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Phytochemical Screening, Total Antioxidant Capacity, and Toxicity Test of Basil Leaf Extract (*Ocimum x africanum* Lour).

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ABSTRACT
High pollutant levels have caused an increase in disease that correlated with Reactive Oxygen Species through a condition that is oxidative stress. Oxidative stress can be stabilized with antioxidants by stabilizing active and unstable radicals to become inactive and stable. Basil leaves (*Ocimum x africanum* Lour) are known to have antioxidant capacity. The aim of this study is to determine the phytochemical profiling and antioxidant capacity of basil leaves extract. This research was conducted with an in vitro experimental study design. Extraction of this research was carried out by maceration method using methanol as solvent. The tests were carried out in the form of phytochemical tests to determine the content of secondary metabolites and antioxidant capacity tests using the DPPH method. In phytochemical test of basil leaf extract, it was found positive for secondary metabolites of alkaloids, anthocyanins, cardio glycosides, coumarins, Flavonoid, glycosides, phenols, quinones, saponins, steroid, terpenoids and tannins and the antioxidant capacity test obtained IC_{50} of 174.04 μg/mL. In this study we found that basil leaves contain secondary metabolites and antioxidants capacity but not as much as vitamin C, even though basil leave antioxidant capacity not as much as vitamin C, basil leave have other compound like Linalool that have antimicrobial and antioxidant activity, beside vitamin C also can induce acid reflux, so it can be concluded that basil leaves have secondary metabolites and the antioxidant capacity.

Keywords: *Ocimum x africanum Lour, Reactive Oxygen Species, Total Antioxidant Capacity.*

1. INTRODUCTION

In the past decade, great attention has been paid to the problem of exposure to air pollution due to vehicle traffic and other combustion processes. Air pollutants such as *Particulate matter (PM)* and gas are considered the most important factors in urban areas [1]. Each air pollutant can cause toxic effects on the respiratory and cardiovascular systems, such as ozone, nitrogen oxides, and suspended particles all of which have the same properties as strong oxidants that are a source of *reactive oxygen species* (ROS) [2], Sources of ROS can result from transition metal chemical reactions (iron, copper, and chromium) that catalyze fenton reactions therefore capable of inducing oxidative damage to DNA [3].

Oxidative Stres due to fenton reaction catalyzed by this transition metal can generate hydroxyl radicals that are the source of ROS in the body's system [4]. thus disrupting systemic organs such as the lungs, heart, liver and kidneys [5]. As a result the human body system needs defenses against ROS, that is antioxidants [6].

Antioxidants are divided into 2 categories such as *endogenous antioxidants* and *exogenous antioxidants. Exogenous antioxidants* can be obtained from nutrients, to support antioxidants that needed by the human body [7]. one of the sources *exogenous antioxidant* derived from Basil (*Ocimum x africanum lour*), *methanolic* extract content of basil leaves has antioxidant properties that neutralize ROS so that it can be hepatoprotective, Anti-carcinogenesis, and Cardioprotective [8].

Basil belongs to the family *Lamiaceae* which is a plant used as a foodstuff. In addition, empirically basil is used for complementary foods, skin diseases, antifungals and antiemetics [9].

Basil leave is a part of basil that known to have a high phenolic content that encourages the author to conduct further research on the antioxidant ability of basil leaves.

2. MATERIAL AND METHOD

This Experimental research using basil leave extract to be test on phytochemical test based on Harborne as reference and antioxidant activity with DPPH method. Basil leave collected as much as 700 grams thereafter this leave be dried to obtain 100 grams of simplicia after that, the simplicia macerated using methanol solvent and then evaporated using rotatory evaporator therefore will produce extract weight as much as 14.59 gram and the yield was 14.59 %. With the equation below:

$$\%\text{Yield} = \frac{\text{Extract weight}}{\text{Dried weight}} \times 100\% \quad (1)$$

$$= \frac{14.59}{100} \times 100\% \quad (2)$$

With the basil leaves extract, we tested the sample to test the phytochemical content that used mayer and dragendroff to find the alkaloid, NaOH to find anthocyanin, betacyanin, and flavonoid, keller-kiliani to find cardio glycosides, NH3 to find coumarin, *Modified* Borntrager to find glycosides, folin ciocalteau to find phenols, H_2SO_4 to find quinone, Foam test to find saponin, Liebermann-Burchard to find steroid, salkowski to find terpenoid, *ferric-chloride* to find tannins.

Antioxidant capacity test (DPPH) using Blois Method. Once the absorbance data of each concentration that has been measured by genesis 30 vis spectrophotometry, the amount of absorption resistance can be calculated through the average absorbance in tubes A and B with the formula:

mean sample absorbance =

$$\frac{\text{Absorbance A+Absorbance B}}{2} \quad (1) \quad (2)$$

% Inhibition is a large percentage of the sample's ability to inhibit the absorption of free radicals, which in this case is used as DPPH radicals.

Control absorption is defined as the result of absorption of DPPH solution with a concentration of 50 µM at maximum wavelength. Meanwhile, the average absorbance of the sample is the result of the absorption of the sample in accordance with the concentration of each sample that has been given radical DPPH 50 µM at the maximum wavelength. The graph (*simple scatter)* is then created according to the data that has been obtained between the concentration (x axis) and %inhibition (Y axis) and note the formation of linear lines, the result of the value R^2 and its linear regression equation (Y = aX + b). After that data is collected by measuring basil leave extract levels through antioxidant activity of DPPH method. Measurement antioxidant activity used Genesys 30 vis spectrophotometer by Collect the sample absorbance value.

Data are expressed as mean standard error of the mean. and presented in the form of tables and graphs. All statistical analysis were performed using GraphPad Prism v. 9.0 (GraphPad Software, San Diego, CA). p-value of <0.05 was considered statistically significant.

3. RESEARCH RESULTS

Phytochemicals of Basil Leave Extract

Based on the results of qualitative phytochemical tests basil leave extract obtained that this extract contains alkaloids, anthocyanins, cardio glycosides, coumarin, flavonoids, glycosides, phenolic, quinine, saponins, steroids, terpenoids, and tannins (Table 1). But no betacyanin compounds were found.

Total Antioxidant Capacity of Basil Leave

Table 1. Phytochemical Screening of Basil Leaf

Phytochemical	Extract	Method/Reagen
Alkaloid	+	Mayer/Wagner
Antosianin	+	NaOH
Betasianin	-	NaOH
Kardio glikosdia	+	Keller Kiliani
Kumarin	+	NaOH & Kloroform
Flavonoid	+	NaOH
Glikosida	+	Modified Borntrager
Fenolik	+	Foil Ciocalteau
Kuinon	+	H_2SO_4
Saponin	+	Foam
Steroid	+	Liebermann Burchard
Terpenoid	+	Salkowski
Tanin	+	*Ferric Chloride*

Extract

The absorbance of the test was determined by using ethanol extract *ocimum* x *africamum lour* weighed as much as 25 mg and dissolved with a solution of methanol in a measuring flask of 25 mL, so that the stock obtained with a concentration of 1000 µg /mL. Then dilute with a solution of methanol so that a concentration of 100, 200, 300, 400 µg/mL in a measuring flask of 10 mL, then stirred until evenly. To find out the required volume according to the specified concentration, the formula M1. V1 = M2. V2 is used. Prepare 4 test tubes and done duplo, so it takes 8 test tubes in total.

Tabel 2. Antioxidant Capacity of Basil Leaf Extract

Concentration (µg/mL)	Absorbance (516 nm)	Inhibition percentage (%)	IC_{50} (µg/mL)
100	0,431	30,484	174,04
200	0,261	58,548	
300	0,112	88,742	
400	0,071	88,226	

Using aluminum *foil* to wrap the entire test tube without exception. Prepare a 2 mL *microtube* tube for each extract sample concentration and then insert it into the *tube* until it is slightly full for easy sampling when DPPH is added. Into the test tube taken as much as 0.5 mL sample from microtube using micropipettes then inserted into the test tube according to their respective concentrations that have been determined earlier. Then added 3.5 mL of DPPH stock solution, after that stirred using *vortex* until well mixed, then incubated in a dark room with room temperature for 30 minutes. After 30 minutes, the absorbance is searched using a 30 vis genesys spectrophotometer. The calibration curve is based on the relationship between the concentration of basil leaves extract and the results of the calculation of the percentage of inhibition.

Maximum wavelength of DPPH is 516 nm with the maximum absorbance obtained is 0.620 nm. The absorbance value of each basil leave extract concentration (*Ocimum x africanum lour*) is read using a Uv-vis spectrophotometer at a wavelength of 510 nm and is calculated percent (%) inhibition of each concentration from (Table 2) then created curves and searched for linear equations. From the percentage value of inhibition obtained, a straight-line graph will be formed so that there is a linear equation y = 0.1974X + 15.645 with R^2 = 0.933 (Fig 1). This equation is used to find IC_{50} capacity of total antioxidant basil leave extract and obtained IC_{50} basil leave extract was 174.04 µg/mL.

Antioxidant Capacity of Vitamin C

The standard antioxidant capacity was determined by using vitamin C (ascorbic acid) by means of ascorbic acid 0.01 grams dissolved in a solution of methanol up to 100 mL. Then a stock with a concentration of 100 µg/mL will be formed. After that the dilution of ascorbic acid is done by taking stock as much as 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL, and 0.6 mL in measuring flask and dissolved with methanol up to 10 mL in all five stocks. Obtained concentrations of 2 µg/mL, 3 µg/mL, 4 µg/mL, 5 µg/mL, 6 µg /mL,respectively. Then take 3.5 mL of DPPH and added as much as 0.5 mL of ascorbic acid is piped on each dilution. Performed incubation for 30 minutes in a dark enclosed room at room temperature. After that, the absorbance is read at optimal wavelengths using a 30 vis genesys spectrophotometer. The calibration curve is based on the relationship between the concentration of ascorbic acid and the result of the calculation of the percentage of inhibition.

Table 3. Standard Levels of Ascorbic Acid

Concentration (µg/mL)	Absorbance (516 nm)	Inhibiton percentage (%)	IC_{50} (µg/mL)
2	0,346	26,85	5,40
4	0,288	39,11	
6	0,213	54,96	
8	0,152	61,86	
10	0,086	81,81	

Each concentration of vitamin C that has been mixed with DPPH is read using uv-vis spectrophotometer (Table 3). Once absorbance is obtained per concentration, it is calculated as percent (%) inhibition then made a standard curve and looked for linear equations. From the result of the percent inhibition obtained, a straight-line chart will be formed so that there is a linear equation y = 6.934X + 12.52 and the value R^2 = 0.9988 (figure 2). This equation is used to find ic value $_{50}$ and obtain IC_{50} of vitamin C was 5.40 µg/mL.

4. DISCUSSION

Phytochemical Screening

In the results of research on basil leaf extract during quantitative phytochemical screening test showed that basil leaves(*Ocimum x africanum lour*) compounds such as alkaloids, anthocyanins, cardio glycosides, coumarins, flavonoids, glycosides, phenolic, saponins, quinone, steroids, terpenoids, and tannins are in line with research conducted ononamadu et al [11], basil with species *Ocimum canum* containing saponins, phenolics, flavonoids, tannins, carotenoids, triterpenes, glycosides and alkaloids. According to Leila et al [12] *Ocimum basilicum L* contains steroids and Anthocyanins. According to Borah et al [13] *Ocimum Sanctum* contains *cardiac glycoside, quinone, and saponins.*

Total antioxidant capacity Test

In this study, researchers conducted a total capacity test of antioxidants using DPPH and the ingredients used as a standard comparison is vitamin C (ascorbic acid). The curve of vitamin C has a value of R2= 0.9983 so that it gets a linear equation Y = 6.935*X + 12.52. From the equation, IC50 vitamin C was obtained at 5.40 µg/mL. Dpph test curve of basil leaves extract has R2= 0.933 so that linear equation Y = 0.1974X + 15.645. From the equation, IC50 was obtained at 174.04 µg/mL. Based on the results of the DPPH scavenging assay test by measuring IC50 basil leaves extract, IC50 of basil leave was 174.04 µg/mL. When compared to IC50 of vitamin C which has a value of 5.40 µg/mL, it can be seen that vitamin C has a higher potential to reduce free radicals because the smaller IC50 indicates the higher the total capacity of antioxidant ingredients. Based on Blois [14] criteria, IC50 values of 150-200 µg/mL indicate weak total antioxidant and active capacity [15]. There is little difference with research conducted by Herani et al [15], antioxidant levels in Ocimum basilicum leave extract of 141.59 µg/mL, this difference is due to different species and different solvents used. Although basil leaves have a lower total antioxidant capacity than vitamin C It must be considered that Vitamin C can induced acid reflux that are not suitable for the people with Gastroesophageal reflux so basil leaves can be the solution for this, beside that basil leave not only contain antioxidant but also contain Linalool and citral which are terpenoid groups that have antioxidants capacity antimicrobials activity and widely used as cosmetic Ingredient [16].

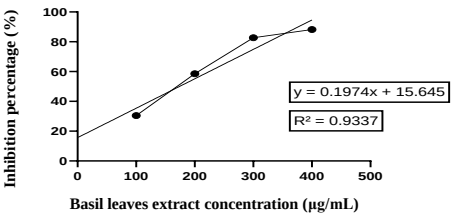

Figure 1 DPPH test of Basil Extract

Vitamin C as comparison standarts

The results of absorbance measurement of each concentration of vitamin C and % inhibition using the UV-Vis spectrophotometer tool and created a linear equation curve to obtain the IC50. From the standard curve that is obtained linear equation Y = 6882*X + 12.62 and value R2 = 0.9988 obtained IC_{50} = 5.40 µg/mL.

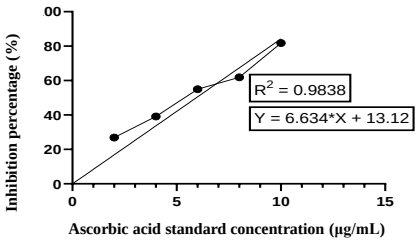

Figure 2. Standard Curve of Ascorbic Acid

5. CONCLUSION AND SUGGESTION

Based on the results and discussion on the study of antioxidant capacity test of basil leaf extract, *Ocimum x africanum lour* then obtained conclusions in the below that:

1. Basil leaves have compounds such as alkaloids, anthocyanins, cardio glycosides, coumarins, flavonoids, glycosides, phenolic, saponins, quinone, steroids, terpenoids, and tannins
2. Basil leaves have Antioxidant capacity with it's *inhibitory concentration* 50 (IC_{50}) was 174.04 µg/mL, that is categorized as active antioxidant capacity.

More research is needed on other parts besides leaves such as stems and roots and in-vivo research is needed.

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AUTHOR CONTRIBUTIONS

The authors contributed equally to all aspects of the article.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest

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