

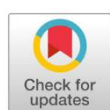
Effect of kecombrang flower (*Etlingera elatior*) and basil leaves (*Ocimum afrinacum*) extracts to the growth of *Streptococcus mutans* ATCC 35668 and *Staphylococcus aureus* ATCC 33591

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Abstract

Utilization of local plants is still not optimal. Experimental research has been carried out in Samarinda City by utilizing kecombrang flower (*Etlingera elatior*) and basil leaf (*Ocimum afrinacum*) extracts which were exposed to *Streptococcus mutans* ATCC 35668 and *Staphylococcus aureus* ATCC 33591. The aim of the study was to determine the effect of kecombrang flower extract (*Etlingera elatior*), basil leaf extract (*O. afrinacum*) on the growth of *Streptococcus mutans* ATCC 35668, and *Staphylococcus aureus* ATCC 33591. The research samples were kecombrang flowers (*E. elatior*) and basil leaves (*O. afrinacum*). The research instrument was a measuring tool (ruler). Results Data analysis showed that kecombrang flower extract (*E. elatior*), basil leaf extract (*O. afrinacum*) significantly affected the growth of *Streptococcus mutans* and *Staphylococcus aureus* ATCC 33591 ($p < 0.00$). Active chemicals (secondary metabolites, namely tannins present in kecombrang flower extract and extract Basil leaves (*O. afrinacum*) can inhibit or kill the growth of *Streptococcus mutans* ATCC 35668 and *Staphylococcus aureus*. The conclusion is kecombrang flower extract (*E. elatior*) and basil leaf extract (*O. afrinacum*) have a significant effect on the growth of *Streptococcus mutans* ATCC 35668 and *Staphylococcus aureus* ATCC 33591.

Keywords: Kecombrang flowers, basil leaves, *Staphylococcus aureus*, *Streptococcus mutans*

Introduction

The health of the human body has always been the goal of life for all human beings. A healthy body condition allows humans to work productively, from the aspect of human life. Efforts to prevent disease in the human body are carried out in various ways. Ways to prevent these diseases include consuming nutritious food, preventing diseases, both generative and infectious.



However, the health condition of the human body can be disrupted by the onset of disease. These diseases can be degenerative or infectious. One of the locations in the human body that becomes a place for bacterial infection is the mouth. Infectious diseases that occur in the mouth can be caused by *Streptococcus mutans*¹. *Streptococcus mutans* bacteria, the main cause of cavities (caries), converts glucose and carbohydrates in food into acids through the fermentation process². In addition, *Staphylococcus aureus*, can also infect the human body³. However, if prevention and treatment efforts are carried out properly and appropriately, these bacteria can be minimized⁴.

The use of various types of medicinal plants is used in traditional medicine. Traditional medicine is a heritage of ancestors that merges with the culture of the community⁵. A survey conducted on people in the Jawa Village, Samarinda City⁶, found that they often consume the flowers of the kecombrang plant (*Etlingera elatior*). Likewise, people in Kelurahan Jawa, Kota Samarinda, often consume basil leaves (*Ocimum afrinacum*), which is done by some ethnic communities. Both of these plants are often consumed by chewing in the mouth. There has been no maximum utilization of basil leaves (*O. afrinacum*) by the community, while if managed properly this plant has good potential to improve the degree of public health⁷.

Kecombrang (*E. elatior*) is empirically used to facilitate breast milk, wound medicine, flavoring, and scientifically proven to have antibacterial and antioxidant activity in it⁸. Basil leaves (*O. afrinacum*) have potential as medicine, vegetable pesticides, and essential oil producers⁹. Basil leaves (*O. afrinacum*) are plants that have antibacterial potential¹⁰.

The use of local plants (kecombrang flowers and basil leaves) is thought to inhibit the growth of *Streptococcus mutans* and *Staphylococcus aureus*. *S. aureus* is referred to as the most common cause of nosocomial infection³. *S. mutans* is facultative bacterium with primary habitats in the oral cavity¹¹. *Streptococcus mutans* includes the normal flora of the oral cavity which plays a role in the occurrence of caries¹². According to Afni et al.¹³, *S. mutans* is a karyogenic bacterium that can leaven carbohydrates and produce acid, grow in an acidic atmosphere, and stick to the surface of the teeth.

One of the spice and medicinal plants that have potential as antioxidants is kecombrang (*E. elatior*)¹⁴. Secondary metabolites in kecombrang flowers (*E. elatior*) and basil leaves (*O. afrinacum*), have the potential to inhibit the growth of *S. mutans* and *S. aureus*. The aim of the study was to determine the effect of kecombrang flower extract (*E. elatior*) and basil leaf extract (*Ocimum afrinacum*) on the growth of *Streptococcus mutans* and *Staphylococcus aureus*.

Materials and Methods

Samples

The research samples were kecombrang flowers (*Etilingera elatior*) and basil leaves (*Ocimum afrinacum*). Kecombrang flowers are taken from people's plantations in Samarinda City. Basil leaves are taken at the Segiri Traditional Market, Samarinda City.

Instrument

The research instrument is a measuring tool in the form of a ruler. A measuring ruler was used to measure the area of the inhibition zone of kecombrang flower and basil leaf extracts. The ruler measuring instrument was obtained from the Microbiology Laboratory, Faculty of Agriculture, Mulawarman University.

Research Procedure

The research carried out followed the following procedure:

1. Determination of research samples, namely kecombrang flowers and basil leaves.
2. Determine the test bacteria. The test bacteria used were *Streptococcus mutans* and *Staphylococcus aureus*.
The test bacteria were obtained from the Microbiology Laboratory, Faculty of Agriculture, Mulawarman University.
3. Carrying out phytochemical analysis. Phytochemical tests were carried out at the Faculty of Forestry, Mulawarman University.
4. Carry out antibacterial test. The antibacterial test was carried out at the Microbiology Laboratory, Faculty of Agriculture, Mulawarman University. Antibacterial testing technique using the well technique.
5. Data analysis. The data analysis process used one-way analysis of variance (ANOVA). Data analysis techniques using the help of SPSS software version 23. Figure 1 shows the flowchart of the research procedure.

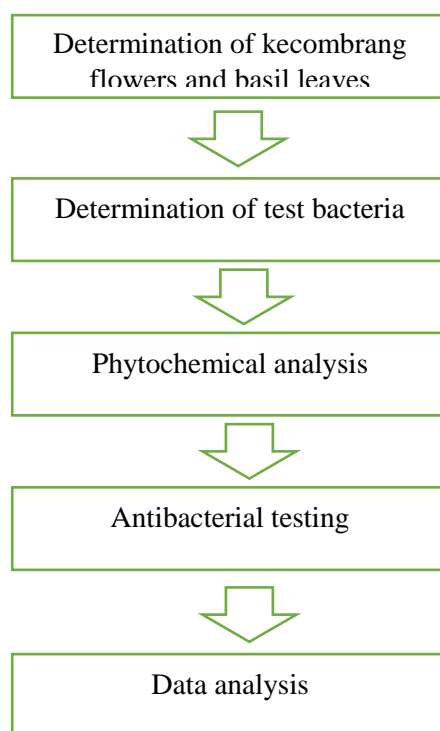


Figure 1. Flow chart of research procedures

Data Analysis Technique

To determine the effects of exposure to extracts of kecombrang flowers and basil leaves, data on the results of measuring the inhibitory zones of extracts of kecombrang flowers and basil leaves were analyzed using one-way analysis of variance (ANOVA). If the ANAVA results show a significant effect, then the Least Significance Difference (LSD) test will be continued. Processing data was using SPSS version 23.

Results

Phytochemical Test Results

The chemical content in kecombrang flowers varies quite a bit. Kecombrang flower extract contains chemicals (secondary metabolites) which have the potential to inhibit or kill the test bacteria¹⁵.

Table 1 shows the secondary metabolites contained in the kecombrang flower extract.

Table 1. Results of the phytochemical analysis of kecombrang flowers (*Etlingera elatior*)

No.	Types of secondary metabolites	Result
1	Flavonoid	(-)
2	Alkaloid	(-)
3	Tanin	(+)
4	Saponin	(-)
5	Steroid	(-)

No.	Types of secondary metabolites	Result
6	Karoteenoid	(-)
7	Kumarin	(-)
8	Triterpenoid	(-)
9	Karbohidrat	(++)

In addition to kecombrang flowers, the study also carried out extracts of basil leaves. The content of chemicals (secondary metabolites) in basil leaf extract is quite varied. Table 2 shows the chemical content (secondary metabolites) in basil leaf extract.

Table 2. Results of phytochemical analysis of basil leaves (*O. afrinacum*)

No.	Types of secondary metabolites	Result
1	Flavonoid	(-)
2	Alkaloid	(-)
3	Tanin	(+)
4	Saponin	(-)
5	Steroid	(-)
6	Karoteenoid	(-)
7	Kumarin	(-)
8	Triterpenoid	(-)
9	Karbohidrat	(++)

Test Results of the Effect of Exposure to Kecombrang Flower Extract on the Growth Inhibition of Test Bacteria

One-way ANOVA results showed that there was a significant effect of exposure to kecombrang flower extract (*E. elatior*) on the growth of *Streptococcus mutans* ATCC 35668 ($p < 0.00$). Thus it can be said that kecombrang flower extract (*E. elatior*), with various levels of treatment has an effect on inhibiting or killing the test bacteria *S. mutans* ATCC 35668 ($p < 0.00$). Table 3 contains a summary of the results of one-way ANOVA for the results of inhibition zone measurements resulting from exposure of kecombrang flower extract (*E. elatior*) to *S. mutans* ATCC 35668.

Table 3. Summary of one-way ANOVA for the average area of the inhibition zone for exposure to treatment of the kecombrang flower extract (*E. elatior*) on the growth of *S. mutans*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2277.632 ^a	5	455.526	617.105	.000
Intercept	5483.712	1	5483.712	7428.826	.000
Kecombrang	2277.632	5	455.526	617.105	.000
Error	17.716	24	.738		
Total	7779.060	30			
Corrected Total	2295.348	29			

a. R Squared = .992 (Adjusted R Squared = .991)

One-way ANOVA results indicate that there is an effect of kecombrang flower extract (*E. elatior*) on the growth of *S. mutans* ATCC 35668. The results of this analysis allow us to carry out further tests using LSD to determine differences in the effect between treatments (concentrations) of kecombrang extract on the growth of *S. mutans* ATCC 35668. Table 4 shows the results of the LSD test on the growth inhibition zone of *S. mutans* ATCC 35668.

Table 4. LSD test results average inhibition zone area for each treatment of exposure to the growth of *Streptococcus mutans* (*E. elatior*) extract of kecombrang flowers

No	Kecombrang flower	Obstacles zone	LSD notation
1	Control	0.000	a
2	25%	9.500	b
3	50%	12.580	c
4	75%	13.880	d
5	100%	15.820	e
6	Positive kontrol (kloramfenikol)	29.340	f

One-way ANOVA results for exposure to kecombrang flower extract (*E. elatior*) on the growth of *Staphylococcus aureus* ATCC 33591, showed that there was a significant effect ($p < 0.00$). One-way ANOVA results show that there is an effect of treatment with various treatment levels on the inhibition zone caused by kecombrang flower extract (*E. elatior*) on the test bacteria *S. aureus* ATCC 33591. Table 5 contains a one-way ANOVA summary for the results of the inhibition zone caused by exposure of kecombrang flower extract (*E. elatior*) to the growth of *S. aureus* ATCC 33591.

Table 5. The results of the inhibition zone caused by exposure of kecombrang flower extract (*E. elatior*) to the growth of *S. aureus* ATCC 33591.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1252.446 ^a	5	250.489	56.107	.000
Intercept	1668.056	1	1668.056	373.627	.000
Kecombrang flower	1252.446	5	250.489	56.107	.000
Error	107.148	24	4.464		
Total	3027.650	30			
Corrected Total	1359.594	29			

The one-way ANOVA results show that there is a significant effect of kecombrang flower extract (*E. elatior*) on the growth of *Staphylococcus aureus* ATCC 33591. The ANOVA results allow us to carry out further tests using LSD to determine differences in the effect of each level of treatment of kecombrang flower extract (*E. elatior*) on the growth of *Staphylococcus aureus* ATCC 33591. **Table 6** contains the results

of the LSD follow-up test to determine the differences in the effect of each level of kecombrang flower extract (*E. elatior*) on the growth of *S. aureus* ATCC 33591.

Table 6. LSD test results mean inhibition zone area for each treatment of kecombrang flower extract (*E. elatior*) exposure to the growth of *Staphylococcus aureus*.

No	Kecombrang flower	Obstacles zone	LSD notation
1	Negative Control	0.000	a
2	50%	3.920	b
3	25%	4.180	b
4	75%	6.260	b
5	100%	10.160	c
6	PositiveControl (kloramfenikol)	20.220	d

Test Results of Exposure Effect of Basil Leaf Extract on the Growth Inhibition of Test Bacteria

Based on the results of one-way ANOVA showed that there was a significant effect of exposure to basil leaf extract (*O. afrinacum*) on the growth of *Streptococcus mutans* ATCC 35668 ($p < 0.00$). The results of the analysis showed that exposure to basil leaf extract (*O. afrinacum*) with its treatment levels had an effect on the inhibition of *S. mutans* ATCC 35668 growth.

One-way ANAVA results for exposure to basil leaf extract (*O. afrinacum*) on the growth of *S. mutans* ATCC 35668, indicating that there is a significant effect of exposure to basil leaf extract (*O. afrinacum*) to *S. mutans* ATCC 35668. To ensure differences in the effect for each level of influence, further LSD test is necessary. Table 8 contains the results of the LSD follow-up test to determine the difference in the effect of each experimental level on *S. mutans* ATCC 35668.

Besides being exposed to the test bacteria, namely *S. mutans* ATCC 35668, basil leaf extract (*O. afrinacum*) was also exposed to *S. aureus* ATCC 33591. The inhibition zones formed varied, from 1.8 mm to 11.8 mm. **Table 9** below contains the average growth inhibition zones of *S. aureus* ATCC 33591 exposed to basil leaf extract.

Table 7. ANOVA summary for the average inhibition zone area for exposure to treatment of basil leaf extract (*O. afrinacum*) on the growth of *S. mutans*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	2932.923 ^a	5	586.585	1628.647	.000
Intercept	2557.633	1	2557.633	7101.249	.000
Kemangi	2932.923	5	586.585	1628.647	.000
Error	8.644	24	.360		
Total	5499.200	30			
Corrected Total	2941.567	29			

a. R Squared = .997 (Adjusted R Squared = .996)

Table 8. LSD test results average inhibition zone area for each treatment of exposure to basil leaf extract (*O. afrinacum*) on the growth of *S. mutans* ATCC 35668

No	Basil leave	Obstacles zone	LSD notation
1	NegativeControl	0.000	a
2	25%	2.000	b
3	50%	4.900	c
4	75%	7.660	d
5	100%	11.000	e
6	PositiveControl (kloramfenikol)	29.840	f

Table 9. Summary of one-way ANOVA for the average inhibition zone area for exposure to treatment of basil leaf extract (*O. afrinacum*) on the growth of *S. aureus*

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1458.103 ^a	5	291.621	1355.324	.000
Intercept	1856.533	1	1856.533	8628.350	.000
DaunKemangi	1458.103	5	291.621	1355.324	.000
Error	5.164	24	.215		
Total	3319.800	30			
Corrected Total	1463.267	29			

a. R Squared = .996 (Adjusted R Squared = .996)

The one-way ANOVA results show that there is an effect of exposure to basil leaf extract (*O. afrinacum*) on the growth of *S. aureus* ATCC 33591. Therefore, it is necessary to carry out further LSD tests. Table 10 below contains the results of the LSD follow-up test for the average inhibition zone area for each treatment level of basil leaf extract (*O. afrinacum*) on the growth of *S. aureus* ATCC 33591

Table 10. Average LSD test results for inhibition zone area for each treatment of exposure to basil leaf extract (*O. afrinacum*) on the growth of *Staphylococcus aureus*

No	Basil leave	Obstacles zone	LSD notation
1	NegativeControl	0.000	a
2	25%	1.820	b
3	50%	5.120	c
4	75%	7.580	d
5	100%	11.760	e
6	PositiveControl (kloramfenikol)	20.920	f

Discussion

Phytochemical Test Results

The chemical content in kecombrang flowers varies quite a bit. Kecombrang flower extract contains chemicals (secondary metabolites) which have the potential to inhibit or kill the test bacteria. In addition to kecombrang flowers, the study also carried out extracts of basil leaves. The content of chemicals (secondary metabolites) in basil leaf extract is quite varied. The use of traditional medicinal plants as anti-bacterial is found in basil leaves (*O. afrinacum*)¹⁶. Basil leaves (*O. afrinacum*) have diverse pharmacological activities such as analgesic, antipyretic, antiseptic, and have antibacterial activity with flavonoids, glycosides, and gallic acid¹⁷.

Test Results of the Effect of Exposure to Kecombrang Flower Extract on the Growth Inhibition of Test Bacteria

One-way ANOVA results showed that there was a significant effect of exposure to kecombrang flower extract (*E. elatior*) on the growth of *Streptococcus mutans* ATCC 35668 ($p < 0.00$). Thus it can be said that kecombrang flower extract (*E. elatior*), with various levels of treatment has an effect on inhibiting or killing the test bacteria *S. mutans* ATCC 35668 ($p < 0.00$).

Summary of one-way ANOVA results (Table 3) showed that there was a significant effect of exposure to kecombrang flower extract outside the zone of inhibition of kecombrang flower growth (*E. elatior*). These results indicate that the active chemicals (secondary metabolites present in kecombrang flower extract (*E. elatior*) can inhibit or kill *Streptococcus mutans* ATCC 35668. In this study, we have investigated the potential of *E. elatior* flower extract as a source of antimicrobial and antifungal agents. The results in this study revealed that the methanol extract of *E. elatior* flowers had great in vitro potential for antimicrobial activity to varying degrees against all tested microorganisms⁶. It was determined that *S. mutans* has a complex regulatory system to respond to changes external environment¹⁸. Related to information on the content of active chemicals (secondary metabolites) in kecombrang flower extracts, there are chemicals in the form of tannins. Tannin secondary metabolites are thought to be able to inhibit or kill *S. mutans* ATCC 35668. Thus, in growth media appeared inhibition zone there but, which is an indicator of the ability of kecombrang flower extract (*E. elatior*) to inhibit or kill the test bacteria.

The results of the LSD follow-up test (Table 4) to determine differences in the effect between treatment levels of kecombrang flower extract on the growth of *S. mutans* ATCC (Table 8) showed that there were significant differences between treatment levels (0%, 25%, 50%, 75%, and 100%) extract of kecombrang flower (*E. elatior*) on the growth of *Streptococcus mutans* ATCC 35668. The results of this LSD test showed that there were different effects for each treatment of extract of kecombrang flowers (*E. elatior*) on the growth of *Streptococcus mutans* ATCC 35668. Each level of treatment applied contains different active chemicals (secondary metabolites). The active chemical in kecombrang flower extract (*E. elatior*) in the form of tannins (Table 1) can inhibit or kill *S. mutans* ATCC 35668. The difference in active

chemical content in kecombrang flower extract (*E. elatior*) at each concentration requested allows for differences in the inhibition zones formed.

One-way ANOVA results for exposure to kecombrang flower extract (*E. elatior*) on the growth of *Staphylococcus aureus* ATCC 33591, showed that there was a significant effect ($p < 0.00$). One-way ANOVA results show that there is an effect of treatment with various treatment levels on the inhibition zone caused by kecombrang flower extract (*E. elatior*) on the test bacteria *S. aureus* ATCC 33591. However, *S. aureus* is also resistant to certain antimicrobials. *S. aureus* is prevalent in both human and animal populations and is known to develop resistance to different antimicrobials¹⁹. This study has shown the occurrence of high penicillin resistance in *S. aureus* ATCC 33591 isolates collected from all host species¹⁹.

The results of the LSD follow-up test to determine differences between treatment levels of kecombrang flower extract (*E. elatior*) against *S. aureus* ATCC 33591 (**Table 6**) show that treatment levels of 25%, 50%, and 75% have the same effect, and the effects are both different from the levels 100%, in inhibiting or killing *S. aureus* ATCC 33591. The results of this LSD analysis show that levels of 25%, 50%, and 75% have the same ability to inhibit or kill *S. aureus* ATCC 33591, and are both different from the 100% level. Therefore, further research is needed to determine the different levels between 25%, 50% and 75%, to obtain more information. Associated with the similarity of the effect for the treatment levels of 25%, 50%, and 75%, this could be due to the fact that the diffusion ability of the active chemical in the treatment concentrations was the same in the growth medium for the tested bacteria (*S. aureus* ATCC 33591), and differed from the rate of diffusion of the active chemicals contained in the 100% treatment concentration. Another possibility is that the amount of the active chemical present in the concentrations is the same, and equally different, from the amount of the active chemical present in the 100% treatment concentration.

Test Results of Exposure Effect of Basil Leaf Extract on the Growth Inhibition of Test Bacteria

Based on the results of one-way ANOVA showed that there was a significant effect of exposure to basil leaf extract (*O. afrinacum*) on the growth of *Streptococcus mutans* ATCC 35668 ($p < 0.00$). The results of the analysis showed that exposure to basil leaf extract (*O. afrinacum*) with its treatment levels had an effect on the inhibition of *S. mutans* ATCC 35668 growth of *S. mutans* ATCC 35668.

Summary of one-way ANOVA results (**Table 7**) shows that there is a significant effect of exposure to basil leaf extract (*O. afrinacum*) on the growth of *S. mutans* ATCC 35668. These results indicate that the active chemical (secondary metabolite present in basil leaf extract (*O. afrinacum*) capable of inhibiting or killing the growth of *S. mutans* ATCC 35668. An indicator of inhibition of the growth of *S. mutans* ATCC 35668 is the formation of an inhibition zone around the test bacteria. The active chemical ingredients present in basil leaf extract (*O. afrinacum*) (**Table 2**) are tannins, capable of inhibiting or killing the test bacteria *S. mutans* ATCC 35668²⁰. The tannins present in kecombrang flower extract (*E. elatior*) are capable of inhibiting or killing *S. mutans* ATCC 35668, and indeed the same tannins

found in basil leaf extract (*O. afrinacum*) can also inhibit or kill the same test bacteria (*S. mutans* ATCC 35668).

The concentration of the treatment in the study contained tannins in different amounts according to the level of concentration. The amount of active chemical that is different in each treatment concentration has an effect on the formation of inhibition zones with different areas. In other words, the difference in the amount of tannin active chemical has a different effect on inhibition or killing power against *S. mutans* ATCC 35668. The active chemicals (secondary metabolites) in basil leaf extract (*O. afrinacum*) are able to inhibit the growth or kill *Staphylococcus aureus* ATCC 33591. The structure of the cell wall of *S. aureus* ATCC 33591 is the same as *S. mutans* 35668. Thus, the mechanism of cell damage in both test bacteria is the same due to exposure to active chemicals (secondary metabolites) tannins. However, to ensure the speed of damage to the cells of these two test bacteria by active chemicals, further research is needed.

The results of one-way ANAVA showed that there was a significant effect of exposure to leaf extract (*O. afrinacum*) on the growth of *S. aureus* ATCC 33591. Thus it is necessary to carry out further tests to find out the differences in the effect between leaf extract treatments (*O. afrinacum*) on the growth of *S. aureus* ATCC 33591 follow-up tests were used is LSD. Table 14 contains the results of the LSD follow-up test on differences in the effect of each treatment level of basil leaf extract (*O. afrinacum*) on the growth of *S. aureus* ATCC 33591. The results of the follow-up test showed that there was a significant difference in effect between treatment levels (0%, 25% , 50%, 75%, and 100%) on the growth of the test bacteria. The amount of tannin active chemical substance that is different at each treatment concentration level, has a different effect on the growth of *S. aureus* ATCC 33591.

Conclusions

The conclusion of the study was that there was a significant effect of giving kecombrang kecombrang flower extract (*E. elatior*) and basil leaf extract (*O. afrinacum*) on the growth of *Streptococcus mutans* and *Staphylococcus aureus*. Similar studies in the future need to increase the concentration of plant organ extracts in order to obtain more information. In addition, it is also necessary to test on other test bacteria.

Acknowledgments

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Conflicts of Interest

The authors declare no conflict of interest in any capacity, including competing or financial.

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