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Phytochemical Screening and *in Vitro* Antibacterial Activity of Sweet Basil Leaves (*Ocimum basilicum* L.) Essential Oil against *Cutibacterium acnes* ATCC 11827

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Abstract. The sweet basil leaves (*Ocimum basilicum* L.) essential oil has antibacterial power against bacteria. The objective of this research was to evaluate the phytochemical components, antibacterial properties of essential oil of sweet basil leaves against *Cutibacterium* (formerly *Propionibacterium*) *acnes*, Minimum Inhibitory Concentration (MIC), and Minimum Bactericidal Concentration (MBC). The research was started by performing gas chromatography-mass spectrometry (GC-MS) analysis. Followed by determining antibacterial activity of solutions which contained 4 % v/v, 6 % v/v, 8 % v/v, and 10 % v/v of sweet basil leaves essential oil using disc diffusion method. The MIC was assessed using the pour plate method. Lastly, the MBC was assessed using the streak plate method. The results revealed that the major components of essential oil of sweet basil leaves namely neral, citral, α-humulene, β-caryophyllene, linalool, and germacrene-d. Furthermore, it showed antibacterial activity against *C. acnes* growth. The MIC and MBC values were 2 % v/v and 3.5 % v/v respectively.

Keywords: *Cutibacterium acnes*, antibacterial activity, essential oil, GC-MS, sweet basil.

INTRODUCTION

Beauty trends always continually evolve from time to time. Nowadays, makeup is not the main thing of beauty. Women are well aware that skin care routine is just as important as makeup. Skincare products are not always set in stone, there are many choices and can be adjusted depending on skin types. One of the hottest trends of skincare is face serum. A serum is a particularly potent and deep-penetrating product that uses high doses of particular ingredients to address specific skincare concerns [1]. Acne is one of the skin problems that women want to eliminate. Serums can be great for acne-prone skin types. Overactive sebaceous glands, clogged pores, the activity of skin bacteria, and inflammation are the factors that can cause acne [2]. Bacteria cause acne are *Cutibacterium acnes*, *Staphylococcus aureus*, and *S. epidermidis*. *C. acnes* is a gram-positive, non-spore-forming, aerotolerant anaerobic bacillus. *C. acnes* is one of the primary factors involved in the pathogenesis of acne vulgaris [3].

One of the main constituents of oil-based face serum is essential oil. The essential oil can be extracted from plants. The essential oil can take part as an antibacterial agent. Therefore, it can inhibit the growth of pathogenic bacteria, i.e., *C. acnes*. Natural products have been used as folk medicine for many years. Among them, sweet basil (*Ocimum basilicum* L.) has attracted increased interest due to its potential antibacterial activity against pathogenic bacteria. Biological activity studies showed that sweet basil essential oil promotes anti-inflammatory properties so it can reduce inflammation that causes by *C. acnes* beside its roles as antibacterial agent [4].

Even though the antibacterial activity of sweet basil essential oil against acne-causing bacteria has been investigated and reported by many researchers for medical purposes, little information is available regarding its efficacy against specific acne-causing bacterium, *C. acnes*. The present study aimed to evaluate phytochemical

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components of sweet basil essential oil and its potential to serve as an antibacterial agent against acne-caused bacterium *C. acnes* using disc diffusion method.

MATERIALS AND METHODS

Bacterial Strains and Culture Conditions

Cutibacterium acnes was obtained from the American Type Culture Collection (ATCC) *C. acnes* ATCC 11827. *C. acnes* was grown at 37 °C in Tryptic Soy Broth (OXOID). Throughout the experiments before use, liquid cultures were transferred using streak plate method onto fresh Tryptic Soy Agar (OXOID) and incubated at 37 °C for 48 h in anaerobic condition using anaerogen. Inocula were prepared by diluting recultures in saline to approximately 10^{11} CFU · L^{-1}. To adjust the turbidity of bacterial suspensions, 0.5 McFarland standard was used as a reference.

Plant Material

Plants were collected from Stan Traditional Market, Tajem, Sleman, Daerah Istimewa Yogyakarta. The sweet basil leaves (from the first branch until forth branch from the top) were air-dried and kept overnight in a place that did not expose to direct sunlight.

Extraction of the Essential Oil

The essential oil was obtained from air-dried plant material by water and steam distillation methods. These methods were specifically used in the fresh or dry botanical material. The air-dried leaves did not directly in contact with water; air-dried leaves were supported above the boiling water on a perforated grid. The steam from boiling water passed through air-dried sweet basil leaves carried its volatile components out through the top of the chamber. The steam and vaporized component condensed to water vapor and essential oil (distillate). The extraction was carried out for three hours, then the distillate was dried over by adding anhydrous sodium sulphate right away. After anhydrous sodium sulphate was added, water vapor would be absorbed by it, and essential oil was separated in a sealed glass bottle at 4 °C until antibacterial activity assay.

Identification of Phytochemical Components

For the identifications of the components, analytical gas chromatography-mass spectrometry (GC-MS) was performed using QP2010S SHIMADZU. The GC-MS was conducted using AB-5MS capillary column, with these conditions: carrier gas:helium; split ratio: 139.0; temperature program: the column was maintained at 50 °C for five min, then raised to 240 °C at the rate of 5 °C · min; EI ion source; electron energy: 70 eV; ion source temperature: 250 °C; interface temperature: 250 °C. The sample was directly injected into GC-MS, and it was performed in the Organic Chemistry Laboratory, Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada.

Antibacterial Susceptibility Assay

The bacterial growth inhibitory potential of sweet basil leaves essential oil was determined using the disc diffusion method. For the disc diffusion assay, 100 µL of bacterial suspension was uniformly spread on a solid Mueller-Hinton Agar (MHA) (OXOID) + 5 % defibrinated goat blood in a Petri dish. Sterile blank discs (5 mm in diameter; OXOID) were impregnated with 20 µL of the diluted sweet basil leaves essential oil (4 % v/v, 6 % v/v, 8 % v/v, and 10 % v/v), clindamycin 0.15 g (NOVELL) as positive control, and Tween 80 as negative control. For each treatment was repeated four times. Plates were incubated at 37 °C for 24 h in anaerobic condition using anaerogen (OXOID AnaeroGen™ 3.5 L Sachet).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MICs of the sweet basil leaves essential oil were determined using the pour plate method. The sweet basil leaves essential oil was made into various concentrations, namely, 3.5 % v/v, 3 % v/v; 2.5 % v/v, 2 % v/v, 1.5 % v/v, 1 % v/v, and 0.5 % v/v, according to the lowest concentration that showed antibacterial activity in the pre-assay antibacterial effect. Each duplication of sweet basil leaves essential oil was taken 0.5 mL using a measuring pipette and then poured into liquid MHA (OXOID) in a Petri dish. Then, 0.5 mL of bacterial suspension was taken using separate measuring pipette and poured into liquid MHA in the same Petri dish. Afterward, the lid was replaced, and the dish was gently rotated to mix the suspension, essential oil, and medium thoroughly. The plates were incubated at 37 °C for 24 h. The MIC was determined as the lowest concentration of essential oil inhibiting the visible growth of *C. acnes* on the surface.

For the determination of MBCs, the plates without any *C. acnes* growth were transferred into new solid MHA. To transfer into the new MHA, streak plate method was chosen and then the plates were incubated at 37 °C for 24 h. Subsequently, the growth of *C. acnes* after the plates were incubated needed to be observed. The lowest concentrations of essential oil that showed no visible growth of *C. acnes* in the Petri dish was interpreted as the MBC.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil of Sweet Basil (*Ocimum basilicum* L.)

The components which composed essential oil of sweet basil leaves were assessed using analytical gas chromatography-mass spectrometry (Fig. 1). Each of peaks in the chromatogram represented the signal created when a component eluted from GC column into the detector. It showed 19 peaks; each peak represented an individual component that was separated from the essential oil of sweet basil.

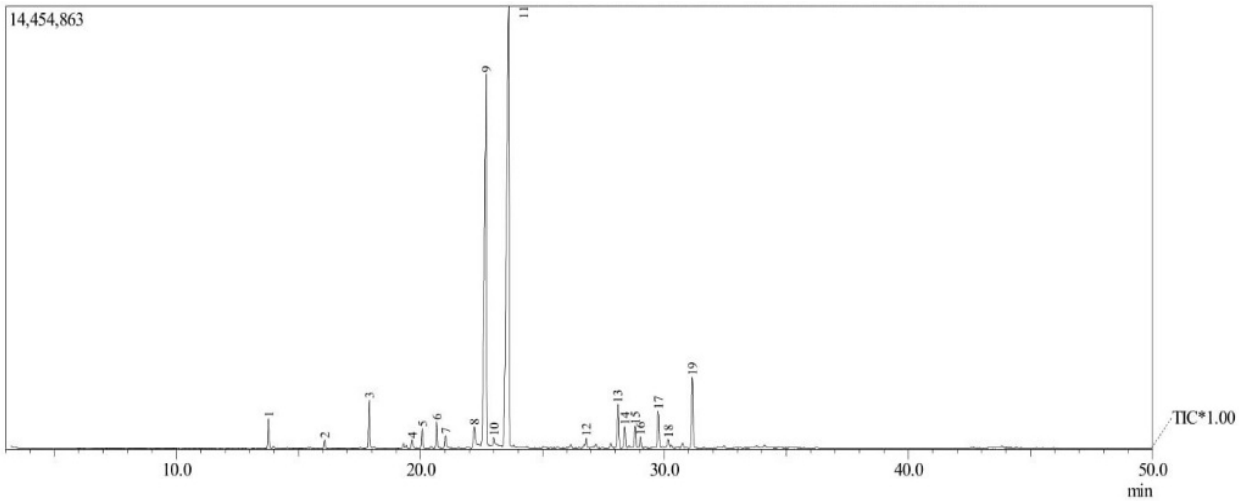

FIGURE 1. Chromatogram of the essential oil of *Ocimum basilicum* L.

Nineteen components were identified in the essential oils of *O. basilicum* L. (Table 1). Essential oil of *O. basilicum* L. was a complex mixture of major compounds, namely neral (41.45 %), citral cis, trans or widely known as citral (33.29 %), α-humulene (4.46 %), β-caryophyllene (2.62 %), linalool (2.45 %), and germacrene-d (2.32 %). Total percentage of all the components were 100 %. Therefore, it indicated that the essential oil of sweet basil was a complex, volatile mixture. Generally speaking, the essential oil is a volatile compound that derived as secondary metabolic products of plants. Secondary metabolites are compounds that are synthesized in a cell, through the pathway of carbohydrate and protein biosynthesis, produced in small quantities and not continuously. The aim of secondary metabolites helps the plants adapt themselves from their habitat and is an act of

self-defence. Its components are unique and can be different because of many factors. Therefore, we noticed an apparent variation in major components essential oil of specific plant could have different odour depending on main part of plant utilized, genotype, the plant ecotype, geographic origins, or adaptive process to some particular and local ecological conditions [5].

A substantial number of studies conducted on the composition of basil essential oil revealed a massive diversity in the components of its oil from many regions of the worlds. In the Brazilian basil leaf, essential oils, linalool, geraniol, and 1,8-cineole are the major compounds corroborating with Oman basil, as well as Poland basil. For the Czech Republic, Guinea, and Reunion, the major compounds of basil essential oil are linalool and eugenol. The presence of linalool, methyl chavicol, and eugenol as main components were found in a mixture of Italian basil essential oil. Additionally, the essential oil from Madagascar, Iran, and Thailand is rich in methyl chavicol [6]. The essential oil that was used in this research belonged to the citral-rich type. It still had linalool like most of basil essential oils, but the presence was not dominant.

TABLE 1. The main constituents of the essential oil of *Ocimum basilicum* L.

Peak No.	Compounds	Retention Time (min)	Area (%)
1.	6-methyl-5-hepten-2-one	13.772	1.50
2.	1,3,7-octatriene, 3,7-dimethyl	16.073	0.48
3.	Linalool	17.903	2.45
4.	Cyclopentane, 1-methyl-1-(2-methyl-2-propenyl)	19.656	0.64
5.	Limonene oxide	20.073	1.02
6.	7-oxabicyclo[4.1.0] heptane	20.665	1.49
7.	α-terpineol	21.029	1.20
8.	Nerol	22.206	1.84
9.	Citral (isomer 1) cis, trans	22.692	33.29
10.	Geraniol	23.005	0.66
11.	Z-Citral	23.612	41.45
12.	Trans-caryophyllene	26.792	0.65
13.	β-Caryophyllene	28.096	2.62
14.	Trans-α-bergamotene	28.376	1.27
15.	β-Farnesene	23.796	1.25
16.	α-Caryophyllene	29.024	0.76
17.	Germacrene-d	29.748	2.32
18.	Bicyclogermacrene	30.154	0.67
19.	α-Humulene	31.149	4.46

Antibacterial Activity of Sweet Basil (*Ocimum basilicum* L.) Essential Oil

In this study, tween 80 was chosen as the negative control because it was a suitable solvent for essential oil of sweet basil leaves without necessarily inhibited the growth of *C. acnes*. Based on the previous study, tween 80 was chosen as chamomile essential oil solvent. It did not inhibit the growth of *C. acnes* [7]. Another underlying study showed it as a suitable solvent for essential oil of sweet basil leaves [8]. In this study, antibacterial activity was determined by measuring the clear zone of inhibition horizontally and vertically, respectively, and then divided by two (Fig. 2). Inhibition zone did not include the diameter of the paper disc. As can be seen in Table 2, active components of sweet basil leaves essential oil revealed that it had strong antibacterial activities against *C. acnes*. The strength of antibacterial activity was determined by inhibition zone. Inhibition zone with diameter >15 mm has strong inhibitory activity, diameter (9–14) mm has moderate inhibitory activity and diameter <8 mm has weak inhibitory activity.

(a) (b)

FIGURE 2. Inhibition zone against *Cutibacterium acnes.*
(a) using *Ocimum basilicum* L. essential oil (10 % v/v, 6 % v/v, 4 % v/v, and 8 % v/v)
(b) using negative and positive control

The highest compound that composed sweet basil leaves essential oil was z-citral or widely known as neral. Neral components individually elicit an antibacterial action on a gram-negative and gram-positive organism. Potential mechanism of antibacterial activity of neral was by damaging the bacterial cell wall. Neral is terpenoid compounds. Neral is cis-isomer of citral or a mixture of terpenoids with the molecular formula $C_{10}H_{16}O$. Terpenoids are the main compounds that composed sweet basil leaves essential oil that can disrupt the permeability of plasma membranes and inactivated the enzymes of a bacterial cell. Another high in concentration compound that composed sweet basil leaves essential oil was citral. The two compounds are double bond isomers. The *trans*-citral is known as geranial or citral A. The *cis*-citral is known as neral or citral B [9].

TABLE 2. Determination of the antibacterial effect of the essential oil.

Treatment		Inhibition Zone (mm)	Category
Essential oil of sweet basil leaves	4 %	16.78^{c}	Strong
	6 %	16.98^{bc}	Strong
	8 %	17.31^{b}	Strong
	10 %	18.13^{a}	Strong
Control	Positive (clindamycin)	13.13	Moderate
	Negative (Tween 80)	0	No inhibition zone

Note: Number which is followed by different alphabets indicate absolute difference according to Least Significant Difference Test at 5-percent level.

The mechanisms citral worked as antibacterial was by reducing intracellular adenosine triphosphate (ATP) significantly. Living organisms generate ATP through respiration and subsequently utilize ATP to carry out cellular functions that are necessary for their survival, growth, replication, and signalling functions [10]. The possible mechanism of citral as the antibacterial agent was after the significant reduction in intracellular ATP pool, the level of ATP extracellular pool increased. The significant reduction in intracellular ATP can be resulted due to the loss of inorganic phosphate across the compromised high permeable cell membrane. Subsequently, leakage of intracellular material is a general phenomenon induced by the antibacterial agent; it was possible citral, results in cell death (bactericidal) [11].

Another compound that took a role as an antibacterial agent was linalool. Linalool showed bacteriostatic properties and reported to inhibit the growth of microorganisms. Linalool may be responsible for its interaction with intracellular components [12]. Terpene alcohols, i.e., Linalool, can a cause damaging effect on bacterial cell membranes. Research had been reported that there were changes in the concentration of K^{+} ions in bacterial suspensions in the presence of terpene alcohols. Thus, terpene alcohols can damage cell membranes depended both on the concentration and the nature of the terpene alcohol. It appears that damage to cell membranes is a common feature of the antibacterial activity of terpene alcohols. Damaged cell membranes can cause leakage of intracellular material. Leakage of cell material can lead to cell death which is a bactericidal effect as an antibacterial agent [11].

Other major constituents of sweet basil leaves essential oil which were found namely α-humulene, β-caryophyllene and germacrene-d. These constituents were categorized as sesquiterpenes. Medium antibacterial effects of the sesquiterpene hydrocarbon α-humulene against gram-positive bacteria was found. Germacrene-d had

been confirmed to have antibacterial activity [13, 14]. Sesquiterpenes are responsible as an antibacterial agent by disrupting the permeability of bacterial cell membranes so that can disrupt the bacterial growth.

Based on the results shown of this study, it can be proven that solutions containing 4 % v/v, 6 % v/v, 8 % v/v, and 10 % v/v sweet basil leaves essential oil had potential as an antibacterial agent that inhibited the growth of *C. acnes*. The higher the concentration of sweet basil leaves essential oil, the stronger inhibitory activity was recorded.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

A new variation of concentrations was made based on the results of antibacterial susceptibility assay. The solution that contained 4 % v/v of sweet basil leaves essential oil indicated as the lowest concentration that inhibited the growth of *C. acnes.* Therefore, the new concentrations to determine MIC were 3.5 % v/v, 3 % v/v, 2.5 % v/v, 2 % v/v, 1.5 % v/v, 1 % v/v, and 0.5 % v/v. The results showed that concentrations of 3.5 % v/v, 3 % v/v, 2.5 % v/v, and 2% v/v of sweet basil leaves essential oil were considered effective in inhibiting the growth of *C. acnes*. Inhibition bacteria growth in MIC test was determined as bacteriostatic because the essential oil derived from sweet basil leaves suppressed the growth of *C. acnes*, while not necessarily killing them. Concentrations of 3.5 % v/v, 3 % v/v, 2.5 % v/v, and 2 % v/v of sweet basil leaves essential oil had potential as bacteriostatic (Fig. 3). From this study, it showed that solution contained 2 % v/v of it was considered as the MIC value because the growth of *C. acnes* was not as dense as the previous concentrations, 1.5 % v/v, 1 % v/v, and 0.5 % v/v.

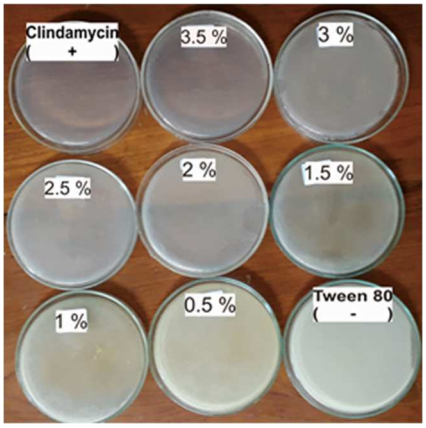

FIGURE 3. Minimum inhibitory concentration of the essential oil of *Ocimum basilicum* L.

MBC test was another subsequent test that was needed to be done as well. MBC test determined the strength of potential solutions as the antibacterial agent. The concentration that used for MBC test was 3.5 % v/v because it showed the least *C. acnes* growth in MIC test. The result proved that the concentration of 3.5 % v/v sweet basil leaves essential oil completely killed *C. acnes* because the result of streak plate showed no growth of *C. acnes*. Therefore, solution that contains 3.5 % v/v of sweet basil leaves essential oil can be categorized as bactericidal concentration.

CONCLUSION

Essential oil of sweet basil leaves (*Ocimum basilicum* L.) had major components which composed it, namely neral (41.45 %), citral (33.29 %), α-humulene (4.46 %), β-Caryophyllene (2.62 %), linalool (2.45 %), and germacrene-d (2.32 %). Those components had their characteristic and displayed high antimicrobial properties towards the gram-positive *Cutibacterium* (formerly *Propionibacterium) acnes*. The higher the concentration of sweet basil leaves essential oil, the stronger inhibitory activity was recorded. The most potent inhibitory activity was recorded on a solution that contained 10 % of sweet basil leaves essential oil with resulted in 18.13 mm of inhibition

zone. The MIC and MBC values for sweet basil leaves essential oil against *C. acnes* were 2 % v/v and 3.5 % v/v, respectively.

A better understanding of the interaction between essential oil compounds and bacteria target is going to be fundamental to work out the best environmental conditions to be used to ensure an effective antimicrobial activity. Sweet basil (*O. basilicum* L.) could be a potential source of new face serum that contains essential oil to prevent acne. Finally, clinical confirmation and pharmacological standardization are required before its application as a face serum.

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