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PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL ACTIVITY OF ETHANOL EXTRACT OF BASIL LEAVES (*Ocimum sanctum* L.) AGAINST *Propionibacterium acne* BACTERIA

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ABSTRACT

Acne is a skin disease often experienced by teenagers and young adults. Basil leaves (*Ocimum sanctum* L.) could be used as an alternative treatment for acne. This study aimed to determine the secondary metabolite content and antibacterial activity of *Ocimum sanctum* ethanol extract against *Propionibacterium acne*. Simplicia was extracted from basil leaves by maceration with 96% ethanol solvent. The extracts were tested for parameters, phytochemical screening, and antibacterial activity using the disc diffusion method. *Ocimum sanctum* ethanol extract was used at concentrations of 20%, 30%, and 40%. *Ocimum sanctum* ethanol extract has a brownish-black color with a distinctive aromatic aroma and a bitter, slightly sour taste, with a moisture content of 5.6%, ash content of 7.71%, and drying shrinkage of 7.1%. *Ocimum sanctum* ethanol extract contains flavonoids, tannins, steroids, and saponins. Antibacterial activity of clindamycin 0.01% and *Ocimum sanctum* ethanol extract concentration of 20%, 30%, and 40% could inhibit *Propionibacterium acne* bacteria as much as 11.95 mm, 4.47 mm, 4.91 mm, and 6.30 mm. The antibacterial activity of the *Ocimum sanctum* ethanol extract was 20%. The 30% and 40% treatments differed significantly from the positive controls. The ethanol extract of basil leaves has antibacterial activity against *Propionibacterium acne* bacteria with the best antibacterial activity at a concentration of 40% as much as 6.30 mm.

Keywords: Basil leaf (*Ocimum sanctum* L.), phytochemical screening, antibacterial activity

INTRODUCTION

Acne is a type of skin disorder that is most often experienced by the public, especially adolescents and young adults (Narulita *et al*., 2019). Ramdani and Sibero (2015) mentioned the highest prevalence of acne sufferers between 80–85% experienced by adolescents aged 15–18 years, 12% occurred in women aged 25 years and over, and 3% experienced by women aged 35–44 years. One of the causes of this disease is bacteria *Staphylococcus aureus*, *Streptococcus mutans*, and *Propionibacterium acne*.

The most commonly given acne treatment is antibiotic class drugs such as clindamycin, erythromycin, and tetracycline can reduce the population of *Propionibacterium acne* bacteria (Afifi *et al*., 2018). Inappropriate choice of antibiotics and excessive use can make sensitive bacteria become resistant so that treatment becomes longer and results in therapeutic failure. The incidence of antibiotic resistance to *Propionibacterium acne* bacteria has increased in various countries, one of which is Indonesia. According to Madelina and Sulistiyaningsih (2018) stated that the resistance of *Propionibacterium acne* bacteria to antibiotics was 12.9% resistance to tetracycline, 45.2% resistance to erythromycin, and

61.3% resistance to clindamycin. In addition to causing resistance, antibiotic treatment has side effects in the form of skin irritation (Wardani *et al*., 2020). Therefore, it is necessary to find alternatives to reduce the incidence. The use of drugs from natural ingredients is an alternative that can be utilized for the treatment of bacterial infections. One of these natural ingredients is basil (*Ocimum sanctum* L.).

Plant basil has long been used by people to relieve fever, treat stomach pain, and overcome bad breath. Basil leaves are effective in treating stomach ulcers, colds, flatulence, convulsions, and body lethargy. This plant, which has a distinctive aroma, can be used to treat diseases caused by bacterial infections including diarrhea and mouth ulcers (Sitohang, 2019). In addition, this plant has other benefits such as antibacterial, antiseptic, analgesic, antipyretic, anti-inflammatory, and antioxidant (Idrus *et al*., 2013).

Research on extracts from basil leaves (*Ocimum basilicum* L.) by Kumalasari and Andiarna (2020) reported the presence of flavonoid, alkaloid, saponin, steroid, and tannin groups of phytochemical compounds. Then according to Naya and Mardiyanti (2021), there are several active substances known to have antibacterial effects such as steroids, saponins, alkaloids, and flavonoids.

Ethanol extract of basil leaves (*Ocimum basilicum* L.) research has been conducted by several researchers showing the presence of antibacterial activity against several bacteria in the extract. The bacteria that can be inhibited include *Staphylococcus aureus, Streptococcus mutans*, and *Propionibacterium acne*. Based on research results Syarifuddin *et al*., (2020) showed antibacterial activity *Streptococcus mutans* on ethanol extract of basil leaves (*Ocimum basilicum* L.) concentrations of 20%, 40%, 60%, and 100%, the largest inhibition area was obtained at a concentration of 100%, which was 10.26 mm. The antibacterial activity of *Staphylococcus aureus* ethanol extract of basil leaves at 100% concentration is 16.75 mm (Solikhah, *et al*., 2016).

RESEARCH METHODS

Tools and Materials

The tools used are a set of glassware (*Pyrex*), analytical balance (*OHAUS*), oven (*Memmert*), macerator, *waterbath, vacuum rotary evaporator* (IKA), vaporizer cup, *moisture analyzer balance* (*OHAUS*), autoclave (model 25 X eledricl 38° max), Petri dish (*Pyrex*), paper discs, incubator *(Memmert*), and a vernier caliper (*Krisbow*).

The material used is ethanol extract of basil leaves (*Ocimum sanctum* L.), isolate *Propionibacterium acne*, clindamycin capsule 150mg (PT Tropica Mas), ethanol 96% (PT. Brataco), nutrient gel (PT. Merck), aquadest (Sanbe), Mayer reagent, Dragendorff reagent, Ferric chloride 1%, Concentrated sulfuric acid (PT. Merck), Hydrochloric acid 2N, Concentrated hydrochloric acid (PT. Merck), Anhydrous acetic acid (PT. Merck), McFarland 0.5 standard solution, Sodium chloride 0.9% (Widatra Bhakti), and magnesium metal powder (CV. Pratama Sains Global).

Research Procedure

1. Plant Determination

   Plant determination was carried out at Laboratory of Biology Study Program at IAIN Syekh NurJati Cirebon. This identification aims to examine and ensure the true identity and avoid errors in the plant samples used for study.
2. Preparation of Basil Leaf Simplicia (*Ocimum sanctum* L.)

   Basil Leaves (*Ocimum sanctum* L.) obtained from Arjawinangun Market Cirebon, were collected and then sorted in fresh condition. Subsequently, the leaves were washed with flowing clean water. The clean sample were weighed and dried using an oven at 40°C. In the end, the dry sorting process is carried out prior to powdering process (Depkes RI, 1979).
3. Preparation of Ethanol Extract of Basil Leaves (*Ocimum sanctum* L.)

   Simplified basil leaves that have been weighed as much as 250 grams, then soaked in a macerator with 96% ethanol as much as 1.875 mL for 3 days in a closed

container and protected from sunlight. Soaking simplicia occasionally stirred. After 3 days, filter and squeeze, then rinse the pulp with 96% ethanol as much as 625 mL. The macerate was concentrated with a rotary evaporator then continued evaporation with a waterbath until a thick extract was obtained.

4. Specific and Non-specific Parameters of Extract
   a. Organoleptics

      Physical examination using the five senses to observe odor, color, shape, and taste (Depkes RI, 2000).
   b. Drying Shrinkage

      Prepare an empty weighing bottle, heat it at 105°C for 30 minutes then cool it in a desiccator and weigh the initial weight. After the porcelain crucible is weighed, then add 1 gram of extract. Heat in an oven at 105°C for 1 hour with the porcelain lid open. Next, the porcelain crucible was put into a desiccator for 15 minutes and then weighed (Depkes RI, 2000).
   c. Water Content

      Weigh 1 gram of extract and put it into a moisture analysis balance. The results are said to be well qualified, namely at a distance of 1 hour until the difference between 2 consecutive weighings is seen with a result of not more than 0.25% (Depkes RI, 2000).
   d. Ash Content

      Weigh 2 grams of extract that has been crushed and then put it into a silicate crust that has been incinerated and then poured and leveled. Incinerate slowly until the charcoal runs out, cool, and then weigh. If the charcoal is not exhausted, add a hot water filter through ash-free filter paper. Then the remaining paper and filter paper are incinerated in the same crucible. The filtrate is put into the crucible and then evaporated. Incinerate to a fixed weight and weigh. Calculate the ash content of the air-dried material (Depkes RI, 2000).
5. Phytochemical Screening

   The ethanol extract of basil leaves was weighed as much as 0.5 grams and then dissolved in a 100 mL volumetric flask with 96% ethanol as much as 10 mL, then added distilled water to 100 mL. The phytochemical screening carried out on ethanol extract of basil leaves includes testing of alkaloid compounds, flavonoids, tannins, saponins, and steroids.
   a. Alkaloid Test

      As much 10 drops of sample is put into 2 test tubes. In tube 1 add 2–3 drops of mayer reagent. Observe the changes that occur, if there is a yellowish-white precipitate it indicates the presence of alkaloid compounds. Then in tube 2 add 2–3 drops of dragendorff, and observe the changes that occur. If there is an orange to red-brown precipitate, it shows flavonoid compounds (Purwati *et al*., 2017).
   b. Flavonoid Test

      A total of 10 drops of sample was put into a test tube. Then 2 mg of magnesium powder and 3 drops of concentrated hydrochloric acid. Then the test tube is shaken and observed for red, yellow, or orange color changes in the solution indicating the presence of flavonoid compounds (Purwati *et al*., 2017).
   c. Tannin Test

      A total of 10 drops of sample was put into a test tube, then added with 2 drops of iron (III) chloride solution. The sample is positive for tannins if it produces a blackish brown color (Pamungkas *et al*., 2016).
   d. Saponin Test

      A total of 10 drops of sample was put into a test tube, added 10 mL of warm water, and shaken vigorously for about 1 minute until foam appeared. The solution was allowed to stand for 10 minutes, then observed the foam formed If the foam formed is 1–3 cm high for not less than 10 minutes, then the extract is positive for saponins (Purwati *et al*., 2017).

e. Steroid Test

A total of 10 drops of sample was put into a test tube, added 10 drops of anhydrous acetic acid and 2 drops of concentrated sulfuric acid were then shaken gently and allowed to stand for a few minutes. Positive extracts containing steroids are characterized by the formation of blue or green (Anastasia *et al*., 2017).

6. Antibacterial Activity Testing

a. Sterilization of Tools

The tools used in this study were sterilized using an autoclave at 121°C for 15 minutes. The ose needle was sterilized by incinerating it.

b. Preparation of Mc. Farland 0.5

$BaCl_2$1% solution was pipetted as much as 0.05 mL and put into a test tube, add sulfuric acid 1% solution as much as 9.95 mL then vortexed until fully mixed.

c. Preparation of *Propionibacterium acne* Bacteria Suspension

The ose wire is burned until the tip of the ose is smoldering, take the *Propionibacterium acne* bacterial culture with an ose wire and then put it in a test tube that has been added with 0.9% NaCl solution and shaken until turbid. The turbidity of the suspension was equalized with McFarland standard solution.

d. Preparation of Nutrient Agar Media

Nutrient agar was weighed as much as 1.4 g, put into a beaker glass then add 70 mL of distilled water and heat until it boils and becomes clear. The mouth of the beaker glass is closed with fatty cotton and then sterilized with an autoclave at 121°C for 15 minutes. After that, the media was poured into 3 Petri dishes of 20 mL each.

e. Antibacterial Activity Test of Ethanol Extract of Basil Leaf

Antibacterial activity testing was carried out by disc diffusion method with 20%, 30%, and 40% extract concentration.

i. 20 % concentration

1 grams of thick extract of basil leaves (*Ocimum sanctum* L.) is then put into a volumetric flask and added with 96% ethanol to 5 mL.

ii. 30 % concentration

1.5 grams of thick extract of basil leaves (*Ocimum sanctum* L.) is then put into a volumetric flask and added with 96% ethanol to 5 mL

iii. 40 % concentration

2 grams of thick extract of basil leaves (*Ocimum sanctum* L.) is then put into a volumetric flask and added with 96% ethanol to 5 mL

0.01% clindamycin as positive control, and 96% ethanol as negative control with 3 repetitions. Paper discs were soaked into each sample for 1 hour. After soaking, the disc paper was placed on agar media that had been inoculated with *Propionibacterium acne* bacteria, let stand for 15 minutes then incubated in an incubator for 24 hours at 37°C. After incubation, the diameter of the clear zone was measured using a caliper.

Data Analysis

The inhibition zone of *Propionibacterium acne* antibacterial activity test results were analyzed using SPSS IBM version 25 with the Kruskal wallis method to see if there were significant differences between sample groups then continued with Mann-Whitney testing.

RESULTS AND DISCUSSION

Extraction

The ethanol extract of basil leaves obtained from the extraction was 30.13 g with a yield of 12.05%. According to Indonesian Herbal Pharmacopoeia Second Edition 2017, the

yield of ethanol extract of basil leaves (*Ocimum sanctum* L.) is not less than 5.6% so it can be said that the extract obtained meets the requirements of the literature.

Specific and Non-specific Parameters of Extract

Table I. Standardization Results of Ethanol Extract of Basil Leaf

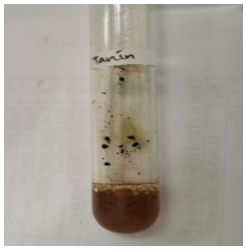
No	Testing	Reference (Kemenkes RI, 2017)	Average Results
1	Moisture content	Not more than 12%	5.6%
2	Drying shrinkage	Not more than 10%	7.1%
3	Total ash content	Not more than 10.7%	7.71%

Extract standardization is the initial stage before antibacterial testing. Organoleptic observation results in ethanol extract of basil leaves (*Ocimum sanctum* L.) has a brownish black color with a distinctive aromatic aroma and a slightly sour bitter taste, this is by the reference. Non-specific parameters include water content, total ash content, and drying shrinkage. The purpose of determining the water content in the extract is to determine the range of water content contained in the extract. The result obtained from this test is 5.6% which is in accordance with the reference of no more than 12%. The total ash content test aims to provide an overview of the external and internal mineral content in the extract. The test results obtained 7.71% which is in accordance with the reference of not more than 10.7%. Drying shrinkage test on extracts is carried out to provide a maximum limit or range of the amount of compounds lost during the drying shrinkage process. The test results obtained the percentage of drying shrinkage of 7.1% which results meet the requirements of drying shrinkage of basil leaf ethanol extract (*Ocimum sanctum* L.) which is not more than 10% (Kemenkes RI, 2017).

Phytochemical Screening

Table II. Phytochemical Screening Results of Ethanol Extract of Basil Leaf

Group of Compounds	Test Method	Theory	Results	Information
Alkaloids	Mayer	Yellowish white precipitate		(-)
	Dragendorf	Orange to red brown precipitate	 Green solution Yellow solution	(-)
Flavonoids	Hydrochloric acid reagent + magnesium powder	Yellow color formed		(+)

Group of Compounds	Test Method	Theory	Results	Information
			Yellow colored solution	
Tannin	Iron (III) chloride reagent	Brown-black	 Brown and slightly black solution	(+)
Saponins	Warm distilled water	Foam remains for not less than 10 minutes	 Slight foam	(+)
Steroids	Glacial acetic acid reagent + concentrated sulfuric acid	Blue or green color formed	 Green colored solution	(+)

Description: (+) = contains secondary metabolites
(-) = does not contain secondary metabolites

Before testing the antibacterial activity, the extract was first tested for phytochemical screening with the aim of identifying the content of secondary metabolites contained in the ethanol extract of basil leaves (*Ocimum sanctum* L.). The secondary metabolites tested in this phytochemical screening include alkaloids, tannins, flavonoids, saponins, and steroids. Alkaloid testing is done with two tests, namely with Mayer and Dragendorff reagents. Positive results of alkaloid testing with Mayer reagent are the formation of yellowish white precipitate and the formation of orange precipitate on positive results with Dragendorff reagent. The results of alkaloid testing on ethanol extracts of basil (*Ocimum sanctum* L.) gave negative results in both testing with Mayer reagents and Dragendorff reagents. This is

not in accordance with the research of Kumalasari and Andiarna (2020), which states that the ethanol extract of basil leaves (*Ocimum sanctum* L.) contains a group of alkaloid compounds.

The difference in the results of this test can be caused by environmental conditions such as the place of growth or the origin of basil (*Ocimum sanctum* L.) in this study which came from the Arjawinangun Market, Cirebon while the previous study of basil (*Ocimum sanctum* L.) leaves used came from the Surabaya area. The difference in plant origin can affect the type and number of groups of phytochemical compounds contained in the plant (Purwati *et al*., 2017).

The results of tannin identification in the ethanol extract of basil leaves (*Ocimum sanctum* L.) showed positive results based on research conducted by Pamungkas *et al*., (2016) where when the sample is added with Ferri Chloride reagent, a brown color is formed, this occurs due to the formation of tannin complex compounds and ions Fe^{3+} (Oktavia and Sutoyo, 2021).

The results of flavonoid testing on ethanol extract of basil leaves (*Ocimum sanctum* L.) also showed positive results based on research conducted by Purwati *et al*. (2017). When the sample was added with magnesium powder and concentrated hydrochloric acid, it produced a yellow color. The color change is caused by the addition of magnesium powder and concentrated hydrochloric acid which reduces the benzopyrone core in the flavonoid structure (Riasari *et al*., 2022).

The next test is a steroid on an ethanol extract of basil leaves (*Ocimum sanctum* L.) which gives positive results based on research conducted by Anastasia *et al*., (2017), where when the sample is added with anhydrous acetic acid and concentrated sulfuric acid produced a green color. The color change is caused by the formation of conjugated double bonds after the steroid compound undergoes oxidation (Oktavia and Sutoyo, 2021).

The next test is saponin on an ethanol extract of basil leaves (*Ocimum sanctum* L.) which also gives positive results based on the results of research conducted by Purwati *et al*., (2017). The results showed that stable foam was formed after shaking with hot water and added with 2N hydrochloric acid. The foam is formed due to a hydrolysis reaction where when saponins are in water, they form foam due to the hydrolysis process (Oktavia and Sutoyo, 2021).

Antibacterial Activity

Table III. Antibacterial Activity Test of Ethanol Extract of Basil Leaf

Replication	Diameter of Inhibition (mm)				
	Positive Control Clindamycin 0,01%	**Negative Control Ethanol 96%**	**Concentration 20%**	**Concentration 30%**	**Concentration 40%**
1	11,95	0,00	4,42	4,82	5,20
2	12,05	0,00	4,52	4,87	7,05
3	11,87	0,00	4,47	5,05	6,65
Total	35,87	0,00	13,41	14,74	18,9
Average	11,95	0,00	4,47	4,91	6,30
SD	± 0,09	± 0,00	± 0,05	± 0,12	± 0,97

Based on Table III the above shows that the ethanol extract of basil leaves has antibacterial activity against *Propionibacterium acnes* as indicated by the presence of inhibition zones around the discs on the agar medium. From the data above, it can be seen that the greater the concentration of the extract used, the greater the inhibitory power against bacterial growth.

The data obtained resulted in an average inhibition zone diameter for the positive control of 11.95 mm, which falls into the strong category. Whereas for the negative control,

there were no inhibition zones around the disc, indicating that the extract solvent does not affect antibacterial activity. Research results at a concentration of 20% showed an average inhibition zone diameter of 4.47 mm, which falls into the weak category. At a concentration of 30%, the average inhibition zone diameter was 4.91 mm, also falling into the weak category. Furthermore, at a concentration of 40%, the results showed an average inhibition zone diameter of 6.3 mm, falling into the moderate category. The inhibitory activity in this study is lower compared to the study conducted by Made *et al*., (2022) which reported the antibacterial activity of *Propionibacterium acnes* using the ethanol extract of basil leaves (*Ocimum basilicum* L.) at a concentration of 15% to be 13.4 mm.

The antibacterial activity in this study can be linked to the content of phytochemical compounds in the ethanol extract of basil leaves (*Ocimum sanctum* L.) which act as antibacterials, including flavonoids that work by forming complexes with extracellular proteins, causing damage to bacterial cell membranes. Tannins, as antibacterials, work by contracting the cell wall, disrupting cell permeability, and leading to cell lysis. Saponins work by reducing surface tension, causing leakage and cell lysis. Steroids, as antibacterials, work by damaging the cell membrane, leading to the release of cytoplasm and cell lysis (Trisia *et al*., 2018).

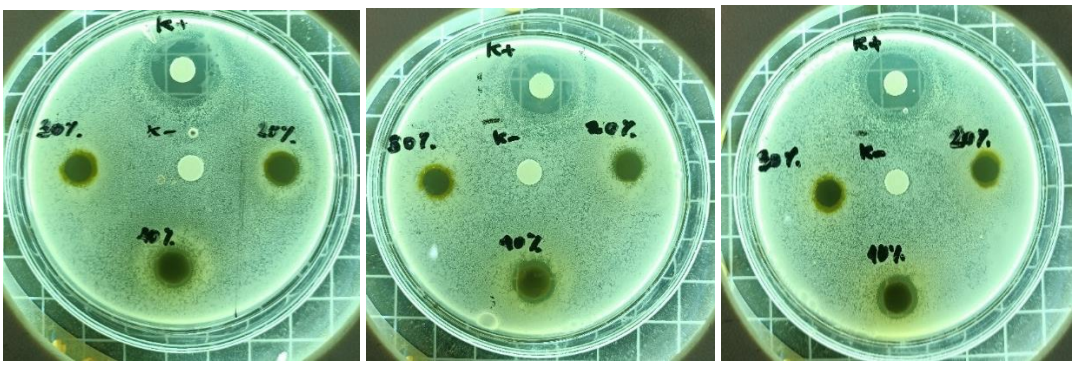


Figure 1. Results of antibacterial activity of ethanol extract of basil leaves Replication 1, 2, and 3

CONCLUSION

The ethanol extract of basil leaves (*Ocimum sanctum* L.) positively contains secondary metabolites such as flavonoids, tannins, saponins, and steroids. Ethanol extract of basil leaves (*Ocimum sanctum* L.) at concentrations of 20%, 30%, and 40% exhibited antibacterial activity against *Propionibacterium acne* with average inhibition zone diameters of 4.47 mm, 4.91 mm, and 6.30 mm, respectively. The higher the concentration of the ethanol extract of basil leaves (*Ocimum sanctum* L.), the greater the antibacterial activity obtained.

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