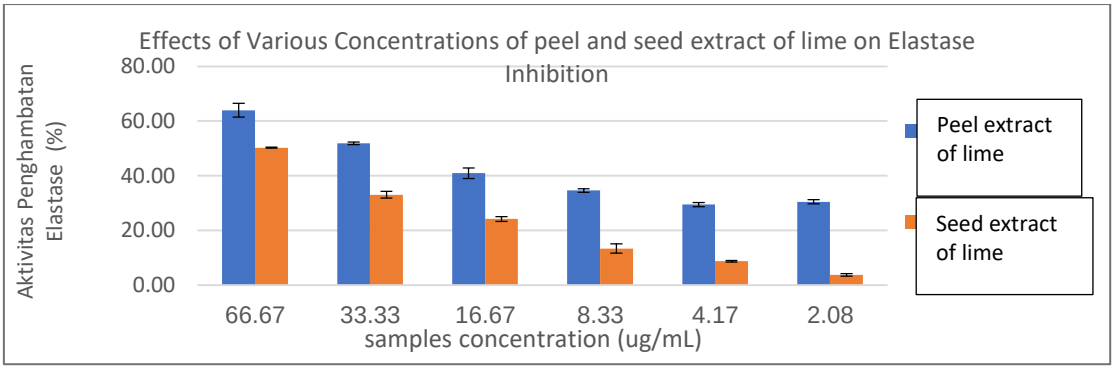

Figure 1: Effects of Various Concentrations of peel and seed extract of lime on Elastase Inhibition

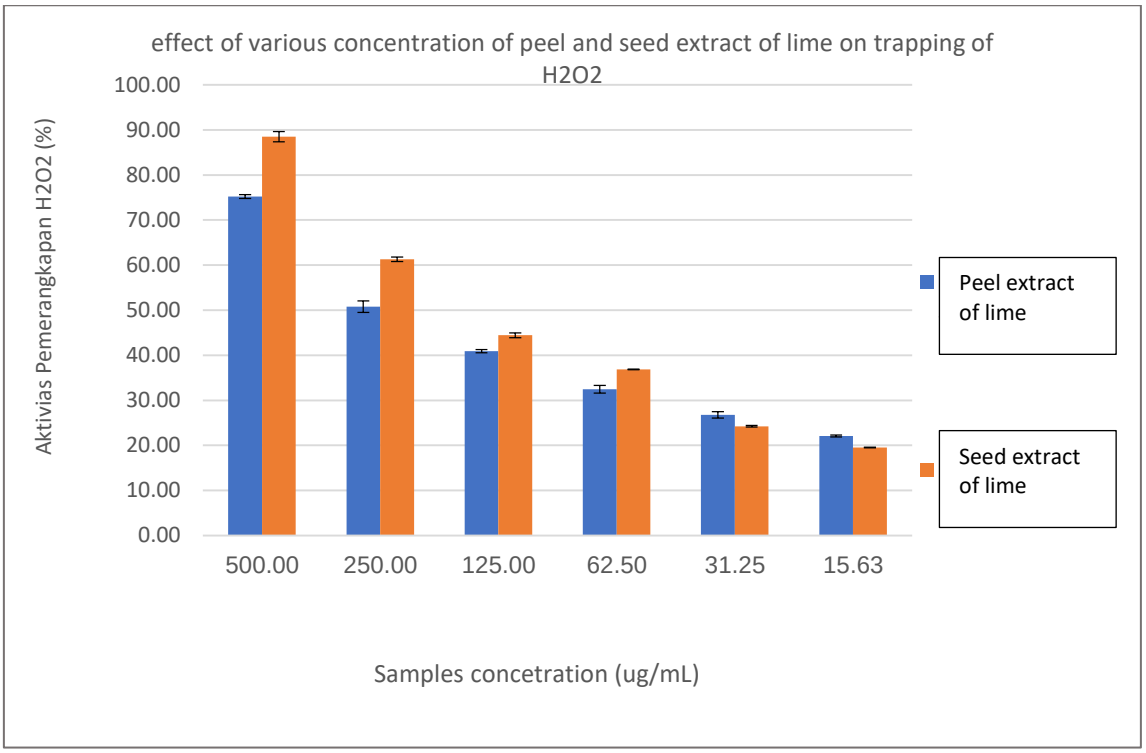

Figure 2: Effects of Various Concentrations of peel and seed extract of lime on trapping H2O2

3.2 Result of antielastase activities of peel and seed extract of lime

Elastase is one of the chymotripsin proteases that play an important role in the damage of elastin, a protein usually found in the extracellular matrix. The method used is measuring Sucla (N-Succinyl-Ala-Ala). The yellow change produced is an indicator of reaction. The higher the ability of the sample to inhibit elastase activity, the less SucAla3 is formed (slightly yellow) [11-13]. Based on the table below, it can be seen that the lime peel and seed extract that antielastase activity in lime peel extract at the highest concentration of 66.67 ug/ml showed an average of 63.95 ± 2.53, and at the lowest concentration 2.08 ug/ml shows the average activity of 30.46 ± 0.73. Whereas the lime seed extract at the highest concentration of 66.67 ug/ml showed an average of 50.25 ± 0.18, and at the lowest concentration 2.08 ug/ml showed an average activity of 3.75 ± 0.47.

Table 4: Results of analysis of post hoc test of tukey HSD test on antielastase activity data on peel and seed extract of lime

Final concentration (ug/ml)	Means of inhibition activity of elastase (%)	
	Peel extract	Seed extract
66.67	63.95 ±2.53^{e}	50.25 ±0.18^{f}
33.33	51.89 ±0.46^{d}	33.05 ±1.23^{e}
16.67	40.91 ±1.96^{c}	24.19 ±0.88^{d}
8.33	34.60 ±0.67^{b}	13.38 ±1.73^{c}
4.17	29.43 ±0.78^{a}	8.74 ±0.28^{b}
2.08	30.46 ±0.73^{a}	3.75 ±0.47^{a}

Data were presented in the form of Mean ± SD. Different lowercase letters in the same column show significance at P <0.05 (Tukey HSD post hoc test)

Table 5: IC50 value of Antielastase from peel and seed extract of

Sampel	Equation	R^2	IC50 (µg/mL)	IC50 (µg/mL)
Peel extract (repeted 1)	Y = 0.5361x+30.81	0.96	35.80	36.94±2.16
Peel extract (repeted 2)	Y = 0.5728x+29.606	0.98	35.60	
Peel extract (repeted 3)	Y = 0.5156x+29.67	0.95	39.43	
Peel extract (Avarege)	Y = 0.5415x+30.029	0.97	36.88	
Seed extract (repeted 1)	Y = 0.6786x+7.2781	0.96	62.96	62.39±0.72
Seed extract (repeted 2)	Y = 0.6942x+7.2496	0.94	61.58	
Seed extract (repeted 3)	Y = 0.6841x+7.1575	0.95	62.63	
(Avarege)	Y = 0.6856x+7.2284	0.95	62.39	

4. Conclusions

The antioxidant and antiaging activity seen from the IC50 value of inhibition of H2O2 from lime peel extract was 246.84 ± 4.06, and IC50 inhibition value of H2O2 from lime seed extract was 194.82 ± 1.49. which means that lime seed extract (*Citrus amblycarpa*) has antioxidant activity through the trapping of H2O2 which is better than that of lime skin extract (*Citrus amblycarpa*). the antioxidant and antiaging activity was seen from IC50 values of inhibition of elastase from lime peel extract by 36.94 ± 2.16, and IC50 value of inhibition of elastase from lime seed extract by 62.39 ± 0.72. which means that lime peel extract has antiaging activity through inhibition of elastase which is better than lime seed extract.

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