

ARTICLE INFO

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Article history

Received	01-07-2025
Revised	11-03-2026
Accepted	13-03-2026
Available online	31-03-2026

Please cite this article in APA 7th edition style as:

Salsabilah, A. P., Budianto, N.E.W., & Sutandio, Y. M. M. (2026). Effect of Basil Seed Extract (*Ocimum basillicum* L) on *Salmonella Typhi* Growth Inhibition. *Jurnal Ilmiah Kedokteran Wijaya Kusuma*, 15(1), 8-16

<https://doi.org/10.30742/jikw.v14i1.4645>

Effect of Basil Seed Extract (*Ocimum basillicum* L) on *Salmonella Typhi* Growth Inhibition

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Abstract

Background: Basil seeds are a traditional spice with the name *Ocimum basilicum* L. Basil seed extract contains secondary metabolite compounds such as alkaloids, flavonoids and polyphenols which have the potential to act as antibacterials for *Salmonella typhi*. The aim of this research was to determine the antibacterial effectiveness of basil seed extract (*Ocimum basilicum* L) against the growth of *Salmonella typhi* bacteria.

Objective: the aim of the research. **Methods:** The experimental research method was a post test only control group design using the well diffusion method. **Result:** The research results showed that the diameter of the inhibition zone formed at successive concentrations of 10%, 20%, 40% and 80% was 27.5 mm, 30.3 mm, 35.6 mm, 40.7 mm against *Salmonella typhi*. In the One-way Anova test, a p value of 0.000 <0.05 was obtained for the inhibition of the growth of *Salmonella typhi* bacteria.

Conclusion: There is a potential anti-microbial effect of *Salmonella typhi* bacteria with an optimal concentration of 80%.

Keywords: Antibacterial, Basil seeds, *Ocimum basillicum*, *Salmonella typhi*

Original Research Article

INTRODUCTION

Infectious diseases are the most common diseases suffered by the population in developing countries. One of them is an infection of the digestive tract that can be caused by bacteria, viruses and protozoa. Several studies in the health field show that infectious diseases in the digestive system are most often caused by *Salmonella typhi* bacteria (Threenesia & Ramadhian, 2019). *Salmonella typhi* bacteria are gram-negative bacteria that can cause typhoid fever. These bacteria are most commonly found through food and cause disorders in the digestive system. This disease is characterized by a persistent increase in body temperature or fever and can be transmitted through food or drinks contaminated with feces or feces from people with typhoid fever (Imara, 2020).

In Indonesia, typhoid fever is usually treated with antibiotics. Improper use of antibiotics can increase antibiotic resistance if taken without a doctor's prescription (Fauziah & Komarudin, 2024). Antibiotic resistance is a serious problem because it can complicate healing and can cause heavier

effects up to the risk of death (Sora et al., 2026). Based on data from the Ministry of Health in 2017, the death rate related to resistance was 700,000 deaths per year (Wahyuni et al., 2024). To reduce the increase in antibiotic resistance, it is necessary to develop innovations in the use of natural ingredients that have antibacterial effects (Rahmasari & Lestari, 2018).

One of the plants that can be used is basil seeds (*Ocimum basilicum* L). Basil seeds (*Ocimum basilicum* L) are one of the parts of the basil plant that contains rich antioxidants such as flavonoids and polyphenols that are able to inhibit the growth of bacteria (Poulios et al., 2025). Basil seed extract (*Ocimum basilicum* L) is also known to contain active compounds that are natural antacids that can neutralize stomach acid and toxins in the stomach (Al salamah, 2022). In Indonesia, basil seeds (*Ocimum basilicum* L) are commonly used as a mixture of drinks and food (Hadipoentyanti, 2008; Dharmawan et al., 2025).

In a study conducted by (Pangaribuan et al., 2019), namely the Effectiveness of the Combination of Basil Seed and Leaf Extract (*Ocimum basilicum* L) as a Natural Antiseptic against Bacteria on the Hand (Guntur et al., 2021). The results were obtained from basil seed and leaf extracts that were able to inhibit the growth of *S. aureus* and *E. coli* bacteria with a concentration of 5%, 10%, 20% and positive and negative controls with a KHM value at a concentration of 10% and a KBM concentration of 20% (Tulandi & Tombuku, 2025; Imran et al., 2021). The higher the concentration given, the larger the barrier zone formed. When the concentration of antibacterial substances is higher, the inhibitory effect on bacterial growth will also increase (Halijah et al., 2021)

Although previous studies have demonstrated the antibacterial activity of basil seed and leaf extracts (*Ocimum basilicum* L.) against bacteria such as *S. aureus* and *E. coli*, research specifically evaluating the effectiveness of basil seed extract against *Salmonella typhi*, the primary causative agent of typhoid fever in developing countries including Indonesia, remains limited. Most existing studies focus on general antiseptic applications or non-typhoidal bacteria, and there is insufficient evidence regarding its potential as an alternative therapy to address antibiotic resistance in typhoid management. Therefore, the novelty of this study lies in investigating the antibacterial effect of basil seed extract specifically against *Salmonella typhi*, aiming to explore its potential as a natural, plant-based therapeutic candidate to reduce reliance on conventional antibiotics and help mitigate the growing problem of antibiotic resistance

Based on the description above, the researcher is interested in conducting a study on how much the antibacterial effect of basil seed extract (*Ocimum basilicum* L) on the diameter of the growth inhibition zone of *Salmonella typhi* bacteria entitled "The Effect of Giving Basil Seed Extract (*Ocimum basilicum* L) on the Growth of *Salmonella typhi*".

MATERIALS AND METHODS

In this study, the sample size was determined using the Federer formula. The experimental design consisted of six groups: four treatment groups and two control groups. The negative control group consisted of *Salmonella typhi*-infected samples treated with sterile distilled water (aquades). The positive control group consisted of *Salmonella typhi*-infected samples treated with chloramphenicol at a concentration of 30 µg per well, in accordance with standard antimicrobial susceptibility testing guidelines. The treatment groups consisted of *Salmonella typhi*-infected samples treated with basil seed extract (*Ocimum basilicum* L.) at different concentrations. After incubation, all plates were removed from the incubator, and the diameter of the inhibition zones formed around each well was measured four times using a caliper. The mean value was then calculated and recorded. The data were classified and tabulated according to the treatment concentrations. Data analysis was performed by observing and comparing the clear zones around the wells to evaluate the antibacterial activity of each treatment.

Sample

The sample in this study was *Salmonella typhi* bacteria which was treated in the form of basil seed extract (*Ocimum basilicum* L) and observed the diameter of the resistance formed. The total number of samples used was 24 samples because each sample had 4 repetitions.

Preparation of Basil Seed Extract (*Ocimum basilicum* L.)

The extraction is done by maceration. Fresh basil seeds (*Ocimum basilicum* L) are dried by aerating, then put in a glass jar and then mashed for 5-10 minutes. Next, basil seed powder (*Ocimum basilicum* L) is soaked in 96% ethanol until completely submerged, then left for 3 days in a lighttight room while stirring. The maceration results were filtered 2 times and obtained ethanol filtrate and dregs, left for 2 days. After that, it is evaporated using an evaporator until the 96% ethanol solvent is gone, then steam using a vacuum rotary evaporator. The result of the heating process will produce a concentrated extract which is then poured into porcelain cups and heated using a water bath heater until it is completely extracted and basil seed extract (*Ocimum basilicum* L) is obtained (Guntur et al., 2021).

Preparation of Variants of Basil Seed Extract Concentrations (*Ocimum basilicum* L.)

In this study, serial dilutions were performed to obtain basil seed extract (*Ocimum basilicum* L.) concentrations of 10%, 20%, and 40% from an 80% stock solution. The dilution process was carried out by adding sterile distilled water (aquades) to the 80% extract using measuring cylinders and micropipettes to ensure accuracy. Using the dilution formula:

$$C1 \times V1 = C2 \times V2$$

Obtained:

- Concentration 10%, mix 12.5 mL of basil seed extract (*Ocimum basilicum* L) 80% with 87.5 mL of aquades.
- Concentration 20%, mix 25 mL of basil seed extract (*Ocimum basilicum* L) 80% with 75 mL of aquades.
- For a concentration of 40%, mix 50 mL of basil seed extract (*Ocimum basilicum* L) 80% with 50 mL of aquades.

Making a suspension of *Salmonella typhi* bacteria

The *Salmonella typhi* bacteria used in this study were bred and obtained at the Microbiology Laboratory, Faculty of Medicine, Universitas Wijaya Kusuma Surabaya. The suspension of the *Salmonella typhi* test colony is made by taking one ose colony from a solid NA medium into a test tube containing 5 mL of physiological NaCl. The turbidity in the test colony suspension was standardized to the standard of 0.5 McFarland (approximately 1.5×10^8 CFU/mL). The suspension should be used as an inoculum within 15 minutes (Sohidullah et al., 2017).

Media creation of Mueller Hinton Agar

The preparation of Mueller Hinton Agar (MHA) medium was carried out by dissolving 19 g of MHA powder in distilled water to a final volume of 500 mL in an Erlenmeyer flask, followed by heating until a homogeneous solution was obtained. The medium was then sterilized using an autoclave at 121 °C for 15 minutes. After sterilization, approximately 25 mL of the medium was poured into sterile Petri dishes and allowed to solidify at room temperature.

For the treatment stage, six 50 mL beaker glasses were prepared and labeled according to the treatment concentrations. A total of 0.1 mL of the bacterial suspension was inoculated onto the surface of the solidified MHA medium, evenly spread using a sterile hockey stick, and allowed to dry. Wells were then made in the agar using a sterile cork borer. Subsequently, 40 µL of basil seed extract (*Ocimum basilicum* L.) was added into each well according to the designated treatment. The plates were then incubated at 37 °C for 24 hours.

Data Analysis

The data obtained in this study was processed with the Kolmogorov-Smirnov Test to find out whether the data has been distributed normally or not. The data is normally distributed if p is more than 0.05. If the data is distributed normally or homogeneously, the one-way ANOVA test is used. By using one-way ANOVA, it will be known whether there is an effect of basil seed extract (*Ocimum basilicum L*) on the diameter of the inhibition zone on the growth of *Salmonella typhi* bacteria. Followed by the Post Hoc Test if the ANOVA gives a H0 result that is rejected, which means that there is a difference between the groups being tested.

If the data is not normally or homogeneously distributed, a non-parametric statistical test will be performed using Kruskal-Wallis. To find out the difference in the inhibition zones formed at each concentration of basil seed extract (*Ocimum basilicum L*).

RESULTS

The research has been conducted at the Microbiology Laboratory, Faculty of Medicine, Wijaya Kusuma University, Surabaya and the manufacture of basil seed extract (*Ocimum basilicum L*) at the Materia Medica Herbal Laboratory of the East Java Provincial Health Office in Batu, Malang. Using the distribution method of sums filled with Basil Seed Extract (*Ocimum basilicum L*) with a concentration of 10%, 20%, 40%, 80% and Chloramphenicol as a positive control and aquades as a negative control. The results of the measurement of the inhibition zone formed in the growth of *Salmonella Typhi* can be seen in table 1 below:

Table 1. Results of Diameter of *Salmonella typhi* Bacterial Growth Inhibition Zone

Repetition	Diameter Zona Hambat (mm)					
	K (+)	K (-)	K3	K4	K5	K6
1	34,85	0	28,05	27,60	34,15	44,60
2	33,10	0	29,50	29,80	36,95	39,05
3	34,15	0	27,60	32,45	37,50	37,20
4	35,60	0	25,20	31,45	33,90	42,20
Total	137,70	0	110,35	121,3	142,5	163,05
Average	34,42	0	27,58	30,32	35,62	40,76
St. Deviasi	0,92	0.00	1,54	1,83	1,61	2,84

Note: The positive control group (+), the negative control group (-), the 10% dose group (K3), the 20% dose group (K4), the 40% dose group (K5), and the 80% dose group (K6).

Based on the table above, it is found that the diameter of the barrier zone formed at a concentration of 10% is 27.58 mm, 20% is 30.325 mm and the concentration is 40% is 35.625 mm while the concentration of 80% has the greatest inhibition force compared to the others, which is 40.76 mm. In the positive control test using the antibiotic Chloramphenicol of 34.425 mm where the positive control inhibition was smaller compared to the inhibition of basil seed extract (*Ocimum Basilicum L*), in the negative control test no inhibition zone was formed which means that there was no inhibition to the growth of *Salmonella typhi* bacteria.

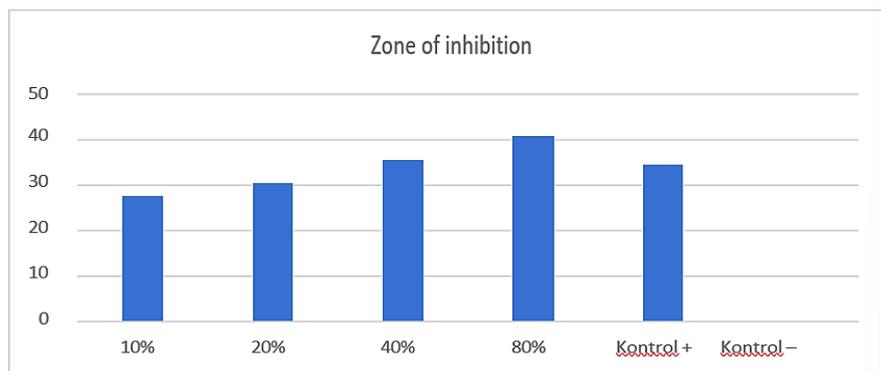


Figure 1. Bar chart of the inhibition zone diameter formed in the negative control group, positive control group, and treatment groups administered basil seed extract (*Ocimum basilicum L.*).

Note: K-: Group treated with distilled water (aquades), K+: Group treated with antibiotics, %: Groups treated with basil seed extract (*Ocimum basilicum L.*)

Table 2. The results of the normality test of the diameter of the barrier zone formed from the treatment of the negative control group, the positive control group and the treatment group given basil seed extract (*Ocimum Basilicum L.*).

Group	Significance (Sig.)
10 %	0,774*
20%	0,813*
40%	0,166*
80%	0,806*
Control (+)	0,968*
Control (-)	-

Note: *Significant

Based on the table above, the normality test results indicate that the data are normally distributed. This can be seen from the significance value (Sig.) > 0.05. Therefore, it can be concluded that the data used in this study are normally distributed.

Table 3 The results of the homogeneity test of the diameter of the barrier zone formed from the treatment of the negative control group, the positive control group and the treatment group given basil seed extract (*Ocimum Basilicum L.*).

Bacteria	Significance (Sig.)
<i>Salmonella typhi</i>	0,087

Based on the above table, the results of the homogeneity test using the levene test above, it is known that the Significance Based on Mean value is 0.087 which is more than (5% or 0.05), then it can be concluded that this data is homogeneous and has met the requirements of *the* Oneway anova test.

Table 4 Oneway *anova* test results of inhibition zone diameter formed from treatment of negative control group, positive control group and treatment group given basil seed extract (*Ocimum Basilicum L.*)

Bacteria	F Calculate	Significance (Sig.)
<i>Salmonella typhi</i>	22,225	0,000

Based on the table above, the results of the analysis of *the Anova One-way Test* obtained a price F calculation of 22.225 with a significance of 0.000 < 0.05, so it can be concluded that there is a

significant difference. This means that the administration of basil seed extract (*Ocimum basillicum L*) has an effectiveness in inhibiting the growth of *Salmonella typhi* bacteria.

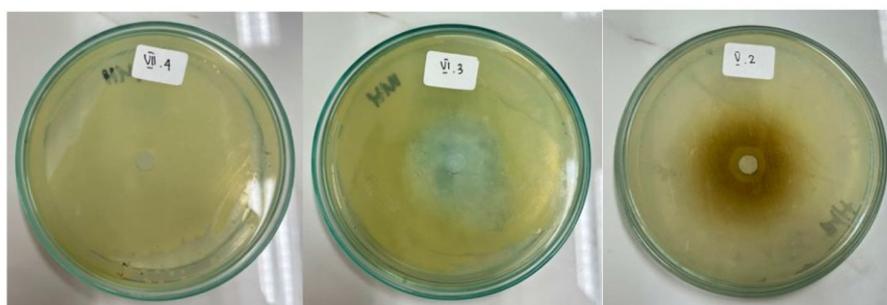
After there is effectiveness in each treatment, the Bonferroni test is performed to see a significant average difference between the treatments that will be compared with positive controls.

Table 5. Bonferroni test analysis

I	J	Sig.	Keterangan
10%	20%	0,093	Not Significant
	40%	0,000	Significant difference
	80%	0,000	Significant difference
20%	40%	0,003	Significant difference
	80%	0,000	Significant difference
40%	80%	0,004	Significant difference
Control (+)	10%	0,000	Significant difference
	20%	0,017	Significant difference
	40%	0,444	Not Significant
	80%	0,001	Significant difference
Control (-)	10%	0,000	Significant difference
	20%	0,000	Significant difference
	40%	0,000	Significant difference
	80%	0,000	Significant difference

Description: I : Comparison extract groups, J : Groups of extracts compared

Based on Table 5, the Bonferroni post hoc analysis shows that most group comparisons demonstrated statistically significant differences ($p < 0.05$). There was no significant difference between the 10% and 20% extract groups ($p = 0.093$) and between the positive control group and the 40% extract group ($p = 0.444$). However, all other comparisons—including 10% vs 40%, 10% vs 80%, 20% vs 40%, 20% vs 80%, 40% vs 80%, as well as comparisons between both control groups and the extract-treated groups—showed significant differences ($p < 0.05$). These results indicate that increasing extract concentrations generally produced significantly different effects compared to lower concentrations and controls.



[VII.4] Aquadest [VI.3] Chloramphenicol [V.2] Ekstrak Biji Selasih

Figure 2. Comparison of the Inhibition Zones of Extract, Chloramphenicol, and Distilled Water (Personal Documentation)

DISCUSSION

Based on the data from the study results, it shows that the diameter of the barrier zone that has the greatest inhibition at a concentration of 80% is 40.7625 mm. In the positive control test using the

antibiotic Chloramphenicol of 34.42 mm where the positive control inhibition was smaller compared to the inhibition of basil seed extract, In the negative control test no inhibition zone was formed which means that there was no inhibition to the growth of *Salmonella typhi* bacteria. In the medium given, basil seed extract (*Ocimum basilicum* L) produces a clear zone that is brownish yellow. For the medium that is dripped with Chloramphenicol antibiotics in the drainage hole, a clear white zone is formed. Meanwhile, in the media that was treated with AQUADES drip as a negative control, the results showed that there was no inhibition zone formed.

The results of the treatment given to *Salmonella typhi* bacteria by giving basil seed extract (*Ocimum basilicum* L) showed that, the higher the concentration given, the larger the barrier zone formed. When the concentration of antibacterial substances is higher, the inhibiting effect on bacterial growth will also increase (Mushemesh et al., 2023). The barrier zone formed around the source of the antibacterial substance will become larger, allowing to control bacterial growth more effectively (Kristiyani et al., 2023). The sumption method has the advantages, namely it is easier to measure the barrier zone that is formed while the disc diffusion method has the advantages of simple processing and faster processing time (Zada & Rahmat, 2021).

This is in accordance with research conducted by (Nurhayat, 2020) & (Muzayyidah et al., 2023), which shows that the higher the concentration of extracts on bacterial growth, the larger the barrier zone formed. Higher concentrations of antimicrobial substances can make them more effective at inactivating bacteria where antimicrobial substances tend to have stronger interactions with bacteria, including stronger adhesion to the cell wall and deeper penetration into bacteria (Zhang et al., 2021).

This research is also in accordance with research conducted by (Halijah et al., 2021), namely Anti-Bacterial Effectiveness Test of Basil Leaf Extract (*Ocimum basilicum* L.) Against *Escherichia coli*. With populations and samples of *Staphylococcus aureus* and *Escherichia coli* bacteria with treatment group variations of 5%, 10%, 20% and negative and positive control groups. The results of the study calculated the average number of bacterial colonies were *Staphylococcus aureus* K(-): 1012, concentration 5%: 121, concentration 10%: 48 and concentration 20% K (+): 0. *Escherichia coli* K(-): 943, concentration 5%: 810, concentration 10%: 372 and concentration 20%, K (+): 0. Conclusion that there was an effect of a combination of basil seed and leaf extract on bacterial growth and KHM value at 10% concentration and KBM concentration at 20% (Mahmudi et al., 2024)

CONCLUSION

Based on the research that has been conducted on the effect of giving basil seed extract (*Ocimum basilicum* L) on the growth of *Salmonella typhi* bacteria, it can be concluded that there is an effect of basil seed extract (*Ocimum basilicum* L) on the antibacterial activity of *Salmonella typhi* growth. The barrier zone for the application of basil seed extract (*Ocimum basilicum* L) against the growth of *Salmonella typhi* was formed at a concentration of 80%, which was 40.76 mm.

CONFLICT OF INTEREST

Author declares no conflict of interest.

ACKNOWLEDGEMENTS

Author declares no acknowledgements.

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