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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF BASIL (*Ocimum basilicum* L.) ESSENTIAL OIL AND LEAVES ETHANOLIC EXTRACT

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Abstrak

Daun kemangi mengandung senyawa metabolit sekunder yang dapat dijadikan sebagai antibakteri terhadap Staphylococcus aureus dan Escherichia coli. Daun kemangi dilakukan ekstraksi menggunakan metode maserasi dengan pelarut etanol 96% dan skrining fitokimia, sedangkan kandungan fitokimia dari minyak atsiri kemangi diidentifikasi dengan menggunakan GC-MS. Aktivitas antibakteri ditentukan dengan menggunakan metode cakram, dan dilanjutkan dengan penentuan MIC dan MBC. Ekstrak daun kemangi mengandung senyawa flavonoid, alkaloid, tanin, saponin, steroid, terpenoid, dan fenol, sedangkan minyak atsiri kemangi mengandung terpenoid, alkohol, hidrokarbon, fenol, asam lemak, dan aldehid. Diameter zona hambat dari ekstrak daun kemangi dan minyak atsiri kemangi terhadap bakteri S. aureus dan E coli yang paling besar diperoleh pada konsentrasi 100% dengan uji MIC ekstrak daun kemangi pada konsentrasi 15% dan minyak atsiri kemangi 10%, sedangkan konsentrasi MBC ekstrak daun kemangi dan minyak atsiri kemangi terhadap kedua bakteri dicapai pada konsentrasi 15%.

Kata kunci: antibakteri, ekstrak daun kemangi, *Escherichia coli,* minyak atsiri kemangi, *Staphylococcus aureus.*

Abstract

Basil (*Ocimum basilicum* L.) leaves have been reported to exert secondary metabolites that inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. This study was conducted by extracting leaf samples by means of maceration method with ethanol 96% as a solvent, and continued with phytochemical analysis, while phytochemical compounds of basil essential oil were identified by employing GC-MS analysis. Antibacterial activity of basil was determined using the disc diffusion method and then followed by determining the MIC and MBC values. The study showed that basil leaf extract contains flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, and phenols, while basil essential oil contains terpenoids, alcohols, phenols and fatty acids, with linalool as the main component. The inhibitory zone of ethanolic extract and essential oil on *S. aureus* and *E. coli* were reached at a concentration of 100% with MIC value obtained at a concentration of 15% for ethanolic extract and 10% for basil essential oil, respectively, while MBC value of both leaves extract and basil essential oil against both bacteria were reached at a concentration of 15%.

Keywords: antibacterial, basil leaf extract, *Escherichia coli*, basil essential oil, *Staphylococcus aureus*

Introduction

Indonesia is an agricultural country with a variety of natural wealth, especially medicinal plants. The use of natural products has begun to be applied in a variety of pharmaceutical and food industries applications [1]. One of the plants that is interesting and might be used as a medicinal plant is basil. Basil is a green plant and has a unique smell [2]. There are several secondary metabolites that are contained in basil leaf extract, such as flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, and phenols. Sweet basil essential oil also contains some compounds such as eugenols, linalools, methyl chavicols, beta-carotenes, phenols, and cineols [3]. Some chemical compounds that contain in basil have been reported to exert antibacterial properties. Antibacterials are needed for treating diseases caused by bacterial infections, and it is often that people in Indonesia are using antibiotics to treat an infection. But, the overuse of antibiotics will lead to antibiotic resistance cases if used for a long period and in the wrong dozes [4]. Antibiotics resistance has become a global problem that can threaten human health. Humans are attempting to find a natural products that can be used for disease treatment. Basil leaves extract and sweet basil essential oil have been applied to treat some various diseases [4], [5]. Several studies have revealed the antimicrobial effects of basil leaves extract and sweet basil essential oil against

gram positive and negative bacteria. *Staphylococcus aureus* is a gram positive bacteria that can be found in water, in the human skin, and as human normal intestinal flora. *Escherichia coli* is a gram negative bacteria that can be found in various habitats, ranging from human intestines, foods and water [6], [7]. It is then the objective of this study to test the antibacterial activity of basil leaves extract and sweet basil essential oil against two pathogenic bacteria, *i.e. S. aureus* and *E. coli*, and then followed by determining the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values of it.

Materials and Methods

Basil Leaf Preparation. Fresh basil leaves were obtained from a wet market in Yogyakarta, namely Pasar Kranggan (Kranggan Market). Basil leaves were cleansed from dirt by washing it with running water. The leaves were then cut and dried at room temperature, and put into the oven at 50°C for 1 hour until a stable weight reached [8].

Maceration and Evaporation. Basil leaves were placed in a blender and then sifted using 60 mesh sieve to obtain a crude simplisia. 209 grams of basil leaves simplicia were put into the glass jar and soaked with ethanol 96% solvent at 1:9 ratio. A rotary vacuum evaporator is then employed to evaporate the solvent until a thick basil leaves extract is obtained at a temperature of 50°C and 60 rpm. After that, put the thick basil leaves extract for 24 hours at room temperature until a paste of basil leaves extract is obtained [9]. After that, put the extract in a refrigerator until use.

Phytochemical Screening of Basil Leaves Extract. Phytochemical screening of the extracts of *Ocimum basilicum* L. leaves were conducted to find the presence of some phytochemicals such as flavonoids, alkaloids, saponins, tannins, steroids, phenols, and terpenoids, by using different standard qualitative methods described by [10], [11], [12], [13], [14], and [15] with some modifications.

Basil Essential Oil GC-MS Analysis. Determination of the chemical compounds of the basil essential oil was carried out by Gas Chromatography-Mass Spectrophotometry (GC-MS) QP2010 (Shimadzu, Japan).

Antibacterial Activity of Basil Leaves Extract and Essential Oil. The antibacterial activity of basil leaves extract and essential oil was determined by disc diffusion method. The crude extract of *Ocimum basilicum* L., basil essential oil, ciprofloxacin as a positive control, ethanol 96% as a negative control for basil leaves extract, and methanol as negative control for basil essential oil were tested against 2 bacteria species, *i.e. S. aureus* and *E. coli*. The test was carried out using an inoculated Mueller Hinton Agar medium of bacterial suspension with the density equal to 0.5 McFarland standard. In each of these plates, there are 8 sterile discs with 5 concentrations (20%, 40%, 60%, 80%, 100%) of basil leaves extract/sweet basil essential oil, positive control and negative control. Sterile discs were soaked in each concentration of leaves extract, essential oil, and control and then air dried. The sterile discs were put above the MHA medium and then were incubated at a temperature of 37°C for 24 hours. Antibacterial activity was measured as inhibition zona that showed around sterile discs and interpreted according to a standard [16].

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Assays. MIC and MBC values were determined using broth dilution methods. Basil leaves extract and essential oil are diluted to concentrations of 30%, 25%, 20%, 15%, 10%, 5%, 2.5%, and 1.25%. The MIC test was performed using microplate 96 well. Each concentration of extract, essential oil, positive control (ciprofloxacin), negative control (methanol and ethanol 96%) were inserted into the well. And then, each well was added with Nutrient Broth (NB) media and bacterial suspension. After that, the microplate is incubated at the temperature of 37°C for 18-24 hours and observed using ELISA Microplate Reader (Thermo Scientific, USA) with a wavelength of 600 nm. The results of MIC are determined on the basis of the smallest concentration that does not indicate the occurrence of bacterial growth. The Minimum Bactericidal Concentration test is done with a similar series of concentration as the MIC test and the MBC value was determined as the lowest concentration which showed no visible bacterial growth on agar plate.

Results and Discussion

Extraction of Basil Leaves. Basil leaves sample for antibacterial test was extracted by maceration using 96% ethanol for three days, and then evaporating using rotary vacuum evaporator at the temperature of 50°C with 60 rpm to obtain a paste extract [17]. The extraction was done by maceration method because it reduces the risk of damage of active compounds that are easily damaged by heat. **Table 1**. Shows the result of the yield percentage of basil leaves crude extract. The results revealed that the basil leaves crude extract yield is 23.54%. This value is much higher than that was reported using 70%

ethanol that gives 0.12% yield [18] and 96% by using 10.67% ethanol [19]. Based on this result, it is recommended to use 96% ethanol for extracting active compounds of basil leaves.

Table 1. The Result of Basil Leaves Crude Extract

Simplicia (gr)	Solvent (mL)	Crude Extract (gr)	Yield Percentage (%)
209	1,881	49.2	23.54

The Chemical Composition of Basil Essential Oil using GC-MS. The gas chromatography-mass spectrophotometry analysis of sweet basil essential oil revealed the presence of 19 compounds as outlined in **Table 2.**

Table 2. Main Chemical Compounds of Basil Essential Oil

Chemical compound	Formula	Area (%)	R. Time (min)	MW	SI
Eucalyptol	C10H18O	0.94	6.422	154	85
Linalool Oxide CIS	$C_{10}H_{18}O_2$	2.37	7.168	170	94
Linalool Oxide Trans	$C_{10}H_{18}O_2$	2.02	7.454	170	93
Linalool L	$C_{10}H_{18}O_2$	22.77	7.761	154	96
Cyclohexanol	$C_6H_{12}OH$	0.68	9.516	156	96
Benzene	C_6H_6O	68.22	10.226	148	96
Z-Citral	$C_{10}H_{16}O$	0.17	11.070	152	95
endo-Isofenchol	$C_{10}H_{18}O$	0.10	11.518	154	70
Benzaldehida	C_6H_5CHO	0.13	11.681	136	96
Trans-Citral	$C_{10}H_{16}O$	0.46	11.851	152	96
Methoxyphenol	$C_7H_8O_2$	0.11	14.428	164	94
α-Cubebene	$C_{15}H_{24}$	0.08	14.671	204	93
Methyl Ester	$C_{19}H_{32}O_2$	0.08	15.108	292	78
α-Bergamotene	$C_{15}H_{24}$	0.69	16.186	204	93
ß-Farnesene	$C_{15}H_{24}$	0.11	16.686	204	92
ß-Funebrene	$C_{15}H_{24}$	0.10	17.519	204	88
ß-Bisabolene	$C_{15}H_{24}$	0.10	18.116	204	95
α-Bisabolene	$C_{15}H_{24}$	0.56	18.959	204	93
Caryophyllene Oxide	$C_{15}H_{24}O$	0.30	20.171	220	85

The similarity index value of the detected compounds are in the range of 70-96 which is considered as high and indicates that these compounds have a similarity and identify as a standard compound in the database library. The highest percentage of chemical compounds achieved by basil essential oil was linalool at 27.16%. It is reported that linalool is a bacteriostatic agent that can interfere with the integrity of the bacterial cell membrane and result in leakage of nucleic acid and proteins in bacterial cells. The impact of cell membrane disruption will cause detrimental effects on the energy metabolism of bacterial cells, and also one of the factors that causes bacterial death [20].

Qualitative Phytochemical Screening of Basil Leaves Crude Extract. The phytochemical compounds in the plants are responsible for many biological activities, including antimicrobial. The research showed that *Ocimum basilicum* L. extract contained all the most bioactive compounds, such as flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, and phenols. The flavonoids compound already reported have the property as an antimicrobial by denaturing the proteins in the cell membrane, and the cell membrane will cause a cell lysis and hinder cell growth [21]. Tannins compounds have an antimicrobial activity by binding the proteins of bacteria, and then it will interfere with the protein synthesis and permeability of bacterial cells [22]. Saponins also have been shown to exert antimicrobial effects so some plants that contain saponins can be used as medical plants [23]. The phenolic compounds in the aromatic plant extract are often used as an antimicrobial agent by interfering membrane permeability which can cause macromolecules and the cations in cells to disappear. Moreover, phenols will interact with proteins and the wall of cell bacteria, and then the cytoplasm membrane will be destroyed [24].

Antibacterial Activity of Basil Leaves Extract and Basil Essential Oil. The result of an antibacterial activity test of basil leaves extract and essential oil against *S. aureus* and *E. coli* is shown in **Table 3** that tells us that leaves ethanolic

extract and basil essential oil have a different antibacterial effect on the growth of *S. aureus* and *E. coli*. The data presented showed the result of antibacterial activity of leaves ethanolic extract with a best inhibition zone of *S. aureus* and *E. coli* achieved at 100% with a diameter of 8 mm and 9 mm, respectively, that could be considered as moderate category. The result of antibacterial activity of essential oil against *S. aureus* and *E. coli* achieved at 100% with a diameter of 16 mm and 17 mm, respectively, that could be considered as a strong category. This result shows the superiority of essential oils compared to leaves ethanolic extract to inhibit the growth of tested bacteria.

Table 3. Antibacterial Activity of Basil Leaves Ethanolic Extract and Essential Oil

Bacteria	Volume of Basil Extract (10 µl)	Inhibition Zone (mm)	Category
Staphylococcus aureus	100%	8	Moderate
	80%	7	Moderate
	60%	7	Moderate
	40%	7	Moderate
	20%	6	Moderate
	Ciprofloxacin 0.005% (+)	22	Very strong
Escherichia coli	100%	9	Moderate
	80%	8	Moderate
	60%	8	Moderate
	40%	7	Moderate
	20%	7	Moderate
	Ciprofloxacin 0.005% (+)	26	Very strong
Bacteria	**Essential Oil Volume (10 µl)**	**Inhibition Zone (mm)**	**Category**
Staphylococcus aureus	100%	16	Strong
	80%	15	Strong
	60%	14	Strong
	40%	10	Moderate
	20%	8	Moderate
	Ciprofloxacin 0.005% (+)	22	Very strong
Escherichia coli	100%	17	Strong
	80%	16	Strong
	60%	15	Strong
	40%	11	Strong
	20%	9	Moderate
	Ciprofloxacin 0.005% (+)	28	Very strong

(+) : positive control

The phytochemical compound detected in the essential oil as reported here, especially linalool, has been known for its biological activity and is also effective for being an antimicrobial [25]. Based on several previous studies, it is known that linalool may act as an antibacterial by reducing the membrane potential; the membrane structure and also the bacterial cell wall will experience leakage of cytoplasmic contents. In general, linalool will inhibit metabolism and energy synthesis

in bacterial cells, as well as inhibiting cellular respiration [26].. Ciprofloxacin is used as positive control and has an inhibition zone of *S. aureus* and *E. coli* with a diameter of 22 and 28 mm that belongs to the very strong category. Ciprofloxacin was chosen because it is a common wide spectrum antibiotic which inhibits bacterial growth by inhibiting the replication of DNA. It is reported that ciprofloxacin may inhibit the action of DNA gyrase and DNA topoisomerase which play a role in the initial stage of DNA replication [27].

MIC and MBC Assay. The inhibitory activity of the essential oil and the ethanolic extract could be further analyzed by conducting the MIC assay. The results of the MIC tests are shown in **Figure 1**, and again, as the result of the antibacterial assay, the MIC value of essential oil was better compared to the MIC value of basil ethanolic leaves extract. The MIC value of basil ethanolic leaves extract was reached at 15% and the MIC value of basil essential oil from both bacteria was reached at 10%.

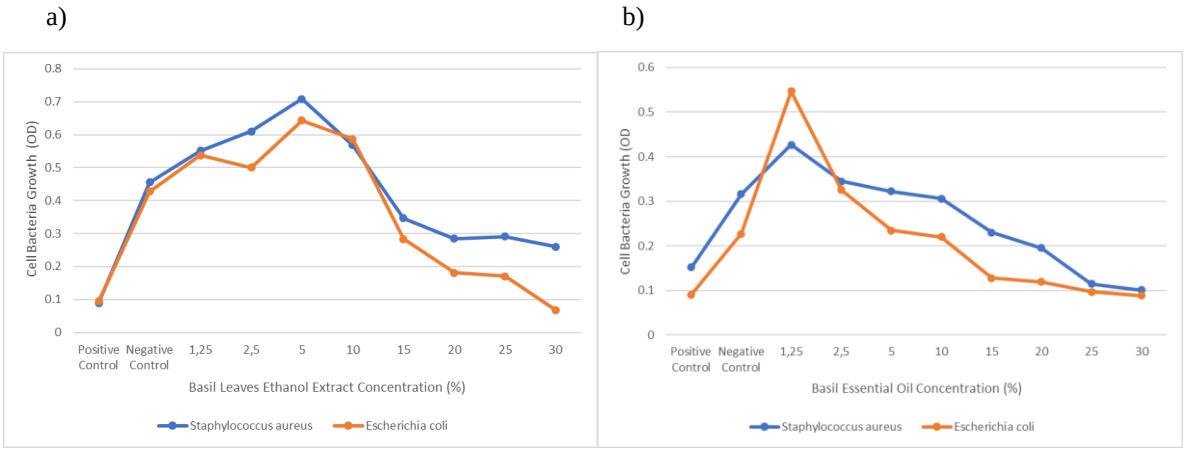

Figure 1. MIC Value of a) Basil Ethanolic Leaves Extract and b) Essential Oil against *S. aureus* and *E. coli*

After determining the MIC results, the study was continued by measuring the MBC value and the results are presented in **Figure 2**. The results of the MBC tests showed that both preparations reached the same value, which is at concentration of 15%.

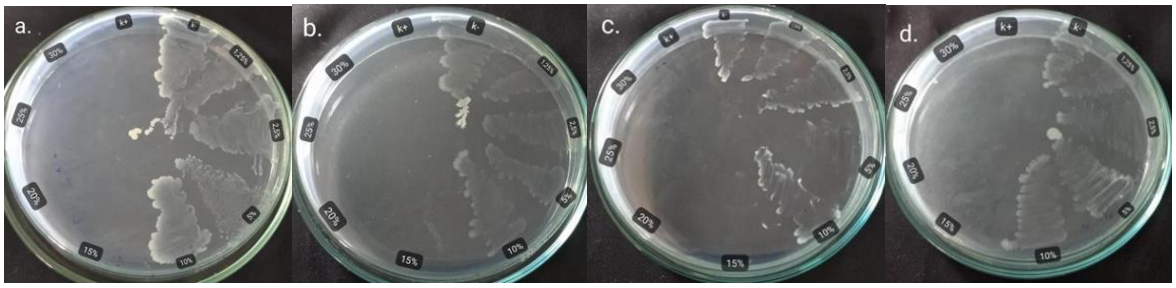

Figure 2. MBC Tests Result of Basil Leaves Extract Against (a) *S. aureus* and (b) *E. coli* and MBC Test Result of Basil Essential Oil Against (c) *S. aureus* and (d) *E. coli*,

(30%-1,25% : Concentration of Basil Leaves Extract and Essential Oil, K(+) = Ciprofloxacin, and K(-) = Ethanol 96% and Methanol as negative controls)

Based on the research results, basil leaves extract and essential oil is able to inhibit *S. aureus* and *E. coli*. The phytochemical analysis results in the knowledge that there is the presence of secondary metabolite compounds in basil. Considering that basil is a popular condiment in Indonesia, further study could be done by testing its antibacterial activity against different bacteria, even the pathogenic ones. However, this study also shows the low resulting yield of the extract and the essential oils. Effort could be considered to improve this yield by adding more basil leaves or optimizing the extraction solvent.

Conclusion

This study showed that basil leaves extract and sweet basil essential oil may be used as an antibacterial agent against *S. aureus* and *E. coli*. The phytochemical compounds contained in the basil leaves extract are flavonoids, tannins, saponins,

steroids, terpenoids, and phenols, while basil essential oil contains terpenoids, alcohols, phenols, fatty acids, and aldehydes. The MIC value of basil ethanolic leaves extract was reached at 15% and basil essential oil at 10%, whereas the MBC value of both preparations were reached at 15%.

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