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Comparison of Antioxidant and Antihyaluronidase Activities in Extract of Basil Leaves (Ocimum americanum L.) with Eugenol Compound

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Abstract

Aging is a slow progressive loss of the ability of tissue to repair or regenerate and maintain its normal structure and function. Free radicals has been one of the aging factor, because free radicals can damage molecule in the body like lipid and protein. Antioxidant can help protect the body from free radicals. Has been known from many studies that there is many fruits and vegetables that contain antioxidant and antiaging (antihyaluronidase). One of that is basil leaves (*Ocimum americanum* L.). And one of the compound which can be found quite much in basil leaves is eugenol. This study aims to reveal comparison of the antioxidant and antihyaluronidase potential possessed in etanol extract of basil leaves compare to Eugenol compound. The hydrogen peroxide (H_2O_2) radicals scavanging antioxidant activity was measured based on the reaction method of ferrous ammonium sulphate and phenanthroline with little modification. The antihyaluronidase activity was detected by measuring the amount of Hyaluronic Acid based on method that has been told by Sigma Aldrich and Tu & Tawata (2015) with little modification. Eugenol had an IC_{50} value for H_2O_2 radicals scavenging activity as of 100,82 ± 8.60 μg/mL and basil leaves extract was 171,68 ± 2.21 μg/mL. While hyaluronidase inhibition activity from eugenol was 26,69 ± 2.89 μg/mL and basil leaves extract was 65,37 ± 3.96 μg/mL. In summary, eugenol compound which can be found in extract of basil leaves (Ocimum americanum L.) has better H_2O_2 radicals scavanging activity and hyaluronidase enzyme inhibitory activity than the extract of basil leaves (Ocimum americanum L.). Thus, present study described maybe eugenol is the main antioxidant and antiaging potential compound in basil leaves extract.

Keywords: Basil leaves extract; Antioxidant; antihyaluronidase.

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1. Introduction

Aging is a process of slowly disappearing the ability of the tissue to repair or replace itself and maintain its normal structure and function, so it cannot survive against injury (including infection) and repair the damage suffered (Santoso 2009). This aging process will occur in all organs of the body including organs in the body, such as the heart, lungs, kidneys, ovaries, brain, and also the outermost and widest organs of the body, namely skin [1-3]. Many factors cause the aging process, namely internal factors, and external factors. Some internal factors include free radicals, hyaluronidase enzymes, reduced hormones, glycosylation processes, methylation, apoptosis, a decreased immune system, and genes. The main external factors are unhealthy lifestyles, wrong habits, environmental pollution and stress [4]. Free radicals are one of the aging factors because free radicals can damage molecules in the body such as fat and protein, so free radicals can accelerate the aging process. Antioxidants can help protect the body from free radicals [5]. It has been known from various studies that there are various fruits and vegetables that contain antioxidants and antiaging. One of them is basil leaves (Ocimum americanum L). Genus Ocimum has more than 50 to 150 species spread in tropical and subtropical regions of Asia, Africa to Central America and South America (Shadia, Aziz, & Omer, 2007; Wossa, Rali, & Leach, 2008). Genus Ocimum has many uses for medicine and as an aromatic plant in many countr In general, basil leaves (Ocimum americanum L.) are medium-sized herbs that have a height of 3-5 cm and have flowers with a size of 8-12 mm can be white, pink, or purple. Basil leaves (Ocimum americanum L.) are plants originating from Africa, India, and the Asian region and are found mostly in Indonesia. Usually, basil leaves are used as vegetables "vegetables" and food tastes especially in Indonesia. Even so, basil leaves are still less popular with many people and the use of basil leaves in other fields, especially in the medical field is still very lacking and tends to be wasted or not utilized properly because of the sharp taste or aroma that stings the nose. In pharmacological studies and in the medical field, basil leaves can also be trusted as antibacterial, antioxidant, antitoxin, and others because they contain essential oils [6-10]. Ocimum americanum L. contains natural chemical compounds, including essential oils, carbohydrates, alkaloids, phenolic compounds, phytosterols, tannins, lignin, starch, saponins, flavonoids, terpenoids and anthraquinones [10-13]. Essential oil is the main component in Ocimum americanum L. The quality of essential oils is influenced by the geographic location of plants planted (related to soil, climate, temperature, irradiation). The main essential oil compounds in Ocimum americanum are camphor, methyl cinnamate, and citral [13-15]. Many studies on the pharmacological activity of basil (Ocimum americanum L.) plants. The hydroalcoholic extract from Ocimum americanum leaves was studied to have antioxidant activity that can prevent ischemia [6,17]. In another study also said petroleum ether extract, methanol, and water from the basil plant (Ocimum americanum L.) have analgesic-anti-inflammatory activity. In addition, in another study also said that water extract from Ocimum americanum can be used as an anti-diabetes Mellitus. While essential oils can show activity against fungi that are pathogenic in humans, fight oral microorganisms, aprotic ipsilon (Lepidoptera: Noctuidae) [17-20]. This research is expected to provide scientific information about the extent to which the ability of ethanol extracts of basil leaves as antioxidants to trap H2O2 free radicals and also as an antiaging of antihyaluronidase. So that the basil leaves not only become vegetables but can be used as natural ingredients of antioxidants and antiaging.

2. Material and Method

2.1 Materials

Multiskan GO Reader (Thermo Scientific 1510), Spatula, Mikropipet (1-10 μl, 50- 200 μl, 100-1000 μl) (Eppendorf), Tips (1-10 μl, 50- 200 μl, 100-1000 μl) (NEPTUNE), 96well-plate (TPP 92096), Falcon tube 15 ml (SPL 50015), Falcon tube 50 ml (SPL 50050), Analytical Balance (AXIS), Rotator (Thermo Fisher Scientific), Alumunium foil , Tube Effendorf 1,5 ml (SPL 60015-1), Vortex (WiseMix VM-10), 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), Tips (1-10 μl, 50- 200 μl, 100-1000 μl) (NEPTUNE), 96well-plate(TPP 92096), Falcon tube 15 ml (SPL 50015), Falcon tube 50 ml (SPL 50050), Analytical Balance(AXIS), Tube Effendorf 1,5 ml (SPL 60015-1), Vortex(WiseMix VM-10), Basil leaves, Ferrous Ammonium Sulfate(Sigma 7783859), Hydrogen peroxide(Merck 1.08597.1000), Asam sulfat (Merck 109981), 1,10-phenanthroline (Sigma 131377), Akuades, Sodium phosphate monobasic (Merck567545), Hyaluronic acid, (Sigma H5542), Hyaluronidase from bovine testes type I-S (Sigma H3506), Sodium chloride(Merck1064040500), Akuades, Bovine Serum Albumin (Sigma A4503), Sodium Acetate(Merck 1062681000), Acetic Acid(Merck 100063), Hydrochloride acid solution (Merck 109057), Sodium hydroxide(Merck 106498)

2.2 Preparation of ethanolic extract of basil leaves

Making basil leaf extract is done by pursuing simplicia, using the maceration method with 70% ethanol. Simplicia powder is weighed approximately 500 gr, put in a jar, plus liquid dancer, namely 70% ethanol as much as 1 L, closed and left for 5 days, protected from light so there is no damage to compound content decomposition (MOH, 1986) while occasionally stirring a minimum of 3 days time. After 5 days, the mixture of simplicia and 70% ethanol was sealed so that the filtrate (macerate) I. Dregs plus 70% ethanol is sufficient to 1 L then closed and left for 2 days, protected from light while stirring occasionally. After 2 days, the mixture of pulp and 70% ethanol is reconstructed and the filtrate (macerate) II is obtained. Filtrate I and II were then mixed and concentrated using a rotary evaporator at a temperature of <50 ° C until a thick ethanol extract was obtained. After the ethanol extract of each simplicia was obtained, it was calculated by extracting the weight formula divided by the weight of the extracted powder then multiplying by 100% [21].

2.3 Phytochemical screening of ethanolic extract of basil leaves

Phytochemical screening of extract ethanol kemangi leaf by using modification of fransworth method consisted of identification of phenol, steroids/terpenoids, saponins, flavonoids, tannin and alkaloid [22].

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:

Tabel 1: Solution

Reagen	Control	Sample test	Blank
Sampel	-	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 hyaluronidase enzyme activity test (in-vitro)

The inhibition of hyaluronidase enzyme activity was measured based on the methods described by Sigma Aldrich and Tu & Tawata (2015) with minor modifications. A mixture of solutions consisting of 25 μL samples (0.78 - 50 μg / mL), 3 μL enzyme hyaluronidase from IS bovine testes (0.02 mg / mL, Sigma H3506), and 12 μL phosphate buffer (300 mM, pH5.35, Sigma 0751) incubated at 37 ° C for 10 minutes. In addition, it was also prepared for controls containing only 3 μL enzymes and 37 μL phosphate buffers and blanks containing only 15 μL phosphate buffers and 25 μL samples. Next, a mixture of 10 μL of the hyaluronic acid substrate was added and re-incubated at 37 ° C for 45 minutes. STOP solution in the form of acid albumin is added as much as 100 μL into the solution and leave it at room temperature for 10 minutes. Oborborbance is measured using a wavelength of 600 nm

Formula of inhibition of hyaluronidase :

$$\% \text{ anti-hyaluronidase} = \frac{C-S}{C} \times 100$$

C : absorbance enzyme without samples

S : absorbance enzyme with samples

2.6 Statistical analysis

Data from the research results of H2O2 and antihyaluronidase free radical scavenging activities were analyzed using the post hoc test ANOVA followed by Post Hoc Test using the Tukey HSD test. P<0.05 was considered as statistical significance. Then the IC50 value of H2O2 and antihyaluronidase free radical trapping was calculated based on linear regression equations.

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 ethanolic extract of basil leaves

Final concentration (ug/ml)	Mean activity of trapping H_2O_2 (%) by samples	
	Basil leaves extract	Eugenol
500	81,87 ± 0,4 e	117,90 ± 13,86 d
250	63,98 ± 0,7 d	69,33 ± 7,29 c
125	49,58 ± 1,5 c	59,59 ± 0,77 bc
62.5	39,94 ± 0,9 b	48,33 ± 1,28 ab
31.25	30,46 ±0,3 a	31,96 ± 2,17 a
15.63	29,23 ±0,2 a	36,94 ± o,53 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)

The final concentration used in testing the trapping of H2O2 for Basil Leaf Extract and Eugenol compounds starts from 500 to 16 μg / ml. The test results showed that the addition of the two compounds was known to be related to the decrease in peroxide levels in the test solution where the highest scavenging activity of radical peroxide possessed by Basil Leaf Extract was found at a concentration of 500 μg / ml while the lowest activity was 16 μg / ml (Table 1). The same thing can be observed in the results of testing the Eugenol compound. Therefore the concentration of phytochemical compounds used in the test solution is directly proportional to the activity of trapping peroxide radicals. IC50 in the H2O2 trapping test is defined as the concentration of the compound needed to trap 50% of the peroxide radical found in the test solution. Linear regression analysis was used to find out the IC50 that was owned by each compound. The results of the analysis showed that the highest peroxide radical scavenging activity was found in Eugenol compounds while the lowest was Basil Leaf Extract (Table 3). Eugenol has an IC50 value of an average of 100.82 ± 8.60 μg / mL, while basil leaf extract is 171.68

± 2.21 μg / mL. The eugenol compound tends to have greater trapping activity than basil leaf extract.

Table 3: IC50 value inhibition of H_2O_2 from ethanolic extract of basil leaves

Samples	equation	R^2	IC_{50} (μg/mL)	IC_{50} (μg/mL)
Eugenol (repeted 1)	Y = 0,1893x + 31,986	0,96	95,15	
Eugenol (repeted 2)	Y = 0,1723x + 33,359	0,98	96,58	100.82 ± 8.60
Eugenol (repeted 3)	Y = 0,108x + 31,527	0,94	110,71	
(Average)	Y = 0,1665x + 33,356	0,97	99,96	
Basil leaves extract (repeted 1)	Y = 0,1092x + 31,451	0,95	169,86	
Basil leaves extract (repeted 2)	Y = 0,1081x + 31,176	0,96	174,14	171.68±2.21
Basil leaves extract (repeted 3)	Y = 0,138 x + 34,722	0,95	171,05	
(Average)	Y = 0,1084x + 31,385	0,95	171,73	

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.

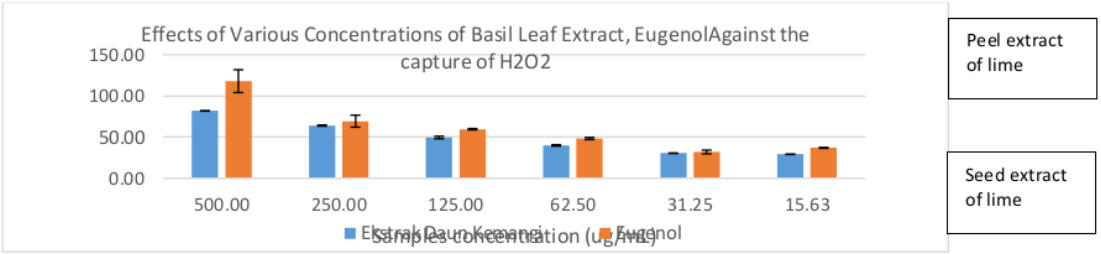

Figure 1: Effects of Various Concentrations of peel and seed extract of lime on Elastase Inhibition

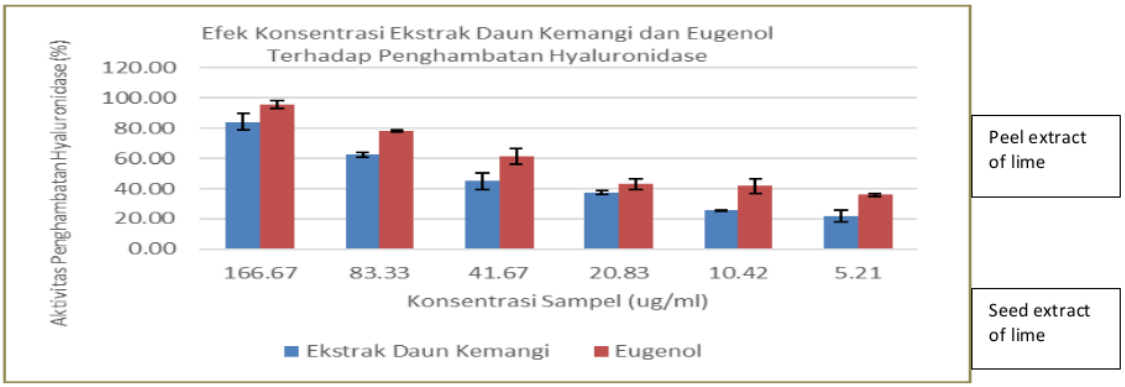

Figure 2: Effects of Various Concentrations of peel and seed extract of lime on trapping H2O2

3.2 Result of antihyaluronidase activity on ethanolic extract of basil leaves

The results of the Post Hoc Tukey HSD test from the antihyaluronidase activity and the average activity at various concentrations of basil leaf extract and eugenol compounds can be seen in Table 3.

Table 4: Results of analysis of post hoc test of tukey HSD test on antihyaluronidase activity data on ethanolic extract of basil leaves

Konsentrasi Akhir (ug/ml)	Mean inhibition acivity of Hyaluronidase (%)	
	Basil leaves extract	Eugenol
166,67	84,01 ± 5,46 [d]	95,60 ± 2,36 [d]
83,33	62,38 ±1,42 [c]	77,96 ± 0,69 [c]
41,67	45,11 ±5,53 [b]	61,41 ± 5,17 [b]
20,83	37,44 ± 1,57 [b]	43,11 ± 3,51 [a]
10,42	25,59 ± 0,47 [a]	41,65 ± 4,64 [a]
5,21	21,88 ± 4,07 [a]	35,88 ± 0,88 [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

Konsentrasi Akhir (ug/ml)	Mean inhibition acivity of Hyaluronidase (%)	
	Basil leaves extract	Eugenol
166,67	84,01 ± 5,46 [d]	95,60 ± 2,36 [d]
83,33	62,38 ±1,42 [c]	77,96 ± 0,69 [c]
41,67	45,11 ±5,53 [b]	61,41 ± 5,17 [b]
20,83	37,44 ± 1,57 [b]	43,11 ± 3,51 [a]
10,42	25,59 ± 0,47 [a]	41,65 ± 4,64 [a]
5,21	21,88 ± 4,07 [a]	35,88 ± 0,88 [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) The final concentration used in testing the antihyaluronidase activity for basil leaf extract and eugenol compounds starts from 166.6 to 5.21 μg / ml. The test results showed that the addition of the two compounds was known to be associated with a decrease in the antihyaluronidase activity of the test solution where the highest antihyaluronidase activity possessed by basil leaf extract was found at concentrations of 166.67 µg / ml while the lowest at concentrations of 5.21 µg / ml (Table 3.). A similar thing can be found in eugenol. So the concentration of basil and eugenol leaf extract used in the test solution is directly proportional to the antihyaluronidase activity.

4. Conclusions

The eugenol compound has a greater antioxidant activity of H2O2 trapping with an average IC50 value of 100.82 µg / ml from basil leaf extract with an average IC50 value of 171.68 µg / ml. The eugenol compound has antiaging anti-hyaluronidase activity with an IC50 value of 26.69 µg / ml which is larger than basil leaf extract with an IC50 value of 65.37 µg / ml. Eugenol compounds are more active as antioxidants and antihyaluronidase than basil leaf extract.

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