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Antioxidants and Antityrosinase Activity of Ethanolic Basil Leaves Extract (*Ocimum americanum* L.) and Eugenol

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ABSTRACT

Harm effect from UV light, both UV-A and UV-B is contributing as a disease trigger and has an impact on human health. To investigate the bioactive compound, antioxidant, and the potential of basil leaves as antiaging sources particularly as the tyrosinase inhibitor. This study used phytochemical screening for the bioactive compound, DPPH scavenging activity for antioxidant assay, and tyrosinase inhibition activity for the antiaging property. The phytochemical screening shows that the basil leaves extract has flavonoid, saponin, phenol, steroid, and alkaloid. The basil leaves extract has lower antioxidant activity (20.55 ± 0.04 μg/mL) compared with eugenol (2.44 ± 0.26 μg/mL) through DPPH scavenging activity. The basil leaves extract (35.59 ± 0.83 μg/mL) has lower antiaging activity particularly as antityrosinase activity compared with eugenol (10.87 ± 0.41 μg/mL). Our findings suggest that basil leaves can be used as an antioxidant and antiaging source, particularly as a tyrosinase inhibitor.
Keywords: Antioxidant; Tyrosinase; Basil; Eugenol; Antiaging

INTRODUCTION

Sunray is an electromagnetic wave that is a source of all kinds of rays. Sunray on the Earth surface is composed of several spectra, i.e. infrared light (> 760 nm), visible light (400-760 nm), ultraviolet light A (UVA) (315-400 nm), UVB light (290-315 nm), and UVC light (100-290 nm) which is very dangerous, have energy very high and are carcinogenic. But nowadays, the thinning of the ozone layer opens opportunities for various diseases and health disorders (D'Orazio *et al.*, 2013). It is known from various studies that there is a variety of fruits and vegetables containing antioxidants and it is believed can be a lightening natural ingredient of the skin because it has the effect of antityrosinase (antihyperpigmentation) or prevent excessive pigment production. One of them is basil leaves *(Ocimum americanum L.)*, which contain phenolic compounds and flavonoids that can serve as antityrosinase that can inhibit the tyrosinase enzyme as a pigment-producing enzyme. In general, the basil leaves are medium-sized plants and herbs which have a high 3-5 cm and have a flower with the size 8-12 mm can be white, pink, or purple (Khair-ul-Bariyah, Ahmed and Ikram, 2012). Basil is an angiosperm that has the seeds and structures of its cell walls rigid and composed of cellulose (Girsang *et al.*, 2015).

The importance of the economic conditions and the spread of basil leaves globally, in both its use as an ingredient or traditional medicine, realized the importance of investigating in terms of pharmacological and toxicologic to see the efficacy and safety of consumption of basil leaves (Güez *et al.*, 2017). According to research conducted by Rasul *et al.*, 2011, that is by using topical cream extract 3% of basil leaves are applied to the skin of the cheeks as control of erythema and melanin skin on 11 voluntary humans with a period of 12 weeks, and the results obtained in the presence of antierythema of the leaf extracts of the basil. According to research conducted by Yonathan, Lintong and Durry, 2016, found in their research believed a compound of flavonoids contained in cocoa beans is believed to be used as an antihyperpigmentation. There is a decrease in the average value of the skin's melanin pigment before and after exposure to sunlight due to the administration of extracts. Based on the problems and the research that supports it and remember it is used less in the medical field, making researchers want to find out what bioactive compound, antioxidant effect, and the potential of basil leaves *(Ocimum americanum L.)* as antiaging sources particularly as the tyrosinase inhibitor.

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METHODOLOGY

Materials

The materials used on the phytochemical screening is Basil leaves extract, Ethanol 70%, Aquades, *2,2 Diphenyl-1-picrylhydrazyl* (Sigma D9132), Methanol absolute (Merck 1060092500), DMSO (Merck 1029522500), *$FeCl_3$ (1% in ddH_2O)* (Merck 103943), *aquabidest (ddH_2O), Glacial acetic acid 100%* (Merck 1000630510), *H_2SO_4*

(Merck 1007310510), *1 N HCl* (Merck 109057), *2 N HCl* (Merck 1090631000), *Amil alcohol* (Merck 1009751000), *Vanillin* (Sigma-Aldrich, W310727), *Mg/Zn powder* (Merck 1058151000, 1087560250), *Dragendorff Reagent (potassium iodide* (Merck 207969), *bismuth nitrate Merck 248592), Potassium dihydrogen phosphate* (Merck 104873), *Dipotassium hydrogen phosphate* (Merck 105104), *Tyrosinase from Mushroom* (Sigma T3824), *L-DOPA (3,4-Dihydroxy-L-phenylalanine)* (Sigma D9628), *Potassium Hydroxyl* (Sigma P5958).

Methods

Preparation of Basil Leaves Extract (BLE)

Fresh basil leaves *(Ocimum americanum L.) were* obtained from the Cigugur Girang Village, Parongpong District, Bandung Regency. The plants were identified by herbarium staff, Department of Biology, School of Biological Science and Technology, Bandung Institute of Technology, Bandung, West Java, Indonesia. The fresh basil leaves were wet sorted then it was washed and dried using a food dehydrator and then into simplicia mashed to obtain simplicia basil leaves powder.

$$\text{\% Drying Shrinkage} = \frac{weightofthedriedsimplicia}{weightofthefreshbasilleaves} \times 100\%$$

Preparation of *Ocimum americanum L.* extract

This procedure was carried out by extraction using the technique of maceration using ethanol solvent 70%, the filtrate was accommodated every 24 hours until the filtrate turned colorless then ethanol filtrate 70% evaporated until ethanol 70% extract shaped paste was obtained.

Phytochemical Screening

In this research, phytochemical screening had been conducted to determine the bioactive component found in extracts of basil leaves *(Ocimum americanum L.).* Identification of the chemical contents of the screening consists of alkaloids, steroids/triterpenoids, saponins, flavonoids, tannins, phenols, and terpenoids (Widowati *et al.*, 2016, 2017, 2018).

Flavonoids Test

The sample of basil leaves extracts as much as 10 mg dissolved in 2 N HCl in the reaction tube. Then added Mg/Zn and heated for 5-10 minutes, then cooled and strain and added amil alcohol as much as 1 ml. If extracts produce a red/orange color, the sample contains a flavonoid compound (Widowati *et al.*, 2016, 2017, 2018).

Alkaloids Test

Sample of basil leaves as much as 10 mg dissolved in *aquabidest* as much as 5 ml was dried in the water bath. The resulting residue was then dissolved with 5 ml 2 N HCl. The acquired solution was divided into 2 reaction tubes. The first tube was added 3 drops of 2 N HCl which functioned as blank. The second tube solution was removed by one drop on a plate of drops, then added 3 drops of Dragendroff reagent. The orange deposits are formed indicating the presence of alkaloids (Widowati *et al.*, 2016, 2017, 2018).

Steroids and Triterpenoids Test

Samples 10 mg in a plate of drops, plus glacial acetic acid until unmerged, left for 10-15 minutes then added one drop H_2SO_4 concentrated. If you produce a blue-green color, the sample contains a compound of steroid class, whereas if it produces a purple/red/orange color, the sample contains a triterpenoid class compound (Widowati *et al.*, 2016, 2017, 2018).

Saponins Test

The basil leaves extract 10 mg dissolved using *aquabidest* in the reaction tube, then simmer in the bath for 5 minutes then filtered and then beaten strongly and added 1 N HCl. If the foam remains stable and still exists after being shed 1 N HCl Then the sample contains a saponin compound (Widowati *et al.*, 2016, 2017, 2018).

Tannins Test

The sample of basil leaves extracts 10 mg dissolved in 2 ml of 2 N HCl in the test tube, then heated in 24 water baths for 30 minutes, then chill and then add amil Alcohol 500 µl. If the amil layer of alcohol is orange/red, the sample contains tannin compounds (Widowati *et al.*, 2016, 2017, 2018).

Phenols Test

Dissolved extracts of basil leaves 10 mg in *aquabidest* as much as 5 ml, added solution $FeCl_3$ 1% as much as 500 µl. If the color is green/red/purple/blue/black, then the sample contains a group of compounds (Widowati *et al.*, 2016, 2017, 2018).

Terpenoids Test

Samples as much as 10 mg into the drop plate added vanillin sufficiently, added H_2SO_4 concentrated one drop then homogenize. If it produces a purple color then the sample contains the terpenoid compounds (Widowati *et al.*, 2016, 2017, 2018).

Table I. Phytochemical Screening Results of BLE

No.	Bioactive Compound	Results	
		Observation	**Conclusion**
1.	Flavonoid	The yellow color is formed	(+)
2.	Saponin	Foam formed as high as ± 1 cm. The addition of 1 drop of HCl does not eliminate the formed foam.	(+)
3.	Fennel	Blacklist Green color is formed	(+)
4.	Tanin	Amyl alcohol layer was not red or orange	(-)
5.	Steroid/Triterpenoid	The green color is formed	(+) Steroids
6.	Terpenoid	Does not produce a purple color	(-)
7.	Alkaloid	Orange color is formed	(+)

(+): Positive/contained; (-): Negative/not contained

DPPH Scavenging Activity Assay

Antioxidant activity was tested by using the DPPH Scavenging method *(2.2-diphenyl-2-picrylhydrazil).* DPPH free radical compound is stable in aqueous or methanol solution and has the purple tape shown by the absorption in solvent methanol at a wavelength of 515-520 nm. DPPH is sensitive to light, oxygen, and pH. However, are stable in a radical form so that the possible measurement of antioxidant activity is quite accurate. Free radical DPPH can capture hydrogen atoms from the component extracts are mixed, then react into reduced shape and marked with the decline in the intensity of the color purple DPPH solution*.* As many as 200 µL DPPH 0.077 mmol in the methanol added to 50 µL basil leaf ethanol extract sample on a microplate. The mix was incubated at room temperature for 30 minutes and then its absorbance values were measured at 517 nm wavelength using a microplate reader. Negative controls were used to DPPH by as much as 250 µL, blank used whereas for DMSO absolute as much as 250 µL (Widowati *et al.*, 2016, 2017, 2018).

$$\text{DPPH Scavenging Activity (\%)} = 1 - \frac{Asample}{Acontrol} \text{ x } 100\%$$

Asample = Sample Absorbance; Acontrol = Negative Control Absorbance.

Tyrosinase Enzyme Inhibitory Activity

Inhibition of tyrosinase enzyme activity is measured by a method that has been elaborated by Sigma Aldrich, (Fais *et al.*, 2009), as well as (Tu and Tawata, 2015) with a little modification. Mix the solution consisting of 20 µL sample (3.13 – 100 µg/mL), 20 µL of the enzyme tyrosinase from mushroom (125 U/mL), and 140 µL potassium phosphate buffer (20 mM, pH 6.8) incubated at room temperature for 15 minutes. It is prepared also to control which only contains 20 µL of enzyme and 160 µL phosphate buffer and the blank contains only 160 µL phosphate buffer and 20 µL sample. Next, mix the solution was added as many as 20 µL substrate l-dopa (1.5 mM) and incubated again at room temperature for 10 minutes. Absorbance is measured using a wavelength of 470 nm.

$$\text{Inhibitory Activity (\%) (Gupta } et\ al.\text{, 2009)} = \frac{C-S}{C} \text{ x } 100$$

C = Absorbance of enzyme activity without the sample; S = Absorbance of enzyme activity with the addition of a tested sample.

IC_{50} value is defined as the concentration of the samples needed to effect a reduction of 50% of the DPPH absorbance. IC_{50} value is then obtained after the relationship test between concentration and % inhibition (Warsi and Sholichah, 2017).

Data Analysis

In this study, the analysis of the test used in this study is a one-way ANOVA (One-way ANOVA) followed by Post Hoc Tukey tests using a Test with a confidence level of 95% (α = 0.05).

RESULT AND DISCUSSION

Phytochemical Screening

The phytochemical screening is a qualitative analysis that includes diverse organic compounds formed and hoarded by living beings (Widowati *et al.*, 2016, 2017, 2018). The results of phytochemical screening can be seen in Table I.

Table I shows the result that Basil leaves extract *(Ocimum americanum L.)* contains flavonoids, saponins, phenols, steroids, and alkaloids, as well as does not contain tannins, triterpenoid, and terpenoids.

Flavonoids are polar compounds because they have some hydroxyl groups. Therefore, the flavonoids are generally soluble in polar solvents such as ethanol that serves as a flavonoid-free of its salt shape. The addition of concentrated hydrochloric acid serves for the flavonoids

Table II. DPPH Scavenging Activity of BLE and Eugenol

Final Concentration (μg/mL)	**Mean of DPPH Scavenging Activity (%)**	
	BLE	**Eugenol**
200	95.36 ± 1.54 [e]	98.69 ± 0.33 [g]
100	94.83 ± 0.34 [e]	77.52 ± 0.17 [f]
50	77.76 ± 5.20 [d]	69.01 ± 0.56 [e]
25	57.26 ± 0.66 [c]	61.33± 0.15 [d]
12.5	42.83 ± 0.14 [b]	55.90 ± 0.27 [c]
6.250	34.50 ±0.63 [a]	52.03 ± 0.17 [b]
3.125		51.12 ± 0.73 [b]
1.563		46.32 ± 1.13 [a]

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

protonation to the flavonoids salts formed. After the addition of magnesium powder, positive results are indicated by the change in the color of the solution to black reddish, yellow, or orange (Rumagit *et al.*, 2015). Foam formed due to the saponins compound has physical properties that are easily soluble in water and will cause foam (Suharto, Edy and Dumanauw, 2012). Discoloration occurs when the addition of a $FeCl_3$ reacts with one of the hydroxyl groups present in the polyphenols (Aini, 2014).

The alkaloid test results show the formation of orange or orange colors. The alkaloid testing is carried out using a reagent/reactant of Dragendroff, where the resulting positive result is an orange precipitate for dragendroff. The deposits are potassium alkaloids. Reagent Dragendorff contains bismuth nitrate and potassium iodide in a solution of glacial acetic acid *(potassium Tetraiodobismutat (III)).* In the manufacture of Dragendorff reagent, the purpose of bismuth nitrate is dissolved in HCl in order not to occur hydrolysis reaction due to easily hydrolyzed salt-salt forming bismuth ions (BiO^+) (Ergina, Nuryanti S and Pursitasari, 2014)

The ingredients that are not contained in BLE, are tannins, triterpenoids, and terpenoids. However, the results of this research are similar to the research results of Ergina, Nuryanti S and Pursitasari, 2014 showing the unenlisted triterpenoid compounds that can be caused due by the use of solvents used in the extraction process are Polar and semi-polar solvents. Because the triterpenoid compound is a compound that is non-polar so these compounds cannot be extracted perfectly on the solvent. Terpenoids compounds are generally nonpolar so that they can be extracted with non-polar n-hexane solvents (Ginting *et al.*, 2017)

According to Lisan, 2015, hydrolyzed tannins are usually found in lower quantities compared to condensing tannins in plants. The tannins are condensed in many woody plants (Monisa *et al.*, 2016). In this study, researchers assume that the types of plants and parts of plants used as samples can also affect the presence or absence and large or small a compound content and in this research used as a sample is Basil plants that do not include woody plants, as well as the parts used, are leaves.

DPPH Scavenging InhibitoryActivity

2.2-diphenyl-2-picrylhydrazil (DPPH) is a compound of hydrogen radicals. DPPH will take a hydrogen atom in a compound. The reaction mechanism of the DPPH is taking place through electron transfer. The DPPH is purple solution gives the maximum absorption at 517 nm. DPPH solution will oxidize compounds in plant extracts. This process is characterized by the purple solution color fading away into yellow color (Widowati *et al.*, 2016, 2017, 2018). The percentage DPPH scavenging activity of *BLE* and eugenol can be seen in Table II and the median inhibitory concentration (IC_{50}) of samples based on DPPH scavenging activity can be seen in Table III.

Table II shows the percentage of DPPH scavenging activity of BLE and eugenol. In the highest concentration, BLE has a lower value (95.36±1.54%) than eugenol does (98.69±0.33%).

Table III shows the result that BLE has higher IC_{50} (20.55±0.04 μg/ml) compared to eugenol (2.44 ± 0.26 μg/mL). This indicates that BLE has lower DPPH scavenging activity than eugenol does.

Antioxidant compounds such as phenolic acids, polyphenols, and flavonoids can reduce or inhibit free radicals by inhibiting oxidative

Table III. IC_{50} Value of DPPH Scavenging Activity of BLEand Eugenol

Sample	Linear Regression	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
BLE	y = 0.6403x + 37.73	0.88	19.16	
	y = 0.6236x + 36.762	0.95	21.23	
	y = 0.6202x + 36.808	0.94	21.27	20.55±0.04
	y = 0.628x + 37.1	0.93	20.54	
Eugenol	y = 0.4154x + 49.205	0.94	1.91	
	y = 0.4176x + 49.094	0.95	2.17	2.44 ± 0.26
	y = 0.4262x + 48.959	0.94	2.44	
	y = 0.4262x + 48.959	0.94	2.44	

Table IV. Antityrosinase Activity of BLE and Eugenol

Final Concentration (µg/mL)	Mean of Antityrosinase Activity (%)	
	Ocimum americanum L.	Eugenol
200	67.98 ±0.44 [e]	92.59 ±1.49 [f]
100	54.89 ±0.15 [d]	69.16 ±0.21 [e]
50	48.11 ±0.80 [c]	59.78 ±0.26 [d]
25	45.62 ±1.00 [b]	54.92 ±0.89 [c]
12.5	39.06 ±0.68 [a]	46.24 ±0.29 [b]
6.25	37.55 ±0.76 [a]	41.71 ±0.39 [a]

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

mechanisms. The main characteristic of antioxidants is the ability to capture or inhibit free radicals because these free radicals can oxidize nucleic acids, proteins, fats, or DNA and can initiate a degenerative disease (Fidrianny, Rizkiya and Ruslan, 2015).

Antioxidant activity test with the DPPH method is based on a reduction of DPPH which is a stable free radical. Free radical DPPH *(2.2-Diphenyl-1-Picrylhydrazyl)* has an electron that can provide maximum absorption at 517 nm (purple color). When antioxidants react with DPPH which is a stable free radical will be paired due to the presence of hydrogen donors (e.g. antioxidant) and transformed into DPPH-H later as a consequence of the absorption of DPPH will be reduced. In the form of DPPH-H, the result obtained is decolorization or color change to yellow due to electron capture. Thus, the more decolorization occurs, the higher the ability to reduce free radicals. In other words, antioxidants can reduce the purple DPPH radicals into yellow diphenyl-picrylhydrazine when the fluid from the DPPH is mixed with the substance that can donate hydrogen atoms, it will occur in the form of non-radical discoloration *(Diphenyl-picrylhydrazine)* with a loss of purple. (Shekhar and Goyal, 2014; Bamidele, Bamidele and Nnate, 2017)

Antityrosinase Activity

Whitening agents play a role in many levels of melanogenesis or melanin formation in the skin and little-known as a competitive inhibitor of tyrosinase. Tyrosinase enzyme is involved in the process of melanogenesis and catalysis of the oxidation process of tyrosine into *dihydroxyphenylalanine* (L-DOPA) and of L-DOPA into L-DOPA-quinone (Kamkaen, Mulsri and Treesak, 2007; Wuttisin *et al.*, 2017). Tyrosinase catalyzes the oxidation reactions that produce chromophore and tyrosine can be detected at wavelengths of up to 510 nm. The reaction of the enzyme tyrosinase substrate with L-DOPA is producing the orange color. The enzyme tyrosinase inhibitory activity was marked by a decline in orange color that is formed or the result of the reaction is colored more light. It also simultaneously mark the presence of antioxidant activity in the reaction (Fais *et al.*, 2009; Tu and Tawata, 2015).

Based on Table IV, the antityrosinase activity of BLE and eugenol. In the highest concentration (200µg/mL), BLE has lower value (67.98 ±0.44 %) than eugenol does (92.59 ± 1.49 %).

Table V shows the result that BLE has higher IC_{50} (35.59 ± 0.83 µg/mL) compared to eugenol

Table V. IC50 Value of Antityrosinase Activity of BLE and Eugenol

Sampel	Persamaan	R^2	IC_{50} (µg/mL)	IC_{50} (µg/mL)
BLE	y = 0.4972x + 44.779	0.97	37.48	
	y = 0.4973x + 44.377	0.97	35.86	
	y = 0.4732x + 44.886	0.97	36.43	35.59 ± 0.83
	y = 0.4892x + 44.681	0.97	36.59	
Eugenol	y = 0.2965x + 38.86	0.97	10.50	
	y = 0.3 x + 39.243	0.95	11.31	10.87±0.41
	y = 0.3013x + 39.023	0.97	10.81	
	y = 0.2993x + 39.05	0.97	10.50	

(10.87±0.41 µg/mL). This indicates that BLE has an activity to inhibit tyrosinase enzyme, but has lower activity than eugenol does.

Hyperpigmentation is characterized by the absence of production and accumulation of melanin or an increase in the number of melanocyte cells. Accumulated hyperpigmentation can be caused by *lentigo* (flat brown spots on the skin), *nevus* (usually congenital nodules), and *ephelis* (red spots), or inflammatory states such as acne and eczema (Di Petrillo *et al.*, 2016). Bleach agents play a role in various levels in melanogenesis or the formation of melanin in the skin and are widely known as competitive inhibitors of tyrosinase. Tyrosinase is an enzyme involved in the process of melanogenesis and the catalyzation of the process of oxidation of tyrosine into *dihydroxyphenylalanine* (DOPA) and from DOPA into DOPA-quinone.

CONCLUSION

Basil leaf extract has lower antioxidant activity through the DPPH scavenging activity and antiaging activity through the inhibition of tyrosinase compared to the eugenol compound. From phytochemical assay obtained that BLE contains flavonoids, saponins, phenols, steroids, and alkaloids as well as negative tannins, triterpenoids, and terpenoids. IC_{50} value of DPPH scavenging activity and tyrosinase inhibition of BLE indicates that BLE has potential as a natural antioxidant and antiaging ingredient for daily consumption.

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