



Components of the essential oil fraction of betel leaf (piper betle linn) and the antibacterial activity test against several types of gram-positive bacteria

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ABSTRACT

Beetle (Piper betle Linn) is a plant belonging to the Piperaceae that is often used in beetle chewing. Essential oil fractions of beetle leaves were given by steam and water distillation and fractionated based on time periods. The results showed that the fractional yields of F₁, F₂, F₃ and F₄ were 0.061%, 0.034%, 0.027% and 0.015%, respectively. Analysis of essential oils by GC-MS showed that 5 main chemical components were chavibetol, caryophyllene, patchouli alcohol, phenol,2-methoxy-4(1-propheyl)-acetate and 4-allyl-1,2-diacetoxybenzene respectively. All of these main chemical constituents were proposed to be responsible for its antibacterial activity. The anti-bacterial activity of essential oils of beetle leaves was evaluated with a microdiluton method. The results showed all of essential oil fractions tested active against Staphylococcus epidermidis and Steptococcus mutant but not active to Bacillus subtilis. The MIC values of Staphylococcus epidermidis for all of essential oil fractions were 0.25%, while Steptococcus mutant was the most sensitive to fourth fraction by 0.25%. Inhibition mechanism their activity was indicated through microbial cell walls vandalism test after treatment 1 MIC and 2 MIC of the oils.

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1. INTRODUCTION

Knowledge of traditional medicine that was widely used by our ancestors in ancient times is currently being explored a lot. This is inseparable from the many obstacles caused by the use of synthetic drugs, such as the high price, the occurrence of resistance if the use is not appropriate and can cause unwanted side effects. People are now more inclined to use medicines made from natural ingredients and carry out traditional medicine as was done in ancient times. Plants as traditional medicines are usually used singly (one type of plant) or compound (a mixture of several types of plants). Parts of plants that are commonly used as traditional medicine are leaves, flowers, fruit, bark, or roots. Its use is directly in a state that is still fresh,

Betel (Piper betle) is a type of plant from the Piperaceae family that is widely known so that it has several regional names, including: sireh, order (Javanese). The most common use of betel is as an ingredient for betel nut. The parts of the betel plant for betel nut are the leaves (generally in western Indonesia) and fruit (generally in eastern Indonesia). Plants from the genus Piper, such as

Piper nigrum, *P. methysticum*, *P. auritum* and *P. betle* have been known for a long time as agricultural commodities for spices, insecticides in the fields.

From previous studies it was reported that betel leaf essential oil has activity as an antibacterial by destroying the bacterial cell wall. In this research, the chemical component fractionation of betel leaf essential oil was carried out and its activity was tested against gram-positive bacteria *B. subtilis*, *S. epidermidis* and *S. mutant*. Therefore, this research aimed at the chemical components of the betel leaf essential oil fraction obtained from variations in distillate taking time on antibacterial activity for several types of gram-positive bacteria *B. subtilis*, *S. epidermidis* and *S. mutant*.

2. RESEARCH METHOD

The method used for the process of separating the chemical components of the essential oil fraction of fresh betel leaves from Balitro, Cimanggu (Bogor), is steam and water distillation (Sugiastuti, 2002; Sulianti and Chairul, 2002). This distillation process was carried out using variations in the time of taking essential oils, namely at 1 hour, 2 hours, 4 hours and 6 hours. The difference in the time of taking this distillate gave different results on the content of the chemical components of the essential oil fraction (table 1). Likewise for the percentage value of the resulting content of each fraction 1,2,3 and 4, namely 0.061%, 0.034%, 0.027% and 0.015%. However, the oil obtained still has the same aroma and color, with a distinctive betel leaf aroma and clear yellow color.

From the results of the GCMS analysis it is known that the four essential oil fractions have different chromatograms from one another (figure 1). The 1st hour fraction has 64 constituent chemical components. Meanwhile, in the 2nd and 6th hour fractions, 56 peaks were detected and 73 peaks for the 4th hour fraction.

3. RESULTS AND DISCUSSIONS

Results

Determination of Chemical Components of Essential Oil Fractions



Figure 1. Betel leaf

The method used for the process of separating the chemical components of the essential oil fraction of fresh betel leaves from Balitro, Cimanggu (Bogor), is steam and water distillation (Sugiastuti, 2002; Sulianti and Chairul, 2002). This distillation process was carried out using variations in the time of taking essential oils, namely at 1 hour, 2 hours, 4 hours and 6 hours. The difference in the time of taking this distillate gave different results on the content of the chemical components of the essential oil fraction (table 1). Likewise for the percentage value of the resulting content of each fraction 1,2,3 and 4, namely 0.061%, 0.034%, 0.027% and 0.015%. However, the oil obtained still has the same aroma and color, with a distinctive betel leaf aroma and clear yellow color.

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chemical components. Meanwhile, in the 2nd and 6th hour fractions, 56 peaks were detected and 73 peaks for the 4th hour fraction.

Identification of each peak on the chromatogram of each essential oil fraction shows that its chemical components consist of monoterpenes, monoterpenes alcohols, sesquiterpenes, sesquiterpenes alcohols and phenylpropanoid derivatives. Then each chemical component of the betel leaf essential oil fraction was grouped based on the compound group as shown in (table 2).

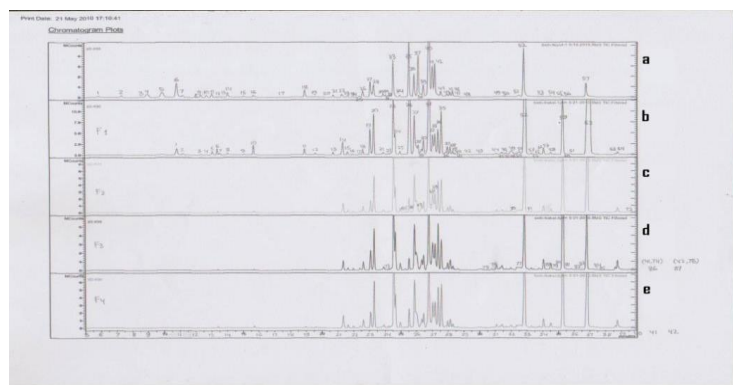


Figure 2. Chromatogram comparison between betel leaf essential oil fractions.

Description: a: Single betel leaf essential oil b: Betel leaf essential oil, 1st hour fraction c: Betel leaf essential oil, 2nd hour fraction d: Betel leaf essential oil, 4th hour fraction e: Betel leaf essential oil, 6th hour fraction

Table 1. Comparison of chemical components between betel leaf essential oil fractions

No	Retenti on Time	Compound Name	Molecular Formula	BM	Relative Content (%)			
					F1	F2	F3	F4
1	10.84	Sabinen	C ₁₀ H ₁₆	136	0.65	-	-	-
2	11.23	2-β-pinene	C ₁₀ H ₁₆	136	0.10	-	-	-
3	12.36	α-Terpinen	C ₁₀ H ₁₆	136	0.06	-	-	-
4	12.73	etc-Limonene	C ₁₀ H ₁₆	136	0.05	-	-	-
5	13.09	β-Felandren	C ₁₀ H ₁₆	136	0.29	-	-	-
6	13.39	3-Karen	C ₁₀ H ₁₆	136	0.39	0.12	0.09	0.12
7	13.55	Not identified	-	-	0.19	-	-	-
8	14.07	γ-Terpinen	C ₁₀ H ₁₆	136	0.10	-	-	-
9	15,10	α-Humulen	C ₁₅ H ₂₄	20	0.05	-	-	-
10	15.69	Linalil Isobutrate	C ₁₄ H ₂₄ O ₂	224	0.59	0.18	0.07	0.18
11	18.93	Origanol	C ₁₀ H ₁₈ O	154	0.37	0.10	0.06	0.10
12	19,62	α-Terpinenyl acetate	C ₁₂ H ₂₀ O ₂	196	0.09	0.06	0.04	0.06
13	20,74	Estragol	C ₁₀ H ₁₂ O	148	0.16	-	-	-
14	21.35	Benzoic acid, 2-hydroxy-, methyl ester	C ₈ H ₈ O ₃	152	1.00	1.28	1.07	1.30
15	21.65	α-Kubeben	C ₁₅ H ₂₄	20	0.20	0.2	0.24	0.29
16	21.98	1-(1-Ethyl-2,3-dimethyl-cyclopene-2-enyl)-etanone	C ₁₁ H ₁₈ O	166	0.06	0.14	0.17	0.15
17	22.40	β-Bourbenen	C ₁₁ H ₁₈ O	166	0.05	0.13	0.17	0.13
18	22,62	α-Kopaen	C ₁₅ H ₂₄	20	0.31	0.69	0.80	0.71
19	23.08	Kavikol	C ₉ H ₁₀ O	134	1.61	1.44	1.90	1.47
20	23,32	β-Element	C ₁₅ H ₂₄	20	2.85	4,41	3.94	4,33
21	23.91	α-Bergamots	C ₁₅ H ₂₄	20	0.16	0.31	0.37	0.32
22	24,24	Trans-α-Bergamot	C ₁₅ H ₂₄	20	0.06	0.12	0.14	0.12

Components of the essential oil fraction of betel leaf (piper betle linn) and the antibacterial activity test against several types of gram-positive bacteria (Raule Kapdo)

23	24.57	caryophyllene	C ₁₅ H ₂₄	4 20	4.82	8.70	8.54	8,61
24	24,66	α-Guaien	C ₁₅ H ₂₄	4 20	1.21	2.63	3.56	2.65
25	24.97	Aromadendren	C ₁₅ H ₂₄	4 20	0.20	0.55	0.73	0.55
26	25,61	Phenol 1,4-(2-propenyl)-acetate	C ₁₁ H ₁₂ O ₂	4 176	10.34	0.02	0.03	0.02
27	25.89	α-Humulene	C ₁₅ H ₂₄	20	3.82	4.97	4.97	4.85
28	26,12	p.s-Eugenol	C ₁₀ H ₁₂ O ₂	4 164	0.59	-	-	-
29	26,33	valensen	C ₁₅ H ₂₄	20	0.50	1.09	1.39	1.09
30	26,46	Naphtalen,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)	C ₁₅ H ₂₄	4 20 4	1.01	1.28	1.46	1.30
31	26,83	Kavibetol	C ₁₀ H ₁₂ O ₂	164	10.61	9.60	10,5 0	9.90
32	27.04	β-Selinen	C ₁₅ H ₂₄	164	0.78	2.97	3.64	2.88
33	27,19	α-Selinen	C ₁₅ H ₂₄	20 4	1.60	2,58	3.02	2.63
34	27,38	Δ- Gurjunena	C ₁₅ H ₂₄	20 4	1.74	3,83	4.63	3.78
35	27,60	Bisiklogermakren	C ₁₅ H ₂₄	20 4	3,11	4,11	3,16	4.08
36	27.85	Benze,1,2-dimethoxy-4-(2-propenyl)	C ₁₁ H ₁₄ O ₂	4 20 4	0.12	0.07	0.09	0.08
37	27.97	α-Pacuelen	C ₁₅ H ₂₄	20	0.47	0.55	0.69	0.54
38	28,13	β-Kadinena	C ₁₅ H ₂₄	4 20	0.43	0.72	1.02	0.72
39	28,30	(-)-α-Panasinsen	C ₁₅ H ₂₄	4 20	0.20	0.29	0.42	0.30
40	28,43	β-Kadinena	C ₁₅ H ₂₄	4 20	0.06	-	0.03	-
41	28.59	Kadina-1,4-diene	C ₁₅ H ₂₄	4 20	0.05	0.08	0.13	0.09
42	29,17	γ-Himakalen	C ₁₅ H ₂₆ O	4 222	0.03	-	-	-
43	29.90	γ-Gurjunena	C ₁₅ H ₂₄	20 4	0.05	-	-	-
44	31.07	Veridiflorol	C ₁₅ H ₂₆ O	20 4	0.07	0.30	0.59	0.30
45	31,29	γ-Gurjunene pokid-(2)	C ₁₅ H ₂₄ O	22 0	0.05	0.14	0.24	0.14
46	31,44	β-Guaien	C ₁₅ H ₂₄	222	0.17	0.37	0.57	0.37
47	31.65	Caryophyllene oxide	C ₁₅ H ₂₄ O	22 0	0.10	0.10	0.14	0.11
48	31.77	(-)-Caryophyllene oxide	C ₁₅ H ₂₄ O	22 0	0.05	0.07	0.10	0.07
49	32.03	Kubenol	C ₁₅ H ₂₆ O	222	0.59	-	0.08	-
50	32.35	2,3,3-Trimethyl-2-(3-methyl-blind-1,3-dienyl)-cyclohexane	C ₁₄ H ₂₂ O	20	0.10	0.22	0.27	0.22
51	32,52	3-Allyl-6-methoxyphenyl acetate	C ₁₂ H ₁₄ O ₃	20 6	0.34	0.51	0.38	0.52
52	32,92	phenol,2-methoxy-4-(1-propenyl)-acetate	C ₁₂ H ₁₄ O ₃	20 6	13.89	12.95	10.77	13.01
53	33,30	β-Guaien	C ₁₅ H ₂₄	20 4	0.12	0.17	0.21	0.17
54	33.45	t-Caddy	C ₁₅ H ₂₆ O	222	0.05	0.08	0.12	0.08
55	33,61	β- Bisabolol	C ₁₅ H ₂₆ O	222	0.05	0.04	0.04	0.04
56	33.78	Toreyol	C ₁₅ H ₂₆ O	222	0.12	0.18	0.20	0.17
57	34.07	junipen	C ₁₅ H ₂₄	222	0.45	0.89	1.52	0.90
58	34,52	Agarospinol	C ₁₅ H ₂₆ O	222	0.12	0.39	0.80	0.40

Description : F1: Fraction of the 1st hour

F2: Fraction of the 2nd hour
 F3: Fraction of the 4th hour
 F4: Fraction of the 6th hour
 - : not identified

Table 2. Classification of chemical components between betel leaf essential oil fractions

No.	Compound Class	Percentage (%)			
		F1	F2	F3	F4
1.	Monoterpene	1.63	0.12	0.09	0.12
2.	Monoterpene Alcohol	0.37	0.10	0.06	0.10
3.	Sesquiterpenes	24,49	44,36	49,21	43,72
4.	Sesquiterpene Alcohol	6.00	11.26	15,15	11.20
5	Phenyl Propanoid Derivatives	66.07	42,54	32,83	43,07
6	Etc	1.16	1.45	2.09	1.61
7	Not identified	0.28	0.17	0.57	0.18
	Total	100	100	100	100

Description: F1: Fraction of the 1st hour

F2: Fraction of the 2nd hour

F3: Fraction of the 4th hour

F4: Fraction of the 6th hour

Testing the Antibacterial Activity of the Betel Leaf Essential Oil Fraction

The antibacterial activity of the betel essential oil fraction against the three test bacteria can be calculated by measuring the diameter of the inhibition area (DDH) of bacterial growth around the paper disc which looks clear. Based on the test results presented in table 3, it can be seen that the betel essential oil fraction at a concentration of 50% can affect the growth of the three bacteria with different levels of inhibition. F2 and F4 betel leaf essential oil had the highest level of sensitivity to the three test bacteria compared to the other three fractions (table.3).

Table 3. Activity of betel leaf essential oil fraction against 3 types of gram-positive bacteria at a concentration of 50%

No	Test bacteria	Inhibition Area Diameter (mm)*			
		F1	F2	F3	F4
1	B. subtilis	4	8	7	7,5
2	S. epidermidis	2	4,5	2,5	5,5
3	S. mutant	5,5	13	4,5	8,5
	Methanol control	-	-	-	-

Description: F1: Fraction of the 1st hour

F2: Fraction of the 2nd hour

F3: Fraction of the 4th hour

F4: Fraction of the 6th hour

* : average of two replicates

- : no diameter of inhibition area

Determination of MIC Fraction of Betel Leaf Essential Oil

Determination of the MIC value is based on the minimum concentration of the essential oil fraction of betel leaf which can inhibit the growth of the three gram-positive bacteria (table 4 and table 5).

Table 4. Determination of MIC of betel leaf essential oil fraction against test bacteria *B. subtilis*

No	ConcentrationM ICs (%)	MIC value of <i>B. subtilis</i>			
		F1	F2	F3	F4
1	17.5	+	+	+	+
2	15.5	+	+	+	+
3	12.5	+	+	+	+
4	10	+	+	+	+
5	7.5	+	+	+	+
6	5	+	+	+	+
7	2,5	+	+	+	+
Solvent control*		-	-	-	-

Description: F1: Fraction of the 1st hour

F2: Fraction of the 2nd hour

F3: Fraction of the 4th hour

F4: Fraction of the 6th hour

“-“: no bacterial growth

+ : there is bacterial growth

*: 0.5% tween 80, 2% absolute ethanol and aquadest

Table 5. Determination of MIC of betel leaf essential oil fraction against test bacteria *S. epidermidis* and *S. mutant*

No	Concentratio nMICs (%)	MIC value <i>S. epidermidis</i>				S. mutant MIC value			
		F1	F2	F3	F4	F1	F2	F3	F4
2	2,5	-	-	-	-	-	-	-	-
3	1	-	-	-	-	+	-	-	-
4	0.5	-	-	-	-	+	+	+	-
5	0.25	-	-	-	-	+	+	+	-
6	0.125	+	+	+	+	+	+	+	+
Solvent control*		-	-	-	-	-	-	-	-

Description: F1: Fraction of the 1st hour

F2: Fraction of the 2nd hour

F3: Fraction of the 4th hour

F4: Fraction of the 6th hour

“-“: no bacterial growth

+ : there is bacterial growth

*: 0.5% tween 80, 2% absolute ethanol and aquadest

Protein and Nucleic Acid Leakage Analysis

Giving the 1st hour fraction of betel leaf essential oil at several doses of MIC resulted in cell damage which was observed by the leakage of protein and nucleic acids from the bacterial cells. The 1st hour fraction of betel leaf essential oil caused cell leakage which was observed with an increase in the absorbance value at a wavelength of 260 nm and 280 nm (figure 3). Compounds that give absorption at a wavelength of 260 nm are nucleic acids (RNA and DNA), while those at a wavelength of 280 nm are identified as proteins.

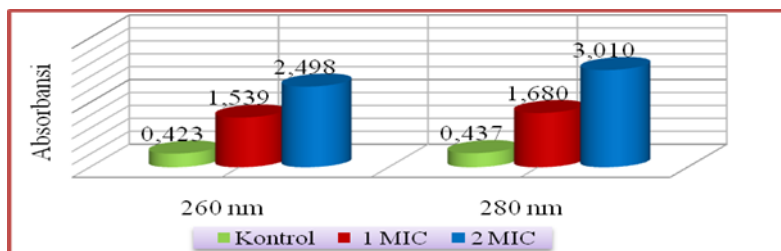


Figure 3. Nucleic acid and protein absorbance values of the 1st hour fraction on *S.epidermidis* cells

Analysis of Ca²⁺ and K⁺ Metal Ions

The 1st hour fraction of betel leaf essential oil can also cause the release of Ca²⁺ and K⁺ ions from *S.epidermidis* bacterial cells. The ions that come out of the cell can be seen in Figure 4.

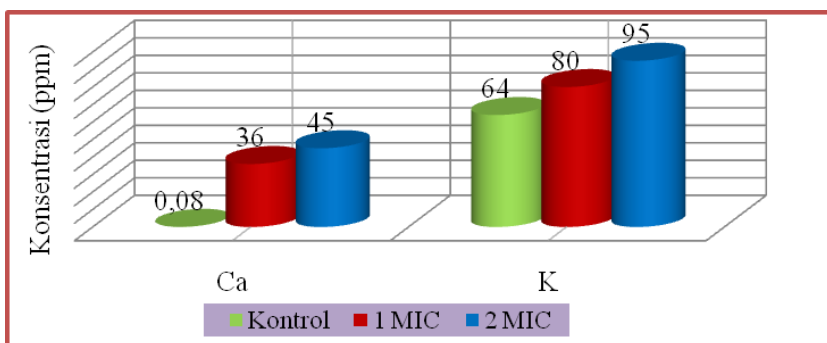


Figure 4. Results of measurements of metal ion concentrations of Ca²⁺ and K⁺ of the 1st hour fraction in the supernatant of *S.epidermidis* culture with concentrations of 1 MIC and 2 MIC

Analysis of Morphological Changes in *S. epidermidis* Bacterial Cells with SEM

The 1st hour fraction of betel leaf essential oil with 1 MIC and 2 MIC treatment can also cause leakage in the cell membrane, this can be seen in Figure 5. From the photo images obtained by the Scanning Electron Microscope it can be seen that there are changes in cell morphology. Changes in cell morphology can be shown from Figure 5(a) where the results show that *S. epidermidis* bacteria are normal, the cells still look compact, and are round in shape. Then there was a cell change as shown in Figure 5(b) and Figure 5(c) where the *S. epidermidis* bacterial cells that had been treated with betel leaf essential oil with 1 MIC and 2 MIC treatments changed the cells to shrink and become hollow.

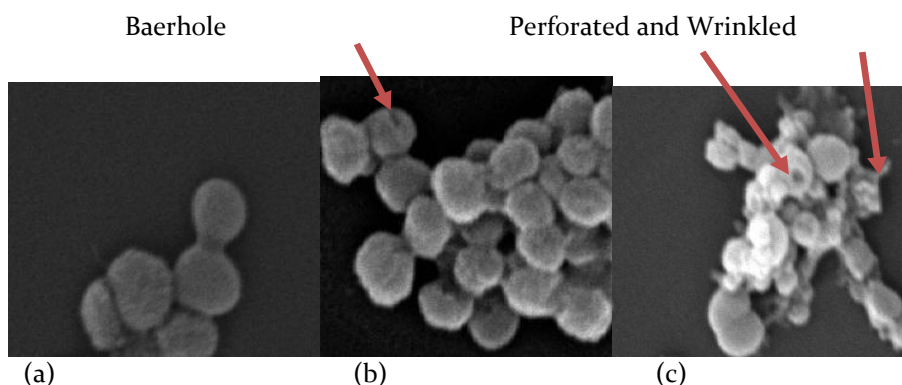


Figure 5. *S.epidermidis* cell morphology. (a) control, (b) *S.epidermidis* cells after 1 MIC treatment, (c) *S.epidermidis* cells after 2 MIC treatments (magnification 10,000x). The arrow indicates a leak.

Discussion

Betel is known because almost all parts of the plant are used as medicine, besides being used as a betel herb. Besides that, it is also considered to be able to strengthen teeth, prevent stomach disorders, increase body endurance, stimulate nerves and others (Pudjiastuti, 1996). In this research, isolation of essential oil fractions, identification of chemical components of the fractions, and testing of the antibacterial activity of betel leaves in the form of essential oil fractions were carried out to obtain antibacterial alternatives. The betel leaves used in this study are fresh betel leaves originating from Balitro, Cimanggu, Bogor and have been determined at the Bogoriense Herbarium, Botany Division, Research Center for Biology LIPI as shown in Appendix 1.

The method used for the process of separating the components of the betel leaf essential oil fraction is the steam and water distillation method (Sugiastuti, 2002; Sulianti and Chairul, 2002). Prior to the distillation process, the betel leaves are first sorted and washed to separate and remove impurities attached to the sample. Then chopped by cutting into small pieces to increase the size of the surface of the particles to facilitate contact between the sample material and water vapor so that the distillation process can take place properly. (Novalny, 2006).

CONCLUSION

Betel leaf essential oil fraction from Balitro, Cimanggu, Bogor has a percentage of grades produced sequentially for fractions 1,2,3 and 4 of 0.061%; 0.034%; 0.027% and 0.015%. and has chemical components of 64 compounds (F1), 56 compounds (F2), 73 compounds (F3) and 56 compounds (F4). A total of 4 chemical compounds as constituents of the major component (> 5%) of F1, and 5 chemical compounds as constituent components for F2, F3 and F4. The highest component of the main compound in F1, F2, and F4 was 4-allyl-1,2-diacetoxybenzene (27.35%; 14.53% and 14.64%), while in F3 was Pacouli alcohol (12.62 %) Fraction Betel leaf essential oil has antibacterial activity against *S. mutant*, *S. epidermidis*, and *B. subtilis* bacteria. The MIC value obtained from each essential oil fraction for *S. epidermidis* was 0.25% (v/v). *B. subtilis* >17.5% (v/v)., while *S. mutant* F1 was 2.5% (v/v), F2 and F3 was 1% (v/v)., and F4 was 0.25% (v/v). The mechanism of inhibition of antibacterial fraction 1 of betel leaf essential oil against *S. epidermidis* is damage to the bacterial cell membrane, which is characterized by an increase in the release of cellular metabolites such as amino acids, proteins, and metal ions Ca²⁺ and K⁺.

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