

Research article

Effectiveness of antioxidant and anticologinase activity of 70% ethanolic extracts of Kemangi leaves (*Ocimum Basilicum*)

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Abstract

Free radicals are one form of reactive oxygen compounds, which are generally known as compounds that have unpaired electrons. Kemangi leaves are used as an aphrodisiac because they contain aragin in which can strengthen sperm resistance and prevent infertility. Besides aragin in, Kemangi leaves also contain other secondary metabolites such as essential oils, phytosterols, alkaloids, phenolic compounds, tannins, lignin, saponins, flavonoids, terpenoids and anthraquinones. Phytochemical screening results of 70% ethanol extract of kemangi herbs themselves showed the presence of secondary metabolites of flavonoids, saponins, tannins and triterpenoids / steroids. In this study, antioxidant activity was tested using the FRAP (Ferric Reducing Antioxidant Power) method, with concentrations of kemangi leaf extract and comparative compounds Eugenol each concentration of 1000 $\mu\text{L/ml}$, 5000 $\mu\text{L/ml}$, 250 $\mu\text{L/ml}$, and 125 $\mu\text{L/ml}$, 62.50 $\mu\text{L/ml}$, and 31.25 $\mu\text{L/ml}$ measured at 745 nm wavelength and collagenase inhibition test with ethanol extract of kemangi leaves with a comparison of eugenol compounds measured at a wavelength of 335 nm. The FRAP antioxidant activity results obtained based on IC_{50} Eugenol value of 261.36 $\mu\text{g/ml}$ and Kemangi leaf extract at 111.32 $\mu\text{g/ml}$, antolagenagenase from Eugenol at 255.32 $\mu\text{g/ml}$ and Kemangi leaf extract at 110.65 $\mu\text{g/ml}$.

Introduction

Free radicals are one form of reactive oxygen compounds, which are generally known as compounds that have unpaired electrons. In other words free radicals are stand-alone atoms, molecules or compounds that have unpaired electrons, therefore they are highly reactive and unstable. Unpaired electrons always try to find new pairs, so that they are easy to react with other substances (proteins, fats and DNA) in the body [1].

Kemangi essential oil is the raw material for making perfumes, cosmetics, and medicines. Studies related to kemangi itself, especially in clinical terms, have been carried out in India. For IC_{50} values in kemangi extract (*Ocimum basilicum*) can inhibit the formation of free radicals with a value of 34.21 $\mu\text{g/mL}$ [2].

Kemangi is very popular in Java, Sumatra and other regions in Indonesia kemangi leaves are often consumed as a supplement to eating and boosting aroma in food. Empirically kemangi is used as an aphrodisiac because it contains aragin in which can strengthen sperm resistance and prevent sterility. Besides aragin in, kemangi leaves also contain other secondary metabolites such as essential oils, phytosterols, alkaloids, phenolic compounds, tannins, lignin, saponins, flavonoids, terpenoids and anthraquinones.

Phytochemical screening results of 70% ethanol extract of kemangi herbs themselves showed the presence of secondary metabolites in flavonoids, saponins, tannins and triterpenoids/steroids [3].

The one of plant that is known to have antioxidant properties and has the ability to fight free radicals is the active compound of kemangi leaves (*Ocimum basilicum*) is the one of 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 contents, namely eugenol, linalol, β -Caryophyllene and other essential oil compounds [4].

Several studies have shown that plant extractive substances have the potential as an active antioxidant compound and inhibitor of tyrosinase enzymes. Natural antioxidant compounds from plant phenolic groups are able to inhibit skin premature aging, kemangi leaf extract (*Ocimum basilicum*) is effective as a bioinsecticide in mosquito repellent preparations against *Aedes aegypti* [5].

Based on the description above, to increase the use of plants as medicine, a study of antioxidant and anticollagenase activity tests of ethanol extract of kemangi leaves compared with eugenol.

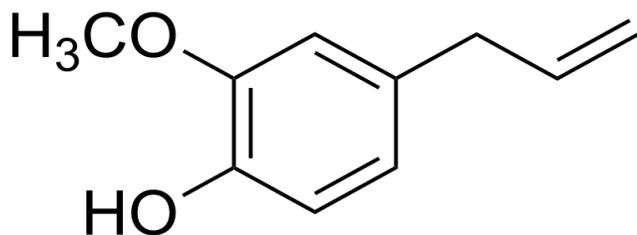


Figure 1. Structure of eugenol.

Materials and methods

Materials

The tools used in this study is Multisize GO Reader (Thermo Scientific 1510), Spatula, Micropipette (1-10 μ l, 50- 200 μ l, 100-1000 μ l) (Eppendorf), Tips (1-10 μ l, 50-200 μ l) , 100-1000 μ l) (NEPTUNE), 96well-plate (TPP 92096), Falcon tube 15 ml (SPL 50015), Falcon tube 50 ml (SPL 50050), Analytical Balance (AXIS), Rotator (Thermo Fisher Scientific), Aluminum foil, 1.5 ml Eppendorf Tube (SPL 60015-1), Vortex (WiseMix VM-10), pH meter (OHAUS Starter300 Portable), Erlenmeyer Tube.

Materials

The ingredients used are TPTZ solution, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ solution, kemangi leaves, Potassium dihydrogen phosphate (Merck 104873), Dipotassium hydrogen phosphate (Merck 105104), Tyrosinase from Mushroom (Sigma T3824), L-DOPA (3,4-Dihydroxy L-phenylalanine) (Sigma D9628), Hydroxyl Potassium (Sigma P5958), Ferrous Ammonium Sulfate (Merck 1.03792.1000), Hydrogen peroxide (Merck 1.08597.1000), Phosphate Buffer, L-Ascorbic Acid (Sigma A5960), Deoxyribose (Sigma 121649), Trichloroacetic Acid (TCA) (Merck 100807), Tiobarbituric Acid (TBA) (Merck 108180), Ferrous Ammonium Sulfate (Sigma 7783859), Sulfuric Acid (Merck 109981), 1,10-phenanthroline (Sigma 131377), Sodium nitroprusside (Sigma 71780), Sulphanilamide (Sigma S9251), Phosphoric Acid (Merck 480939), N- (1-naphthyl) ethylenediamine dihydrochloride (Sigma N9125), Ethanol (Merck 100983), PBS, distilled water.

Extract preparation

Making kemangi leaf extract is done by pursuing dry leaves using maceration method with 70% ethanol. Dry leaves 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 for a minimum of 3

days time. After 5 days, the mixture of dry leaves and 70% ethanol was sealed so that the filtrate I (macerate) is obtained. Pulp is added to 1 L 70% ethanol, 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 is then mixed and concentrated using a rotary evaporator at a temperature of $<50^\circ\text{C}$ until a thick ethanol extract was obtained. After obtaining the ethanol extract of each dry leaves, the yield was calculated by the weight formula extract obtained divided by the weight of the extracted powder then multiplied by 100% [5].

Phytochemical screening

In phytochemical tests using a modified Farnsworth method consisting of identification of phenols, steroids / triterpenoids, terpenoids, saponins, flavonoids, tannins and alkaloids [6-8].

Test antioxidant activity

The antioxidant activity test was carried out, namely the FRAP method activity test.

Antioxidant activity test of ferric-reducing/antioxidant power (FRAP) method on ethanol extract of kemangi leaf and eugenol

A total of 7.5 μ L of samples of various concentrations of kemangi and eugenol leaf extract (1000, 500, 250, 125, 62.5, 31.25 μ g / mL and 142.5 μ L FRAP solutions were added to the well samples and added DMSO to well blank and well control Microplate is closed, then incubated 37°C for 6 minutes. Absorbance is measured with microplate reader at a wavelength of 745 nm. Standardization is done with Ferrous sulfate. Ferrous sulphate solution is prepared by dissolving 0.03 grams of FeSO_4 in 100 mL and H_2O [9].

Anticollagenase test/inhibition of collagenase enzyme activity (In Vitro)

The inhibition of collagenase enzyme activity was measured based on the method described by Sigma Aldrich and Wittenauer *et al.* (2015) with slight modifications (Widowati *et al.*, 2016; 2017; 2018; Utami *et al.*, 2018). A mixture of solutions consisting of 30 μ L samples (0.78-50 μ g/mL), 10 μ L collagenase from clostridium histolyticum enzyme and 60 μ L tricine buffer incubated at 37°C for 20 minutes. In addition, it was also prepared for controls containing only 10 μ L enzymes and 90 μ L phosphate buffers and blanks containing 10 μ L enzymes, 80 μ L phosphate buffers and 30 μ L samples. Next, a mixture of 20 μ L of the FALGPA substrate is added except the blank. Absorbance is measured at a wavelength of 335 nm.

Data analysis

The research data was processed using the SPSS program with One Way ANOVA test, followed by Post Hoc Test using the LSD test. Qualitatively, the phytochemical test results of kemangi leaves were not analyzed using statistical analysis but through observation.

Results and discussion

Authentication of plant

The results of the identification of plants carried out by Hans jaya (2019) at the Medanese Herbarium (MEDA) the Universitas Sumatera Utara, the fruit used in this study was Kemangi (*Ocimum basilicum*) Kingdom: Plantae, Subkingdom: Tracheophytes, Super Division: angiosperm, Division: Eudicots, Subclass: Asteridae Class: Lamiales, Family: Lamiaceae, Genus: *Ocimum*, Species : *Ocimum basilicum* L.

Phytochemical tests

The results of phytochemical screening qualitatively in Kemangi extract are shown in table 1.

Table 1. Phytochemical screening of kemangi extract.

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

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

Antioxidant effectiveness analysis with FRAP

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

Table 2. Antioxidant activity FRAP of kemangi leaf extract, eugenol (On average, Post Hoc Test Results for Tukey HSD Test).

Final Concentration ($\mu\text{g/ml}$)	Activity Average FRAP ($\mu\text{M Fe(II)}/\mu\text{g}$)	
	Kemangi Leaf Extract	Eugenol
50	150.32 \pm 0.63 ^e	406.37 \pm 2.08 ^f
25	100.33 \pm 0.81 ^d	307.75 \pm 0.98 ^e
12.5	72.37 \pm 2.89 ^c	256.10 \pm 1.15 ^d
6.25	37.83 \pm 6.30 ^b	104.73 \pm 1.80 ^c
3.125	29.27 \pm 3.76 ^b	55.33 \pm 1.30 ^b
1.5625	16.00 \pm 2.58 ^a	25.33 \pm 0.58 ^a

Based on the table 2 it can be seen that the more potent antioxidant activity in the eugenol compound samples from kemangi leaves was compared with kemangi leaf extract in each concentration tested.

In the ethanol extract of kemangi leaves, the most potent antioxidant activity was at a concentration of 50 $\mu\text{g/ml}$ of 150.32 \pm 0.63^e. While at the lowest concentration of 1.5625 $\mu\text{g/ml}$ showed the weakest antioxidant activity as well which was 16.00 \pm 2.58^a. While at in eugenol compounds from kemangi leaves the most potent antioxidant activity was 406.37 \pm 2.08^f at the highest concentration of 50 $\mu\text{g/ml}$, and the weakest activity was 25.33 \pm 0.58^a at the lowest concentration of 1.5625 $\mu\text{g/ml}$.

The results of the post hoc turkey HSD test showed a p value of <0.05 , which means that in various concentrations of kemangi and eugenol leaves ethanol extract from kemangi leaves significantly increased antioxidant activity in line with changes in the concentration of each kemangi leaf extract tested.

Measurement of antioxidant activity using this FRAP test with ascorbic acid solution as standard. The addition of TCA aims to make the potassium ferrosianide complex settle. The addition of FeCl_3 also aims to form green complex to be piled up by plants in their chemical structure, biosynthesis, scientific distribution and biological functions.

In table 3, IC_{50} Value FRAP reduction from ethanol extract of kemangi leaves was 111.17 \pm 6.40, IC_{50} value FRAP reduction Eugenols compound was 259.84 \pm 49.94. 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 kemangi leaves has IC_{50} value of 110.65 $\mu\text{g/ml}$ and the eugenol compound has an IC_{50} value of 259.84 $\mu\text{g/ml}$. 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 FRAP by 50% at a concentration of 111.17 $\mu\text{g/ml}$, while the eugenol compound was able to reduce FRAP by 50% at a concentration of 259.84 $\mu\text{g/ml}$.

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

Table 3. IC_{50} Anticolagenase value from kemangi leaf extract, eugenol.

Sample	Equation	R ²	IC ₅₀ (µg/mL)	IC ₅₀ (µg/mL)
EDK (test 1)	Y = 0.1131x + 37.382	0.97	111.56	111.17 \pm 6.40
EDK (test 2)	Y = 0.093x + 40.253	0.94	104.81	
EDK (test 3)	Y = 0.063x+42.591	0.96	117.69	
EDK (average)	Y = 0.0897x+40.075	0.97	110.65	
Eugenol (test 1)	Y = 0.071x + 33.98	0.95	225.63	259.84 \pm 49.94
Eugenol (test 2)	Y = 0.0501x + 34.047	0.90	318.42	
Eugenol (test 3)	Y = 0.0688x + 33.487	0.98	240.01	
Eugenol (average)	Y = 0.0633x + 33.838	0.95	255.32	

Effectiveness of anticolagenase in 70% ethanol extract of kemangi leaves

Anticolagenase activity in the ethanol extract of kemangi leaves and eugenol compounds from kemangi leaves was analyzed by jenny method. The inhibition of collagenase enzyme activity was measured by the method described by Sigma Aldrich with slight modifications [10-11]. The data from the analysis of anticolagenase 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 more potent antielastase activity in the eugenol compound sample from kemangi leaves was compared with the ethanol extract of kemangi leaves at each concentration tested.

Difference in the activity of ethanol extract of kemangi leaves in the FRAP antioxidant test

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 solvent. Eugenol contains several functional groups, namely allil, phenol and ether.

In this study in figure 2 and figure 3 it can be seen that the antielastase and antioxidant activity of the kemangi leaves eugenol compound shows increased activity in line with the increase in concentration.

From figure 2 and figure 3, it can be seen that the activity of kemangi leaf extract has antioxidant activity and anticolagenase is not as potent as the eugenol compound of kemangi leaf extract.

Differences in percentage of anticoligenase activity in kemangi leaf extract

In the ethanol extract of kemangi leaves, the most potent anticoligenase activity was at a concentration of 250.00 µg/ml at 61.55 \pm 3.79%. Whereas at the lowest concentration of 7.81 µg/ml showed the weakest anti-cholagenase activity as well, which was 41.03 \pm 3.26%. While at the eugenol compound from kemangi leaves the most potent antioxidant activity was 48.62 \pm 2.89 % at the highest concentration of 250.00 µg/ml, and the weakest activity was 33.49 \pm 0.15% at the lowest concentration of 7.81 µg/ml.

This study showed that the eugenol compound had a higher anti-cholagenase activity compared to the ethanol extract of kemangi leaves. The anticolagenase activity showed an activity that was directly proportional to the concentration of the sample, the higher the concentration of the sample the greater the anti-collagenase activity.

To determine the IC_{50} value of the ethanol extract of kemangi leaves and eugenol compounds on inhibition of collagenase linear regression analysis was used followed to determine IC_{50} . The linear regression equation, R² and IC_{50} value of collagenase inhibition activity can be seen in table 3.

Table 4. Anticolagenase activity of kemangi leaf extract, eugenol (On average, Post Hoc Test Results for Tukey HSD Test).

Final Concentration (µg/ml)	Average Collagenase Inhibition Activity (%)	
	Kemangi Leaf Extract	Eugenol
250.00	61.55 \pm 3.79 ^c	48.62 \pm 2.89 ^d
125.00	53.86 \pm 1.45 ^b	43.70 \pm 1.65 ^c
62.50	44.77 \pm 2.53 ^a	38.74 \pm 0.26 ^b
31.25	41.87 \pm 2.17 ^a	34.54 \pm 0.61 ^{ab}
15.63	41.51 \pm 1.70 ^a	35.09 \pm 0.37 ^a
7.81	41.03 \pm 3.26 ^a	33.49 \pm 0.15 ^a

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

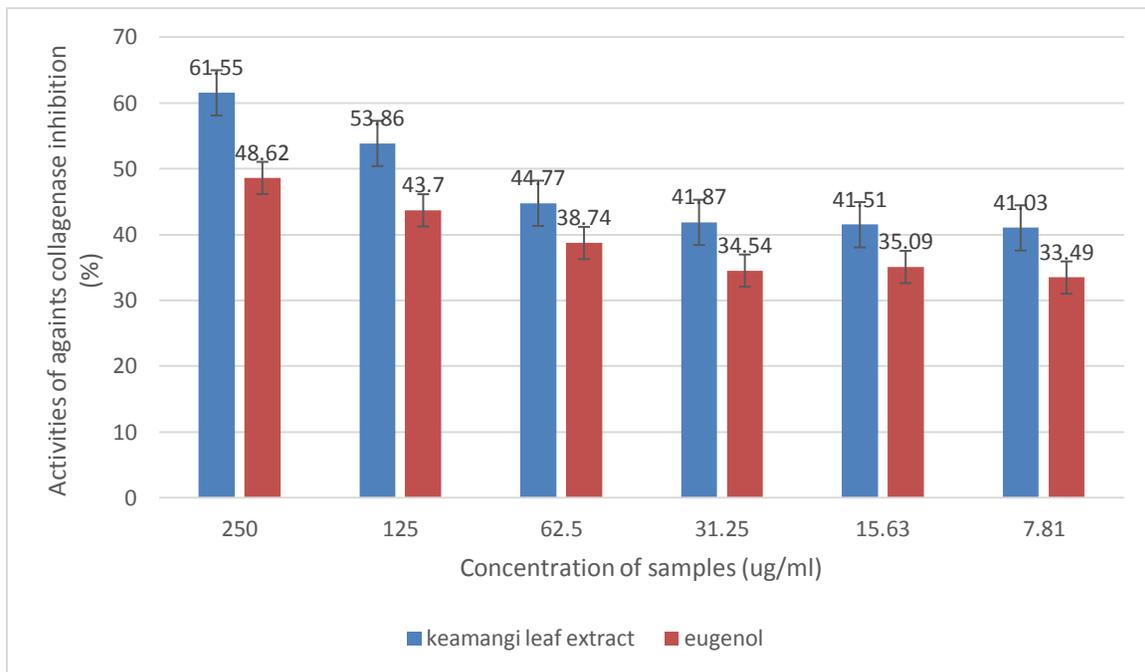


Figure 2. Effects of various concentrations of kemangi leaf extract, eugenol on collagenase inhibition.

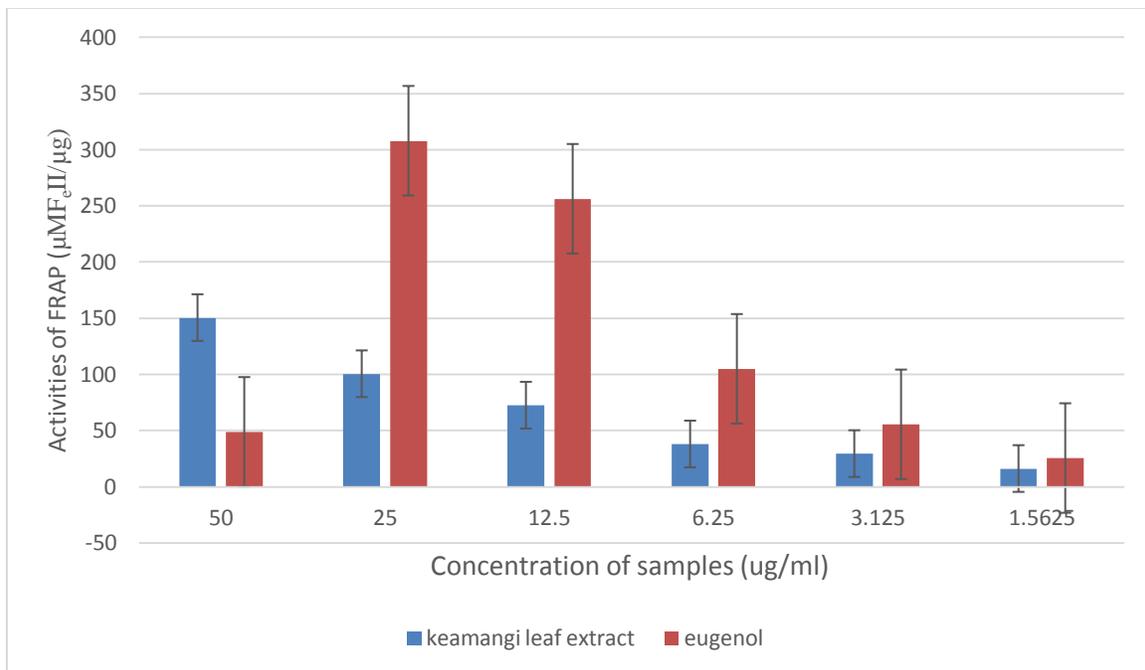


Figure 3. Effects of various concentrations of kemangi leaf extract, eugenol on FRAP activities.

Conclusion

Eugenol compounds from Kemangi Leaf extract (*Ocimum basilicum*) have antioxidant activity through the activity of FRAP method and antioxidant activity through anticollagenase activity which is better than the compounds of Kemangi Leaf extract (*Ocimum basilicum*) the most potent anti-collagenase activity was at a concentration of 250.00 µg/ml at 61.55 ± 3.79%. Whereas at the lowest concentration of 7.81 µg/ml showed the weakest anti-collagenase activity as well, which was 41.03 ± 3.26%. While at the eugenol compound from

kemangi leaves the most potent antioxidant activity was 48.62 ± 2.89 % at the highest concentration of 250.00 µg/ml, and the weakest activity was 33.49 ± 0.15% at the lowest concentration of 7.81 µg/ml.

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