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Research article

Potential (2,2-azinobis (3-ethylbenzatiazolin) -6-sulfonic acid) reducing and anti-elastase of ethanol extract of KEMANGI leaves (*Ocimumbasilicum L.*) and Eugenol

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Key words: ABTS, Eugenol, Antioxidant, Anti-elastase.

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Abstract

Aging is a complex process characterized by a progressive decrease in the physiological functions of the body, including the skin, followed by dysfunction, and death. Kemangi leaves (*Ocimumbasilicum L.*) have been used empirically and are scientifically proven to have a variety of pharmacological activities, including analgesic, sedative, anti-inflammatory, antioxidant, antiaging, antimicrobial, antifungal, antiviral and contains chemical compounds eugenol, linalool, β-Caryophyllene. The purpose of this study was to determine the antioxidant activity and anti-elastase of the ethanol extract of kemangi leaves and eugenol. In this study, antioxidant activity was tested by ABTS reduction method (2,2-Azinobis (3-ethylbenzatiazolin) -6-sulfonic acid) and elastase inhibition tested by ethanol extract of kemangi leaves with a comparison of eugenol compounds. The results of ABTS reduction antioxidant activity based on IC_{50} Eugenol value of 2.09 µg / ml and ethanol extract of kemangi leaves of 18.27 µg / ml, anti-elastase from Eugenol at 37.49 µg / ml and ethanol extract of kemangi leaves at 43.02 µ / ml.

Introduction

Aging is a complex process characterized by a progressive decrease in the physiological functions of the body, including the skin, followed by dysfunction, and death. Many factors cause the aging process, including internal factors, and external factors. Some internal factors include free radicals, tyrosinase enzymes, elastase enzymes, collagenase enzymes, reduced hormones, glycosylation processes, methylation, apoptosis, a decreased immune system, and genes [1].

Free radicals can increase the levels of collagenase enzymes and increase the activity of the elastase enzyme which increases collagen degradation which caused skin shrinkage which speeds up the aging process. Free radicals are molecular atoms that have high reactivity; this is due to the presence of unpaired electrons [2].

The increase in a population experiencing premature aging and psychosocial effects has created a demand to fight aging in the skin, one of which is anti-aging cosmeceutical products. Anti-aging products that are used to fight aging are those caused by free radicals containing antioxidants as their active ingredients [3].

In Indonesia, traditional medicinal plants are able to prove the importance of natural ingredients for various processes of human medicine. In recent years, there has been an increase in researcher's interest in the use of natural materials as natural biological compounds in the manufacture of drugs.

One plant that is known to have antioxidant properties and has the ability to fight free radicals is the active compound of kemangi leaves (*Ocimumbasilicum L*). Kemangi leaves is one plant that is rich in essential oils, its properties have long been used empirically and are scientifically proven to have a variety of pharmacological activities, including analgesics, sedatives, anti-inflammatory, antioxidant, antiaging, antimicrobial, antifungal, and antiviral. These activities both in vitro and in vivo have been proven and caused by various chemical ingredients, namely eugenol, linalool, β-Caryophyllene, and other essential oil compounds [4].

In recent years the use of natural ingredients as an antidote to free radicals is very popular. Most of these natural ingredients have been shown to contain active antioxidant compounds. Several studies have shown that compounds in plants have the ability as antioxidants which can also inhibit the enzyme elastase, hyaluronidase, collagenase, and tyrosinase. Phytochemical compounds such as polyphenols contained in plants can counteract free radicals that have the potential to be anti-aging [5-7].

Based on the description above, to increase the use of plants as medicine, a study of the antioxidant activity and anti-

elastase of the ethanol extract of kemangi leaves compared with eugenol was conducted.

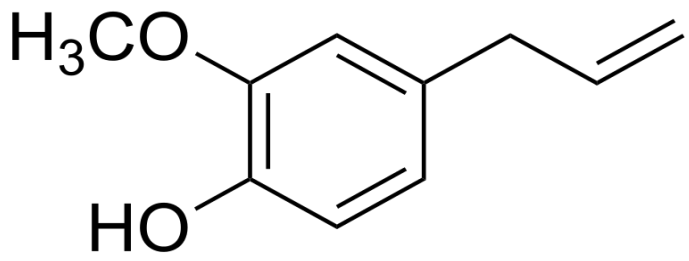

Figure 1. Structure of eugenol.

Materials and method

Material

Microplate Reader, pH meter (OHAUS Starter300 Portable) Beaker glass (IWAKI CTE33), Multiskan Go Reader (Thermo Fisher Scientific 1510), analytic measure, Eppendorf tube, Vial 1 ml, Spatula, Micropipette (1-10 µL, 50-200 µL, 100-1000 µL) (Eppendorf), Termometer, Rotary vacuum evaporator, Maserator, Tube 2 ml, Tips (1-10 µL, 50-200 µL, 100-1000 µL) (NEPTUNE), Multichannel pipette 2-200 µL, 96 well-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), Incubator (ESCO IFA-32-8), Waterbath, Tanur, Vortex (WiseMix VM-10), distillation tools, Orbital Shaker.

Kemangi leaves, eugenol from *Chengdu* Biopurify Phytochemical, Tiongkok, 2,2-Azinobis(3-etilbenzatiazolin)-6-sulfonat (ABTS) included Potasiumpersulfat, Phosphate-buffered saline (PBS) buffer (pH 7.4) [$Na_2HPO_{4.12}H_2O$, NaH_2PO_4, NaCl, Distilled water] (Sigma P-4417), Elastasedariporcine pancrease (Sigma 45124), N-Sucanyl-Ala-Ala-Ala-p-nitroanilide, elastasesubstrat, (Sigma 54760), Trizma base, Phamacia Biotech, 17-1321-01, Hydrocholic acid solution (Merck 109057).

Preparation of ethanol extract of kemangi leaves

Air-dried leaves of kemangi leaves (*Ocimumbasilicum L*) (500g) were extracted with 70% ethanol (12L) three times (2h each) using a soxhlet under reflux. The ethanol extract was concentrated under vacuum to give a crude extract (100g).

Phytochemical screening of ethanol extract of kemangi 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 alkaloids [8-9].

Antioxidants activity test

ABTS reduction activity test (asam 2,2-Azinobis(3-etilbenzatiazolin)-6-sulfonat) on ethanol extract of kemangi leaves

Put the ABTS solution into the well (on plate maping) as many as 198 µL containing 2 µL samples of ethanol extract of kemangi leaves with concentrations of 10.00 µL / ml, 5.00 µL / ml, 2.50 µL / ml, and 1.25 µL / ml. while the well control was filled with ABTS and then closed the microplate. The microplate reader is fixed at 30°C and the wavelength is 745nm and is measured every 1 minute for 8 minutes.

Formula of inhibition of ABTS:

% Reducing Activity = [(A absorbance of control – Absorbance of extract)/ absorbance of control x 100]

ABTS reduction activity test (asam 2,2-Azinobis(3-etilbenzatiazolin)-6-sulfonat) on eugenol

Put the ABTS solution into the well (on plate maping) as many as 198 µL containing 2 µL samples of scutellarein with concentrations of 10.00 µL / ml, 5.00 µL / ml, 2.50 µL / ml, and 1.25 µL / ml. while the well control was filled with ABTS and then closed the microplate. The microplate reader is fixed at 30°C and the wavelength is 745 nm and is measured every 1 minute for 8 minutes.

Formula of inhibition of ABTS :

% Reducing Activity = [(A absorbance of control – Absorbance of eugenol)/ absorbance of eugenol x 100]

Inhibition of elastase enzyme activity test (In vitro)

Inhibition of elastase enzyme activity was measured based on the method described by Thring *et al.* [11] 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 :

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

C : absorbance enzyme without samples

S : absorbance enzyme with samples

Statistical analysis

The measured data are ABTS reduction trapping activity and antielastase activity (%). 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.

Result and discussion

Phytochemical result

The results of phytochemical screening qualitatively in kemangi leaves extract are shown in the table 1.

Table 1. Results of phytochemical screening of kemangi leaves extract.

Chemical Component	Result
Tanin	+
Saponin	+
Flavonoid	+
Steroid	+
Glikosida	+
Alkoloid	+

Phytochemical screening of ethanol extract of kemangi leaves showed the positive result of flavonoids, tannins, saponins, glycosides, alkaloid, and steroids.

Analysis of antioxidant activities test by using ABTS method

The antioxidant activity in the ethanol extract of kemangi leaves and eugenol compounds was analyzed by the ABTS method. Data from the analysis of antioxidant activity were analyzed by the Post Hoc Test Turkey HSD test, as shown in the table 2.

Based on the table 2 above, it can be seen that the more potent antioxidant activity in the eugenol compounds was compared with ethanol extract of kemangi leaves in each concentration tested. In the ethanol extract of kemangi leaves, the most potent antioxidant activity was at a concentration of 50 µg / ml at 97.20 ± 0.18%. Whereas at the lowest concentration 1.56 µg / ml showed the weakest antioxidant activity as well which was equal to 22.15 ± 1.59%. Whereas the eugenol compound the most potent antioxidant activity is 99.86 ± 0.25% at the highest concentration of 50 µg / ml, and the weakest activity is 49.56 ± 0.06% at the lowest concentration of 1.56 µg / ml.

The results of the post hoc turkey HSD analysis showed a p-value of <0.05, which means that in various concentrations of extract ethanol kemangi leaf and eugenol significantly increased antioxidant activity in line with changes in the concentration of each basil leaf extract tested. The results of ABTS reduction antioxidant activity were then calculated IC50 values based on linear regression equations between concentrations.

The IC_{50} value with the lowest value shows the higher the free radical scavenging activity, which means that the lower the IC_{50} value of a test sample it can be said that the sample has the highest antioxidant activity and is the best antioxidant [8].

To determine the IC_{50} value of ethanol extract of kemangi leaves and eugenol compounds, linear regression analysis was used between concentrations. The results of IC_{50} ABTS reduction from the ethanol extract of kemangi leaves and eugenol compounds on basil leaf extract can be seen in table 3.

Table 2. Results of analysis of post hoc test of tukey HSD test on antioxidant activity data with ABTS method on ethanol extract of kemangi leaves.

Final concentration (ug/ml)	Means of ABTS reduction activities (%)	
	Ethanol extract of kemangi leaves	Eugenol
50.00	97.20±0.18^{f}	99.86±0.25 e
25.00	63.00±0.59 e	72.46±0.94 d
12.50	41.29±0.27 d	63.50±0.39 c
6.25	32.62±0.86 c	53.67±0.80 b
3.13	26.60±3.06 b	50.10±0.22 a
1.56	22.15±1.59 a	49.56±0.06 a

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

Table 3. IC_{50} value reduction of ABTS from ethanol extract kemangi leaf and eugenol compounds.

Sample	Equation	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
Extracts (repeated 1)	Y = 1.5397x + 21.782	0.99	18.33	18.27 ± 0.11
Extracts (repeated2)	Y = 1.5451x + 21.654	0.99	18.35	
Extracts(repeated3)	Y = 1.5126x + 2.559	0.99	18.14	
Extracts (Means)	Y = 1.5325x + 21.999	0.99	18.27	
Eugenol (repeated 1)	Y = 1.0338x + 47.858	0.99	2.07	2.09 ± 0.27
Eugenol (repeated 2)	Y = 1.0472X + 47.519	0.99	2.37	
Eugenol (repeated 3)	Y = 1.0336x +48.1	0.99	1.84	
Eugenol (Means)	Y = 1.0382x + 47.826	0.99	2.09	

In table 3, the IC_{50} value of ABTS reduction from the ethanol extract of kemangi leaves is 18.27 ± 0.11, IC_{50} value Reduction of eugenol compound ABTS is 2.09 ± 0.27. In table 3 it can be seen that the eugenol compound has the highest antioxidant activity compared to the ethanol extract of kemangi leaves. The compound of ethanol extract of basil leaves has an IC_{50} value of 18.27 µg / ml and the eugenol compound has an IC_{50} value of 2.09 µg / ml. The antioxidant activity of the eugenol compound was better than the ethanol extract of kemangi leaves. Ethanol extract of kemangi leaves was able to reduce ABTS by 50% at a concentration of 18.27 µg / ml, while the eugenol compound was able to reduce ABTS by 50% at a concentration of 2.09 µg / ml.

Analysis of anti-elastase test

Anti-elastase activity in the ethanol extract of kemangi leaves and eugenol compounds from basil leaves was analyzed by method. The inhibition of elastase enzyme activity was measured based on the method described by Sigma Aldrich with slight modifications [8-10]. Data from the analysis of antielastase activity were analyzed by the Post Hoc Test Turkey HSD test, as shown in the table 4.

Based on the table 4 it can be seen that the anti-elastase activity is more potent in the eugenol compound compared to the ethanol extract of kemangi leaves leaves at each concentration tested. In the ethanol extract of kemangi leaves, the most potent anti-elastase activity was at a concentration of 66.67 µg / ml amounting to 69.10 ± 1.60%. Whereas the lowest concentration of 2.08 µg / ml showed the weakest anti-elastase activity, which was 8.28 ± 0.84%. Whereas the eugenol compound the most potent antioxidant activity was 74.58 ± 2.77% at the highest concentration of 66.67 µg / ml, and the weakest activity was 5.59 ± 0.97% at the lowest concentration of 2.08 µg / ml.

This study showed that the eugenol compound had a higher anti-elastase activity compared to the ethanol extract of kemangi leaves. The antie-lastase activity showed an activity that was directly proportional to the sample concentration, the higher the concentration of the sample the greater the anti-elastase activity. To find out the IC_{50} value of the ethanol extract of kemangi leaves and eugenol compounds on inhibition of elastase, linear regression analysis was used to determine IC_{50}. The linear regression equation, R^2 and IC_{50} value of collagenase inhibition activity can be seen in Table 5.

In table 5, IC_{50} Anti-elastase value of ethanol extract of kemangi leaves is 43.20 µg / mL and IC50 Anti-elastase value of eugenols is 37.49 µg / mL. In this study showed that the eugenol compound had a higher elastase inhibitory activity compared to the ethanol extract of kemangi leaves. Eugenol can inhibit 50% elastase at a concentration of 37.49 µg / mL, while the ethanol extract of kemangi leaves can inhibit 50% elastase at a concentration of 43.02 µg / mL. Eugenol is a methoxyphenol with a short hydrocarbon chain that has another name 2-methoxy-4-propenylphenol and has properties such as volatile, colorless or rather yellow in color, slightly soluble in water but easily soluble in organic solvents. Eugenol contains several functional groups, namely allil, phenol and ether [11-12].

The potential of antioxidant constituents of plants materials for the maintenance of health and protection from coronary heart disease and cancer and antioxidant compound that can delay or inhibit the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions this also can be inhibit the elastase process [13].

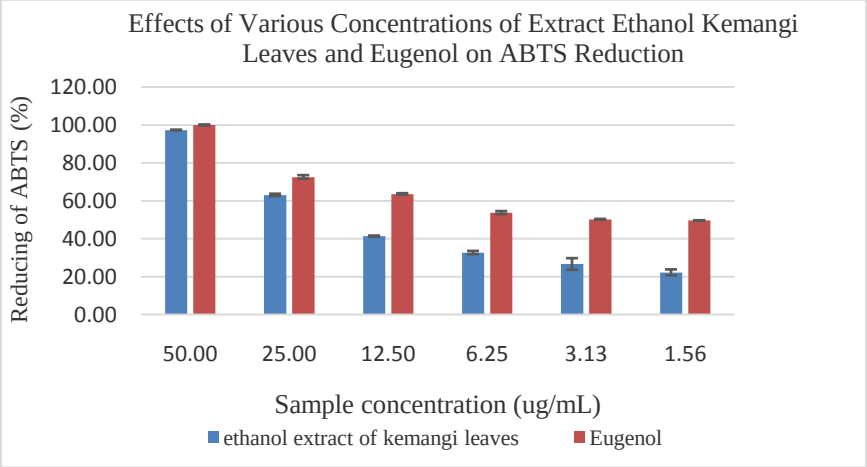

Figure 2. Diagram of effects of various concentrations of extract ethanol kemangi leaves and eugenol on ABTS reduction.

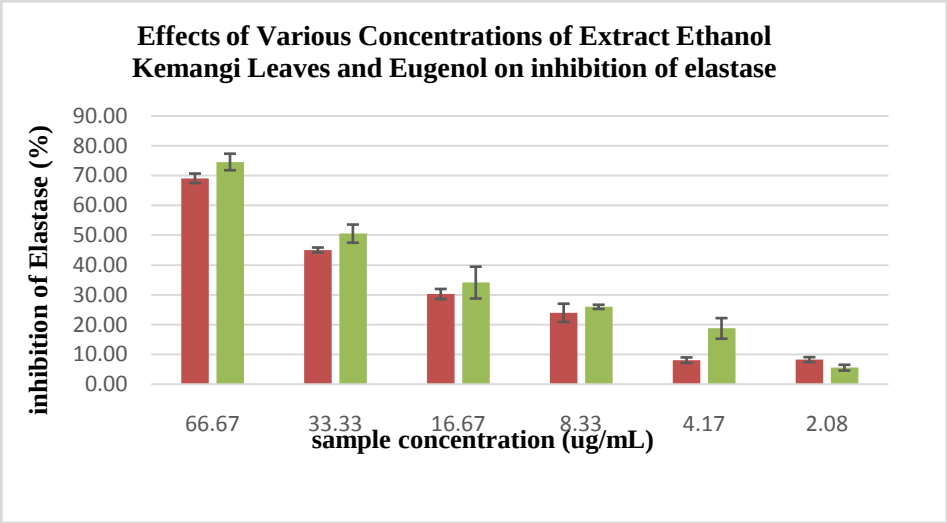

Figure 3. Diagram of Effects of Various Concentrations of Extract Ethanol Kemangi Leaves and Eugenol on inhibition of elastase.

Table 4. Results of analysis of post hoc test of tukey HSD test on antielastase activity data on in kemangi Leaves Ethanol extract and eugenol.

Final concentration (µg/ml)	Means of inhibition activity of elastase (%)	
	Extract ethanol kemangi leaf	Eugenol
66.67	69.10 ±1.60 ^{e}	74.58 ±2.77 ^{e}
33.33	45.05 ±0.84 ^{d}	50.54 ±3.05 ^{d}
16.67	30.28 ±1.70 ^{c}	34.12 ±5.34 ^{c}
8.33	23.98 ±3.06 ^{b}	25.98 ±0.70 ^{bc}
4.17	8.09 ±0.93 ^{a}	18.75 ±3.46 ^{b}
2.08	8.28 ± 0.84 ^{a}	5.59 ±0.97 ^{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. IC_{50} value of Antielastase from ethanol extract of kemangi leaves and eugenol compounds.

Samples	Equation	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
Extracts (repeated 1)	Y = 0.9181x + 10.629	0.96	42.88	
Extracts (repeated 2)	Y = 0.9172x + 9.7826	0.96	43.85	
Extracts (repeated 3)	Y = 0.937x + 11.329	0.93	41.27	43.02 ± 0.76
Extracts (Means)	Y= 0.9241x + 10.58	0.95	42.66	
Eugenol (repeated 1)	Y = 0.9323x + 14.74	0.90	37.82	
Eugenol (repeated 2)	Y = 0.9924x + 12.748	0.93	37.54	
Eugenol (repeated 3)	Y = 0.9712x + 13.944	0.96	37.13	37.49 ± 0.35
Eugenol (Means)	Y = 0.9653x + 15.811	0.94	37.49	

Conclusions

Eugenol compounds have antioxidant activity through ABTS activity and anti-aging activities through better anti-elastase activity compared to the compounds of ethanol extract of kemangi leaves (*Ocimumbasilicum L*) ABTS reduction antioxidant activity based on IC_{50} Eugenol value of 2.09 µg / ml and Kemangi Leaves extract was 18.27 µg / ml, IC_{50} of anti-elastase from eugenol was 37.49 µg / ml and ethanol extract of kemangi leaves was 43.02 µ / ml.

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