

Test of Antibacterial Activity of Kicked Oranges (*Citrus Hystrix*) and Basil Leaves (*Ocimum Basilicum L*) Against *Staphylococcus Epidermidis* Bacteria in Spray Deodorant Preparations.

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ABSTRACT

This study tested the antibacterial activity of essential oils of kaffir lime peel (*Citrus hystrix*) and basil leaves (*Ocimum basilicum L.*) against *Staphylococcus epidermidis* bacteria in deodorant spray preparations. Essential oils were obtained through steam distillation, analyzed using GC-MS, and tested by the pitting diffusion method. The results showed that the combination of essential oils contained active compounds such as limonene and linalool, which effectively inhibited bacterial growth. The formula with 6% concentration showed the largest inhibition zone (24.74 mm) and was stable during storage. In conclusion, deodorant spray made from kaffir lime and basil essential oils has strong and stable antibacterial activity, so it has the potential to be developed as a natural product.

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1. INTRODUCTION

Body odor is caused by the activity of bacteria such as *Staphylococcus epidermidis* that break down sweat, especially in the underarm area. The use of deodorant is an effective solution to reduce body odor. As public awareness of the use of natural ingredients increases, the combination of kaffir lime peel essential oil and basil leaves is an attractive option due to its bioactive content that has antibacterial effects.

Kaffir lime peel essential oil contains active compounds such as linalool, limonene, and geranyl acetate that are known to be effective against various bacteria. Meanwhile, basil leaf essential oil is rich in compounds such as estragole and linalool that have antimicrobial properties. This study aims to identify the chemical components of essential oils from both plants using GC-MS and test their antibacterial activity in deodorant spray formulations. Cleanliness and body odor are the main and important things in a person's hygiene and appearance. A person will have higher self-confidence if his body smells fragrant and refreshing (Erviainingsih and Razak, 2019).

Solutions to reduce body odor can be addressed with soap. The use of soap as a body cleanser at bath time can be a solution to reduce body odor, but this is considered less effective and efficient to prevent body odor. Therefore, many individuals have used other more practical alternative measures, such as using deodorants (Zulfa, 2016).

Deodorant is a cosmetic preparation containing antiseptics to resist or reduce bacterial decomposition so as to control body odor (Sitompul, 2015). In addition, the large number of herbal plants in Indonesia that are easily obtained and utilized as active substances in deodorant preparations and the popularity of the back to nature concept has caused people to return to using natural ingredients as an alternative to health and cosmetics because they are considered safer, easier, cheaper (Zulfa, 2016).

2. RESEARCH METHOD**a. Materials and Tools**

The main ingredients used were essential oils of kaffir lime peel and basil leaves, *Staphylococcus epidermidis*,

and additional ingredients such as 96% ethanol, propylenglycol, and distilled water. The tools used include GC-MS, autoclave, hot plate stirrer, and laminar air flow.

b. Spray Deodorant Formulation

Three spray deodorant formulas were made with kaffir lime peel and basil leaf essential oil concentrations of 2%, 4%, and 6%, respectively. Each formula was tested for physical stability, pH, and organoleptic.

Table 1. Deodorant Spray Preparation Formulation

material	Formula		
	F1 (%)	F2 (%)	F3 (%)
Kaffir lime peel essential oil	2	4	6
Basil leaf essential oil	2	4	6
Niacinamide	5	5	5
alum	5	5	5
Propylene glycol	5	5	5
Methyl Praben	0,1	0.1	0,1
Aquades	Ad 60 ml	Ad 60 ml	Ad 60 ml

c. Antibacterial Activity Test

The pitting diffusion method was used to measure the zone of inhibition. Petri dishes that had been inoculated with *Staphylococcus epidermidis* were given wells containing deodorant spray samples. After incubation for 24 hours at 37°C, the diameter of the inhibition zone was measured.

3. RESULT AND DISCUSSION

a. Chemical compound identification of Kaffir Lime Peel Essential Oil (*Citrus hystrix*)

The chemical compounds of kaffir lime peel essential oil can be seen organoleptically in the table below.

Tabel 2. Organoleptical examination results of kaffir lime peel essential oil

No	Parameter	Inspection Result	Source Reference Parameter
1.	color	colorless	Colorless or yellow, green, blue Parameters of black pepper essential oil (International Organization for Standardization, 2008).
2.	Smell	Typical of kaffir lime peel essential oil	Typical of citronella essential oil
3.	Solubility in Alcohol	late	Parameter of the solubility of the highest compound content (β -pinene) late 1:6 in alcohol 90% (Moellhausen, 2008).

Based on the data in table 2, the organoleptic test of kaffir lime peel essential oil meets the requirements after passing the organoleptic test. Source Reference Parameters are specific references for the quality standards of kaffir lime peel essential oil. According to the Source Reference Parameters, organoleptically kaffir lime peel oil has no color with a refractive index of 1.4710 at 20°C, and is easily soluble in alcohol, appears clear onwards. The smell of kaffir lime peel essential oil has a distinctive and fresh odor. (Fadzil Latifah, 2023)

After organoleptically testing the standards, the process of testing the chemical compounds possessed by kaffir lime peel essential oil using GC-MS was carried out. Through the identification results using GC-MS, the chromatogram and chemical components of kaffir lime peel essential oil are found in table 3.

Analysis of essential oil components with GC-MS states that kaffir lime peel essential oil contains about 25 components that have been interpreted based on the Wiley Standard Library which can be seen in Table 4 and Figure 4. The results of the three highest compound content tests with the literature are almost the same, but the number of compounds detected is much more than the research of Warsito et al. (2018), so the distilled essential oil content is more complete. Distillation of essential oils from the same raw material can have different compound content, this depends on the type of plant, environmental factors during the process of secondary metabolite formation such as climate, soil, harvest age (Jailani et al., 2015). The results obtained in this study totaled 65.56%. Where the identification of the 3 highest percentage compounds are sabinene, β -pinene, limonene. In table 3., the results of the identification of chemical compound components of kaffir lime peel essential oil are as follows.

Table 3. Components of kaffir lime peel essential oil by GC-MS. Description: Hydrocarbon Total of the three highest compounds (sabinene, β -pinene, limonene) = 65.56%.

No	Compound Name	% Area	R, Time
1.	α - Thujene	0,28	3,911
2.	α - Pinene	2,52	4,018
3.	Camphene	0,22	4,212
4.	Sabinene	15,17	4,497
5.	β - Pinene	32,04	4,582
6.	β - Myrcene	1,22	4,638
7.	1- Phellandrene	0,25	4,875
8.	α - Terpinene	1,17	5,032
9.	β - Cimene	1,58	5,146
10.	Limonene	18,35	5,220
11.	γ - Terpinene	2,69	5,606
12.	Linalool Oxide	1,47	5,807
13.	Allo Ocimene	1,42	6,033
14.	Linalool	1,92	6,155
15.	Cis- Sabinene hydrate	0,18	6,550
16.	Citronellal	9,55	6,927
17.	Terpinene-4-ol	6,30	7,392
18.	α - Terpineol	2,07	7,575
19.	Cyclopentyl acetate	0,15	7,894
20.	Citronellyl acetate	0,29	9,748
21.	Geranyl acetate	0,28	10,181
22.	α - Copaene	0,30	10,239
23.	Germacrene- D	0,12	10,420
24.	γ - Caryophyllene	0,19	10,886
25.	δ - Cadinene	0,27	12,191
	TOTAL	100,00	

Based on the results of the identification test using GC-MS, it is identified that it contains 25 compounds, of which there are 3 high content compounds namely sabinene (15.17%), β -pinene (32.04%) and Limonene (18.35%). In the identification of chemical compounds, there are 3 chemical compounds that make up most of the essential oil, namely sabinene, β -pinene, and limonene.

a. Identification of chemical compounds of essential oil of basil leaves (*Ocimum Basilicum L.*)

Can be seen organoleptically identification of basil leaf essential oil with comparison parameters at The International Organization for Standardization (ISO) in the table below.

Table 4. Comparison of the results of the examination of essential oil of basil leaves and The International Organization for Standardization (ISO).

No	Parameter	check up result	ISO 11043 – 1998
1.	color	light yellow	Yellow or pale yellow
2.	smell	Typical essential oil of basil leaves (basil)	Typical essential oil of basil leaves (basil)
3.	Solubility in alcohol	late	1:1 soluble in alcohol

Based on the data in table 4. basil leaf essential oil (*Ocimum basilicum L.*) tested organoleptically is considered to meet the requirements after passing the organoleptic test. ISO 11043-1998 is a specific reference for basil leaf essential oil quality standards. According to ISO 11043-1998, organoleptically, basil leaf essential oil has a pale yellow color with a refractive index of 1.510-1.520 and is easily soluble in alcohol at a ratio of 1:1. The odor of basil leaf essential oil is distinctive and fresh. (ISO Standard, 1998).

After organoleptically testing the standards, the process of testing the chemical compounds possessed by basil leaf essential oil using GC-MS was carried out. Through the identification results using GC-MS, the chromatogram and chemical components of basil leaf essential oil are found in table 5. There is a percentage

difference in the results obtained in the study with the ISO 11043-1998 standard. This difference can be explained by comparing several factors related to the growth and development of these plants such as plant age, genetics, season or location of the plant. Essential oil of basil leaves tested by (T, Tanrisanah, et al., 2023) is a basil leaf obtained from Sungguminasa, Gowa Regency which was tested in the laboratory. While the essential oil of basil leaves in this study at PT Syai.

Table 5. Chemical compound components of basil leaf essential oil

No	Retention time (RT)	Area %	Compound	Qual
1	6.444	0.09	(1S)-2,6,6-Trimethylbicyclo	93
2	8.108	0.30	Eucalyptol	99
3	8.713	2.21	2-Furanmethanol, 5-Ethenyltetrahyd	91
4	8.965	1.91	Ethyl2-(5-methyl-5-vinyltetrahydr ofuran	91
5	9.179	22.35	Linalool	97
6	10.414	0.70	Levomenthol	91
7	10.578	0.70	dl-Menthol	91
8	10.578	0.48	3,7-Octadiene-2,6-diol, 2,6-dimethyl-	72
9	10.767	55.80	Estragole	99
10	11.258	0.40	Citral	58
11	11.258	0.40	Neral	95
12	11.397	0.17	Geraniol	76
13	11.561	0.59	Benzaldehyde, 4-methoxy-	97
14	11.561	0.59	Benzaldehyde,4methoxy	97
15	11.674	1.03	2,6-Octadienal,3,7 Dimethyl	97
16	11.989	0.26	Anethole	98
17	11.989	0.26	Benzene, 1-methoxy-4-(1-propenyl)	98
18	12.178	0.23	Benzene,1-methoxy-4-Propyl	87
19	13.250	0.33	cis-3-Hexenyl cis-3- hexenoate 71411 5Heptenal,2,6dimethyl	74
20	13.993	1.26	Bergamotene	99
21.	13.993	1.26	Bicyclo hept-2-ene, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-	98
22	14.661	0.18	(4-methylpent-3-en-1- yl)bicyclo heptane	96
23	14.661	0.18	Farnesene	97
24	14.926	0.27	Bisabolene	96
25	15.317	1.62	Cyclohexene, 4-[(1E)-1,5-dimethyl- 1,4-hexadien-1- yl]	99
26	15.707	3.76	Methoxycinnamaldehyde	99
27	15.959	1.36	Caryophyllene oxide	90
28	16.287	0.61	(1R,3E,7E,11R)-1,5,5,8-Tetramethyl	99

Based on the results of the identification test using GC-MS, essential oil obtained from PT Syailendra Agropreneur Karanganyar Regency, identified as containing 29 compounds, of which there are 2 high content compounds namely Estragole (55.80%), Linallol (22.35%). In the identification of chemical compounds, there are 2 chemical compounds that make up most of the essential oils, namely Estragole, Linallol. The identification results in other researchers also found that of the many compounds in essential oil (*Ocimum basilicum* L.) Neral (55.97%), Sitral (46.19%), and Geraniol (2.99%) (T, Tanrisanah, et al., 2023).

b. Antibacterial Activity Test

Inhibition zone test is done by measuring the circle around the disk.

Table 6. Antibacterial activity test of deodorant spray of kaffir lime peel essential oil (*Citrus hystrix*) and basil leaf essential oil (*Ocimum basilicum* L).

concentration	diameter of the inhibition zone		average (mm)	category
	Vertical Diameter	Horizontal Diameter		
Negative Control	8.02	8.95	8,48 mm	currently
Positive Control	25.85	23.00	24,42 mm	Very strong
F1 (2%)	15.90	20.90	18,4 mm	strong
F2 (4%)	20.90	23.50	22,2 mm	very strong
F3 (6%)	24.23	25.25	24.74 mm	very strong

In testing the antibacterial properties of deodorant spray formulations using formulations with kaffir lime peel essential oil and basil leaf essential oil at cold and room temperatures. according to (Winastri, Muliastri, and Hidayati, 2020) the category of antibacterial activity, namely the weak inhibition zone is ≤ 5 mm, moderate is in the range of 6-10 mm, strong is in the range of 11-20 mm, and very strong when in the range ≥ 21 mm. Antibacterial activity test data on spray deodorant formulations, the average data shows that antibacterial activity on negative control with moderate strength (8.48 mm). but on average positive control and formulas 2 and 3 there is antibacterial activity with very strong category (≥ 21 mm) and formula 1 with strong strength (18.4 mm). Through the average data obtained, it is known that formulas 1, 2 and 3 with concentrations of 2%, 4% and 6% have very strong inhibition against *Staphylococcus epidermidis* bacteria. The data obtained were then analyzed using a normality test, if the data were considered normally distributed, then continued the homogeneity of variances test, and if the data were considered homogeneous, the data were analyzed using One Way ANOVA and Post Hoc Tukey.

c. Organoleptical Test

Through the organoleptic test, there are 3 parameters assessed in this test, namely the odor, color, and texture of the formula. Based on our findings, it appears that the formula has stable organoleptic criteria both at cold temperature (4°C) and room temperature (35°C) as evidenced by no change in aroma, color, taste and texture from week 0 to week 4.

Table 7. Organoleptic test of deodorant spray of kaffir lime peel essential oil (*Citrus hystrix*) and basil leaf essential oil (*Ocimum basilicum* L) for 1 month at cold and room temperature.

Deodorant Spray	Organoleptic Test	Cold temperature (4°C)				
		Treatment				
		Week 0	Week 1	Week 2	Week 3	Week 4
Negative Control	Smell	odorless	odorless	odorless	odorless	odorless
	colour	clear	clear	clear	clear	clear
	Texture	Liquid + bubbles				
Formula 1	Smell	Typical MA Kaffir lime skin +				
	colour	It's a bit murky				
	Texture	liquid	liquid	liquid	liquid	liquid
	Smell	Typical MA Kaffir lime peel	Typical MA Kaffir lime			

Cold temperature (4°C)						
Deodorant Spray	Organoleptic Test	Treatment				
		Week 0	Week 1	Week 2	Week 3	Week 4
Formula 2		+ basil leaves	peel + basil leaves	peel + basil leaves	peel + basil leaves	peel + basil leaves
	colour	a bit murky				
	Texture	liquid	liquid	liquid	liquid	liquid
	Smell	Typical MA				
		Kaffir lime peel + basil leaves				
Formula 3						
	colour	a bit murky				
	Texture	liquid	liquid	liquid	liquid	liquid
	Smell	Typical MA				
		Kaffir lime peel + basil leaves				
Room temperature (35°C)						
Deodorant Spray	Organoleptic Test	Treatment				
		Week 0	Week 1	Week 2	Week 3	Week 4
Negative Control	Smell	odorless	odorless	odorless	odorless	odorless
	colour	clear	clear	clear	clear	clear
	Texture	Liquid + air bubbles				
	Smell	Typical MA				
		Kaffir lime skin + Basil leave				
Formula 1	colour	a bit murky				
	Texture	liquid	liquid	liquid	liquid	liquid
	Smell	Typical MA				
		Kaffir lime skin + Basil leave				
	Smell	Typical MA				
Formula 2	colour	a bit murky				
	Texture	liquid	liquid	liquid	liquid	liquid
	Smell	Typical MA				
		Kaffir lime skin + Basil leave				
	Smell	Typical MA				
Formula 3	colour	a bit murky				
	Texture	liquid	liquid	liquid	liquid	liquid
	Smell	Typical MA				
		Kaffir lime skin + Basil leave				
	Smell	Typical MA				

In organoleptical testing of the formula at cold temperature (4°C), there were 3 parameters evaluated, namely odor, color, and texture of the formula for one month. Based on the findings, the negative control obtained the results of odorless, clear color, liquid texture with air bubbles. Then in formulas 1, 2 and 3, the results obtained were typical odor of kaffir lime peel and basil leaf essential oils, slightly cloudy color, liquid texture. It can be seen that these formulas are stable in their organoleptic criteria at cold temperatures (4°C), as evidenced by the absence of changes in odor, color, and texture from week 0 to week 4. It should be noted that the aroma of kaffir lime peel essential oil and basil leaf essential oil has a strong and distinctive aroma.

In testing at room temperature (35°C), the same treatment as at cold temperature was carried out, there were 3 parameters evaluated, namely the odor, color, and texture of the formula for one month at room temperature (35°C). Based on the findings, the negative control obtained odorless results, clear color, and liquid texture with air bubbles. Then in formulas 1, 2 and 3, the results obtained had a distinctive smell of kaffir lime peel essential

oil and basil leaf essential oil, a slightly cloudy color, and a liquid texture. It can be seen that these formulas remain stable in their organoleptic criteria at room temperature (35°C), as evidenced by the absence of changes in odor, color, and texture from week 0 to week 4. The aroma of kaffir lime peel essential oil and basil leaf essential oil has a strong and distinctive aroma.

d. Normality Test

Through the normality test carried out using the one sample kolmogrov-smirnov test, it is obtained

Table 8. Normality Test

	Kolmogorov-Smirnov ^a			Shapiro-Wilk		
	Statistic	Df	Sig.	Statistic	df	Sig.
diameter_horizontal	.020	5	.020*	.931	5	.604
diameter_vertikal	.331	5	.078	.760	5	.037

The normality test can assess whether your data meets the assumption that the data is normally distributed. If this assumption is violated, the violation can affect the validity and reliability of the statistical analysis results. In the normality test, the modified Kolmogorov-Smirnov test was used. The Kolmogorov-Smirnov test with Lilliefors significance correction is based on the greatest difference between the cumulative sample distribution and the cumulative normal distribution, used for samples with $n \geq 50$. This test has proven to be less powerful than other tests in most situations and is included in statistical programs due to its popularity. Based on the results of the Essential Oil Antibacterial Activity Test Data, a significance value of $0.020 > 0.05$ was obtained, it was concluded that the group's bacterial data was normal, with the interpretation that the data was normally distributed and could be used for analysis.

e. Uji ANOVA

Table 9. Uji *One Way* ANOVA
ANOVA

Horizontal diameter					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	.353	1	.353	.008	.933
Within Groups	372.357	8	46.545		
Total	372.711	9			

Based on the results of the data in the One Way ANOVA test, the sig value is $0.933 > 0.05$, it is concluded that there is no bacterial difference between the antibacterial essential oil of kaffir lime peel and essential oil of basil leaves.

4. CONCLUSION

From this study, it can be concluded that the deodorant spray formulation of kaffir lime peel essential oil and basil leaf essential oil is considered to have met the standards and can prevent the growth of *Staphylococcus epidermidis* bacteria. Through the identification of chemical compounds in kaffir lime peel essential oil, it is identified that it contains 25 compounds, of which there are 3 high content compounds namely sabinene (15.17%), β -pinene (32.04%) and Limonene (18.35%) and basil leaf essential oil is identified to contain 29 compounds, of which there are 2 high content compounds namely Estragole (55.80%), Linallol (22.35%). The formula with 6% concentration showed the largest inhibition zone against *Staphylococcus epidermidis*. These results indicate the potential of the essential oil combination as a natural deodorant active ingredient.

In the inhibition zone test, formulas 1, 2 and 3 are considered to have strong inhibition. Formula 1 had an average inhibition of 12.4 mm, formula 2 had an average inhibition of 16.02 and formula 3 had an average inhibition of 18.74. In the formulation evaluation test, the negative control and formulations 1, 2 and 3 which were

given cold temperature treatment (4 ° C) and room temperature (40 ° C) were considered to have met the requirements with no organoleptical changes in the physical preparation, homogeneous, having a pH range of 6 at cold temperature (4 ° C) and room temperature (40 ° C). On the One Way ANOVA test obtained sig value of 0.933 > 0.05, it is concluded that there is no difference in the antibacterial bacteria between the antibacterial essential oil of kaffir lime peel and essential oil of basil leaves and for the Essential Oil Antibacterial Activity Test, a significance value of 0.020 > 0.05 was obtained, so it was concluded that the group's bacterial data was normal.

The combination of essential oils of kaffir lime peel and basil leaves is effective as an antibacterial against *Staphylococcus epidermidis*. The formula with 6% concentration gives the best antibacterial activity and good stability. These results support the potential of essential oils as active ingredients in natural deodorant products.

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