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Effectiveness Test of Potassium Sorbate and Sodium Benzoate Preservatives *Against Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* in Herbal Cough Medicine (OBH) Preparations

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ABSTRACT (9)
Preservatives are food additives that are permitted for use to prevent or inhibit fermentation, acidification, decomposition, and other damage to food caused by microorganisms. Herbal cough medicines generally contain plant materials that are sensitive to microbiological hazards, so they are at greater risk of microbial contamination. The purpose of this study was to assess the effectiveness of Potassium sorbate and Sodium benzoate preservatives in inhibiting the growth of *Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* bacteria in herbal cough medicine preparations with menthol and strawberry variants. The preservative effectiveness test method refers to the Indonesian Pharmacopoeia Edition VI, carried out on days 0, 14 and 28. Based on the study, Potassium sorbate and Sodium benzoate effectively inhibit the growth of *Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* bacteria in OBH preparations with menthol and strawberry variants. In the group OBH menthol variant colonies of 5.5 Log Reduction from the initial count on day 14 and the number of colonies did not increase from the initial count until days 14 and 28. In the group OBH strawberry variant colonies by 5.6 Log Reduction from the initial count on day 14 and the number of colonies did not increase from the initial count until the 14th and 28th days.

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1. INTRODUCTION

Coughing is a defense effort of the lungs against various existing stimuli and a physiological reflex that protects the lungs from mechanical, chemical, and temperature trauma. Coughing is often an indication of a disease in or outside the lungs, and sometimes an early symptom of a disease. Coughing is a reflexaction of the respiratory tract used to clear the upper respiratory tract (Mathur et al., 2019). The prevalence of cough in Indonesia is quite high, considering that cough is one of the common symptoms that often occurs due to various health conditions, including upper respiratory tract infections (URTIs), asthma, tuberculosis (TB), and other lung diseases. The prevalence of cough is around 15% in children and 20% in the adult population. One in ten patients who visit a doctor's practice each year have complaints of cough (Ministry of Health of the Republic of Indonesia, 2023).

Based on BPOM Regulation No. 11 of 2009 (Food and Drug Supervisory Agency of the Republic of Indonesia, 2019), preservatives are one of the Food Additives (BTP) to prevent or inhibit fermentation, acidification, decomposition, and other damage to food caused by microorganisms. Herbal cough medicines generally contain plant materials that are sensitive to microbiological hazards, so they are more at risk of microbial contamination compared to non-herbal cough medicines. In addition, they can also be contaminated by hazardous chemicals that are toxic such as pesticide residues and heavy metals (Safriansyah, 2002). For that, preservatives are added to the formulation to inhibit microbial growth.

Potassium Sorbate and Sodium Benzoate are preservatives that are permitted for use by BPOM. Research on the levels of benzoate and sorbate has previously been conducted on samples of kepok banana chili sauce by

(Mia, 2022) and on jelly agar by (Revelation Utami & Darmawan, 2020). The results of the study indicate that benzoate and sorbate are used as a combination of preservatives. Currently, there has been no study that describes the effectiveness of Potassium Sorbate and Sodium Benzoate preservatives in herbal cough medicine preparations. Therefore, this study aims to determine the effectiveness of Potassium Sorbate and Sodium Benzoate preservatives in herbal cough medicine preparations with menthol and strawberry variants against *Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* bacteria.

2. RESEARCH METHOD

The type of research used is experimental. This experimental research is a research conducted to test the effectiveness of Potassium sorbate and Sodium benzoate preservatives in herbal cough medicine preparations with menthol and strawberry variants. The product samples used were samples from Company X located in East Jakarta. The analysis was also completed with positive and negative control tests. Negative control is a herbal cough medicine preparation without Potassium sorbate and Sodium benzoate preservatives. Positive control is a herbal cough medicine on the market with other brands that use preservatives Potassium sorbate and Sodium benzoate. The effectiveness test of this preservative was carried out on 3 bacteria and 2 fungi, namely *Eschericia coli* bacteria, *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans fungi* and *Aspergillus brasiliensis.* Analysis method referring to the Indonesian Pharmacopoeia, VI Edition, chapter 61, Antimicrobial Effectiveness Test.

3. RESULT AND DISCUSSION

On The effectiveness test of this preservative, 4 samples were used, namely negative control (cough medicine without preservatives Potassium sorbate and Sodium benzoate), positive control (herbal cough medicine on the market with other brands that use preservatives Potassium sorbate and Sodium benzoate), OBH menthol variant and OBH strawberry variant. Observations were made on days 0, 7, 14, and 28. The microbes used were *Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* bacteria. The concentration of preservatives used in the menthol variant of OBH is 1723.26 mg/kg, while in the strawberry variant of OBH is 1728.27 mg/kg. The maximum limit for preservative use based on BPOM Regulation No. 29 of 2023 is 2000 mg/kg. The results of the preservative effectiveness test are as follows:

Descriptive Analysis

Table 1. Descriptive Analysis of Sample Groups (Log Reduction)

Group	Day	N	Mean	Minimum	Maximum	Std. Deviation
Negative Control	To-0	5	5.7	5.6	5.8	0.084
	The 7th	5	6.6	6.5	6.7	0.084
	The 14th	5	6.7	6.6	6.8	0.084
	The 28th	5	7.0	7.0	7.0	0.000
Positive Control	To-0	5	5.6	5.5	5.7	0.084
	The 7th	5	4.5	4.0	4.8	0.356
	The 14th	5	0.0	0.0	0.0	0.000
	The 28th	5	0.0	0.0	0.0	0.000
OBH Menthol Variant	To-0	5	5.5	5.4	5.7	0.114
	The 7th	5	4.8	4.6	5.0	0.158
	The 14th	5	0.0	0.0	0.0	0.000
	The 28th	5	0.0	0.0	0.0	0.000
OBH Strawberry Variant	To-0	5	5.6	5.5	5.7	0.084
	The 7th	5	5.0	4.8	5.1	0.114
	The 14th	5	0.0	0.0	0.0	0.000
	The 28th	5	0.0	0.0	0.0	0.000

Based on the results of the table above, the N value of 5 is the number of types of microbes tested in this study, namely *Eschericia coli* bacteria, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans fungi and *Aspergillus brasiliensis*. In the Negative Control group, the average Log Reduction was seen to

increase every week. Testing on day 0 averaged 5.7, on day 14 the Log reduction was -1.0 from the initial count, and the colonies increased from day 14 to day 28. In the Positive Control group, the average Log Reduction was seen to decrease every week. Testing on day 0 averaged 5.6, on day 14 the Log reduction was 5.6 from the initial count, and the colonies did not increase from day 14 to day 28. A significant decrease occurred from day 7 to day 14. In the group OBH Menthol Variant. It can be seen that the average Log Reduction decreases every week. Testing on day 0 averaged 5.5, on day 14 the Log reduction was 5.5 from the initial count, and the colonies did not increase from day 14 to day 28. A significant decrease occurred from day 7 to day 14. In the group OBH Strawberry Variant, it can be seen that the average Log Reduction decreases every week. Testing on day 0 averaged 5.6, on day 14 the Log reduction was 5.6 from the initial count, and the colonies did not increase from day 14 to day 28. A significant decrease occurred from day 7 to day 14.

In the observation for 28 days, both for menthol and strawberry cough medicine preparations, it was seen that the preservatives Potassium sorbate and Sodium benzoate were effective in inhibiting the growth of microorganisms. In the negative control sample, there was an increase in Log reduction from day 0 to day 7. The negative control did not contain preservatives, so that microorganisms had a supportive environment to grow. This shows that the use of preservatives plays an important role in inhibiting the growth of microorganisms. When viewed as a whole based on the five types of microbes, the effectiveness of preservatives in menthol cough medicine preparations is slightly higher than in the strawberry variant. This is due to the presence of natural compounds in menthol cough medicine, namely menthol itself which has antimicrobial properties as supported by the literature (Smith et al., 2020). Menthol has antibacterial activity, both against gram-positive and gram-negative bacteria according to research conducted by Zhao et al (2019). While strawberries contain natural sugars that can increase the risk of microbial contamination.

Normality Test

Table 2. Normality Test

Group	Shapiro Wilk		
	Statistics	df	Sig.
Negative Control	.817	20	.002
Positive Control	.721	20	.000
OBH Menthol Variant	.701	20	.000
OBH Strawberry Variant	.690	20	.000

a. Lilliefors Significance Correction

The normality test in this study uses the Shapiro-Wilk Normality Test method, because in this study the amount of data is relatively small. The basis for decision making is if the Sig value> 0.05 then it is stated that the data is normally distributed and vice versa if the Sig value <0.05 then it is stated that the data is not normally distributed.

Based on the results of the Sig value test in the Negative Control group (0.002), Positive Control (0.000), OBH Menthol Variant (0.000), and OBH Strawberry Variant (0.000), the Sig value in all groups was less than 0.05, so it can be concluded that the four groups are not normally distributed.

Homogeneity Test

Table 3. Homogeneity Test

Group		Levene Statistics	df1	df2	Sig.
Negative Control	Based on Mean	3,631	3	16	0.036
Positive Control	Based on Mean	20,424	3	16	0.000
OBH Menthol Variant	Based on Mean	7,318	3	16	0.003
OBH Strawberry Variant	Based on Mean	7,774	3	16	0.002

The basis for decision making in the Homogeneity Test is if the Sig. value > 0.05 then the data is declared homogeneous, while if the Sig. value < 0.05 then the data is declared non-homogeneous.

Based on the results of the Sig value test in the Negative Control group (0.036), Positive Control (0.000), OBH Menthol Variant (0.003), and OBH Strawberry Variant (0.002), the Sig value in all groups is less than 0.05, so it can be concluded that the four groups above are not homogeneous.

Difference Test (Anova)

The results of the normality and homogeneity tests show that the data is not normally distributed and is not homogeneous, so the difference test uses a non-parametric test method, namely the Mann-Whitney Test and the Kruskall Wallis Test. The Mann-Whitney Test is used to determine the average difference of two unpaired samples, while the Kruskal-Wallis Test is a non-parametric statistical test used to compare two or more groups of sample data.

1. Negative Group

Table 4. Mann-Whitney Difference Test for Negative Group

Day	Mean	Day	Mean	GAP	P-Value Mann Whitney	Information
To-0	5.7	The 7th	6.6	0.86	0.008	Significant Increase
To-0	5.7	The 14th	6.7	1.00	0.008	Significant Increase
To-0	5.7	The 28th	7.0	1.28	0.005	Significant Increase
The 7th	6.6	The 14th	6.7	0.14	0.041	Significant Increase
The 7th	6.6	The 28th	7.0	0.42	0.005	Significant Increase
The 14th	6.7	The 28th	7.0	0.28	0.005	Significant Increase

The test results showed that in the negative group on day 0 the average value was 5.7 Log Reduction, after day 7 the average increased to 6.6 Log Reduction, there was an increase of 0.86 Log Reduction, with a P-Value of 0.008 <0.05. From these data, it can be concluded that there is a significant difference in testing on day 0 and day 7 which can be interpreted that bacterial growth increased significantly from day 0 to day 7. This result also occurs in comparisons of other days. Based on the test table, it shows a significant increase, namely day 0 to day 28, with a GAP of 1.28 Log Reduction.

Table 5. Kruskal Wallis Difference Test for Negative Groups

	Negative Control
Kruskal-Wallis H	17,524
Df	3
Asymp. Sig.	.001

a. Kruskal Wallis Test
b. Grouping Variable: Day

Based on the results of the Kruskal Wallis test, the Sig value of 0.001 is smaller than 0.05, so it can be stated that in the negative group of testing on days 0, 7, 14 and 28 there is a significant difference. so it can be concluded that in the negative group the growth of colonies of *Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* bacteria increased significantly from days 0, 7, 14 to 28.

2. Positive Group

Table 6. Mann-Whitney Difference Test for Positive Groups

Day	Mean	Day	Mean	GAP	P-Value Mann Whitney	Information
To-0	5.6	The 7th	4.5	-1.10	0.008	Significantly Decreased
To-0	5.6	The 14th	0.0	-5.62	0.005	Significantly Decreased
To-0	5.6	The 28th	0.0	-5.62	0.005	Significantly Decreased
The 7th	4.5	The 14th	0.0	-4.52	0.005	Significantly Decreased
The 7th	4.5	The 28th	0.0	-4.52	0.005	Significantly Decreased
The 14th	0.0	The 28th	0.0	0.00	1,000	No difference

The test results showed that in the Positive group, the average value of the test on day 0 was 5.6 Log Reduction, after day 7 the average decreased to 4.5 Log Reduction, there was a decrease of -1.10 Log Reduction, with a P-Value of 0.008 <0.05. From these data, it can be concluded that there is a significant difference in testing on day 0 and day 7, which can be interpreted that bacterial growth decreased significantly from day 0 to day 7. On day 14, bacterial growth had stopped so that the Log Reduction value was 5.6 from the initial count. On day 28 of the test, there was no increase in the number of colonies from day 14. Based on the test table, the highest significant decrease was from day 0 to day 14.

Table 7. Kruskal Wallis Difference Test for Positive Groups

	Positive Control
Kruskal-Wallis H	18,395
Df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test
b. Grouping Variable: Day

Based on the results of the Kruskal Wallis test, the Sig value of 0.000 is smaller than 0.05, so it can be stated that in the positive group, the test on days 0, 7, 14 and 28 had significant differences, so it can be concluded that in the positive group, the growth of *Eschericia coli* bacterial colonies, *Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* fungi decreased significantly from days 0, 7, 14 to 28. The most effective decrease was on day 7 to day 14.

3. OBH Group Menthol Variant

Table 8. Mann-Whitney Difference Test for Menthol Variant OBH Groups

Day	Mean	Day	Mean	GAP	P-Value Mann Whitney	Information
To-0	5.5	The 7th	4.8	-0.74	0.009	Significantly Decreased
To-0	5.5	The 14th	0.0	-5.54	0.005	Significantly Decreased
To-0	5.5	The 28th	0.0	-5.54	0.005	Significantly Decreased
The 7th	4.8	The 14th	0.0	-4.80	0.005	Significantly Decreased
The 7th	4.8	The 28th	0.0	-4.80	0.005	Significantly Decreased
The 14th	0.0	The 28th	0.0	0.00	1,000	No difference

The test results showed that in the Menthol Variant OBH group, the average value of the test on day 0 was 5.5 Log Reduction, after day 7 the average decreased to 4.8 Log Reduction, there was a decrease of -0.74 Log Reduction, with a P-Value of 0.009 <0.05. From these data, it can be concluded that there is a significant difference in testing on day 0 and day 7, which can be interpreted that bacterial growth decreased significantly from day 0 to day 7. On day 14, bacterial growth had stopped so that the Log Reduction value was 5.5 from the initial count. On day 28 of the test, there was no increase in the number of colonies from day 14. Based on the test table, the highest significant decrease was from day 0 to day 14.

Table 9. Kruskal Wallis Difference Test for Menthol Variant OBH Groups

	OBH Menthol Variant
Kruskal-Wallis H	18,363
Df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test
b. Grouping Variable: Day

Based on the results of the Kruskal Wallis test, the Sig value of 0.000 is smaller than 0.05, so it can be stated that in the OBH Menthol Variant group, testing on days 0, 7, 14 and 28 has a significant difference. From these data, it can be concluded that in the OBH Menthol Variant group, the preservatives Potassium sorbate and Sodium benzoate can inhibit the growth of colonies of *Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* bacteria, decreasing significantly from days 0, 7, 14 to 28. The most effective decrease was on day 7 to day 14.

4. Strawberry Variant OBH Group

Table 10. Mann-Whitney Difference Test for Strawberry Variant OBH Groups

Day	Mean	Day	Mean	GAP	P-Value Mann Whitney	Information
To-0	5.6	The 7th	5.0	-0.62	0.008	Significantly Decreased
To-0	5.6	The 14th	0.0	-5.58	0.005	Significantly Decreased
To-0	5.6	The 28th	0.0	-5.58	0.005	Significantly Decreased

Day	Mean	Day	Mean	GAP	P-Value Mann Whitney	Information
The 7th	5.0	The 14th	0.0	-4.96	0.005	Significantly Decreased
The 7th	5.0	The 28th	0.0	-4.96	0.005	Significantly Decreased
The 14th	0.0	The 28th	0.0	0.00	1,000	No difference

The test results showed that in the OBH Strawberry Variant group, the average value of the test on day 0 was 5.6 Log Reduction, after day 7 the average decreased to 5.0 Log Reduction, there was a decrease of -0.62 Log Reduction, with a P-Value of 0.008 <0.05. From these data, it can be concluded that there is a significant difference in testing on day 0 and day 7, which can be interpreted that bacterial growth decreased significantly from day 0 to day 7. On day 14, bacterial growth had stopped so that the Log Reduction value was 5.6 from the initial count. On day 28 of the test, there was no additional colony from day 14. Based on the test table, the highest significant decrease was from day 0 to day 14.

Table 11. Kruskal Wallis Difference Test for Strawberry Variant OBH Groups

	OBH Strawberry Variant
Kruskal-Wallis H	18,395
df	3
Asymp. Sig.	.000

a. Kruskal Wallis Test
b. Grouping Variable: Day

Based on the results of the Kruskal Wallis test, the Sig value of 0.000 is smaller than 0.05, so it can be stated that in the OBH Strawberry Variant group, testing on days 0, 7, 14 and 28 has a significant difference. So it can be concluded that in the OBH Strawberry Variant group, the growth of *Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and Aspergillus brasiliensis bacterial colonies decreased significantly from days 0, 7, 14 to 28. The most significant decrease was on day 7 to day 14.

4. CONCLUSION

Based on research, Potassium sorbate and Sodium benzoate are effective in inhibiting the growth of *Eschericia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus brasiliensis* bacteria in OBH preparations with menthol and strawberry variants. In the group OBH menthol variant colonies of 5.5 Log Reduction from the initial count on day 14 and the number of colonies did not increase from the initial count until days 14 and 28. In the group OBH strawberry variant colonies by 5.6 Log Reduction from the initial count on day 14 and the number of colonies did not increase from the initial count until days 14 and 28.

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