ISSN XXXX-XXXX (Online) | XXXX-XXXX (Print) | Vol. 1, No. 1, November (2022), pp.9-18

Published by: IARN Institute

International Journal of Nursing and Midwifery Research

Journal homepage: https://journals.iarn.or.id/index.php/ners/index

Analysis of the chemical components of the essential oil fraction of betel leaf (piper bettle linn.) and the antibacterial activity test against several types of gram-positive bacteria

Alec Baldwin
Washington state University, Pullman, WA, Amerika Serikat

Article Info

Article history:

Received Nov 02, 2222
Revised Nov 16, 2022
Accepted Nov 30, 2022

Keywords:

Antibacterial activity;
essential oils;
Fractions;
gram positive bacteria;
Piper betle.

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-prophenyl)-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.

Corresponding Author:

Alec Baldwin,
Pharmacy study program,
Faculty of Medicine and Health Sciences,
Washington state University, Pullman, WA, Amerika Serikat
E-mail: alecwin@gmail.com

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 from natural ingredients and carry out traditional treatments as they were 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

1. Results

Determination of Chemical Components of Essential Oil Fractions

Image 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.

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.

Identification of each peak on the chromatogram of each essential oil fraction shows that its chemical components consist of monoterpenes, monoterpenes alcohols, sexuiterpenes, 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).

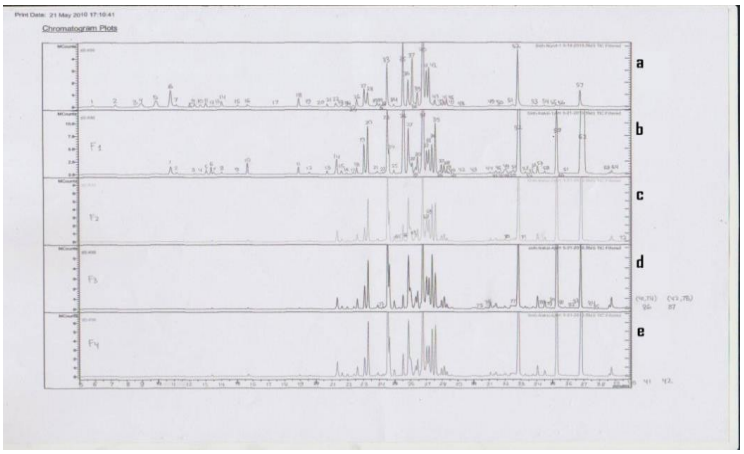

Figure 2. Chromatogram comparison between betel leaf essential oil fractions.

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	Retention 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	$C_{15}H_{24}$	204	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	$C_8H_8O_3$	152	1.00	1.28	1.07	1.30
15	21.65	*α*-Kubeben	$C_{15}H_{24}$	204	0.20	0.29	0.24	0.29

16	21.98	1-(1-Ethyl-2,3-dimethyl-cyclopene-2-enyl)-etanone	C11H18O	166	0.06	0.14	0.17	0.15
17	22.40	*β*-Bourbenen	C11H18O	166	0.05	0.13	0.17	0.13
18	22,62	*α*-Kopaen	C15H24	204	0.31	0.69	0.80	0.71
19	23.08	Kavikol	C9H10O	134	1.61	1.44	1.90	1.47
20	23,32	*β*-Element	C15H24	204	2.85	4,41	3.94	4,33
21	23.91	*α*-Bergamots	C15H24	204	0.16	0.31	0.37	0.32
22	24,24	*Trans*-α-Bergamot	C15H24	204	0.06	0.12	0.14	0.12
23	24.57	caryophyllene	C15H24	204	4.82	8.70	8.54	8,61
24	24,66	*α*-Guaien	C15H24	204	1.21	2.63	3.56	2.65
25	24.97	Aromadendren	C15H24	204	0.20	0.55	0.73	0.55
26	25,61	Phenol 1,4-(2-prophenyl)-acetate	C11H12O2	176	10.34	0.02	0.03	0.02
27	25.89	*α*-Humulene	C15H24	204	3.82	4.97	4.97	4.85
28	26,12	*p.s*-Eugenol	C10H12O2	164	0.59	-	-	-
29	26,33	valensen	C15H24	204	0.50	1.09	1.39	1.09
30	26,46	Napthalen,1,2,3,4,4a,5,6,8a-octahydro-7-methyl-4-methylene-1-(1-methylethyl)	C15H24	204	1.01	1.28	1.46	1.30
31	26,83	Kavibetol	C10H12O2	164	10.61	9.60	10.50	9.90
32	27.04	*β*-Selinen	C15H24	164	0.78	2.97	3.64	2.88
33	27,19	*α*-Selinen	C15H24	204	1.60	2.58	3.02	2.63
34	27,38	Δ- Gurjunena	C15H24	204	1.74	3.83	4.63	3.78
35	27,60	Bisiklogermakren	C15H24	204	3,11	4,11	3,16	4.08
36	27.85	Benze,1,2-dimethoxy-4-(2-propenyl)	C11H14O2	204	0.12	0.07	0.09	0.08
37	27.97	*α*-Pacuelen	C15H24	204	0.47	0.55	0.69	0.54
38	28,13	*β*-Kadinena	C15H24	204	0.43	0.72	1.02	0.72
39	28.30	(-)-α-Panasinsen	C15H24	204	0.20	0.29	0.42	0.30
40	28,43	*β*-Kadinena	C15H24	204	0.06	-	0.03	-
41	28.59	Kadina-1,4-diene	C15H24	204	0.05	0.08	0.13	0.09
42	29,17	*γ*-Himakalen	C15H26O	222	0.03	-	-	-
43	29.90	*γ*-Gurjunena	C15H24	204	0.05	-	-	-
44	31.07	Veridiflorol	C15H26O	204	0.07	0.30	0.59	0.30
45	31,29	*γ*-Gurjunene pokid-(2)	C15H24O	220	0.05	0.14	0.24	0.14
46	31,44	*β*-Guaien	C15H24	222	0.17	0.37	0.57	0.37
47	31.65	Caryophyllene oxide	C15H24O	220	0.10	0.10	0.14	0.11
48	31.77	(-)-Caryophyllene oxide	C15H24O	220	0.05	0.07	0.10	0.07
49	32.03	Kubenol	C15H26O	222	0.59	-	0.08	-

50	32.35	2,3,3-Trimethyl-2-(3-methyl-blind-1,3-dienyl)-cyclohexane	C14H22O		0.10	0.22	0.27	0.22
51	32,52	3-Alyl-6-methoxypenyl acetate	C12H14O3	206	0.34	0.51	0.38	0.52
52	32,92	phenol,2-methoxy-4-(1-prophenyl)-acetate	C12H14O3	206	13.89	12.95	10.77	13.01
53	33,30	*β*-Guaien	C15H24	204	0.12	0.17	0.21	0.17
54	33.45	*t*-Caddy	C15H26O	222	0.05	0.08	0.12	0.08
55	33,61	*β*- Bisabolol	C15H26O	222	0.05	0.04	0.04	0.04
56	33.78	Toreyol	C15H26O	222	0.12	0.18	0.20	0.17
57	34.07	junipen	C15H24	222	0.45	0.89	1.52	0.90
58	34,52	Agarospirol	C15H26O	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.	Monoterpenes	1.63	0.12	0.09	0.12
2.	Monoterpene Alcohol	0.37	0.10	0.06	0.10
3.	Sesquiterpene	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	Concentratio nMICs (%)	MIC value of B. subtilis			
		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 *S. epidermidis*				S. mutant MIC value			
		F1	F2	F3	F4	F1	F2	F3	F4
1	5	-	-	-	-	-	-	-	-
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.

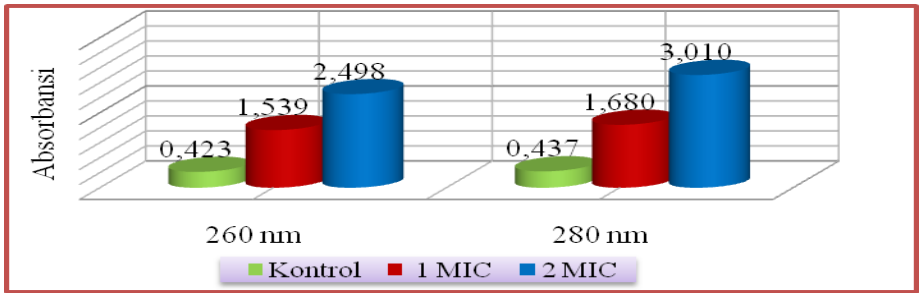

Figure 3. Nucleic acid and protein absorbance values of the 1st hour fraction on S.epidermidis cells

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.

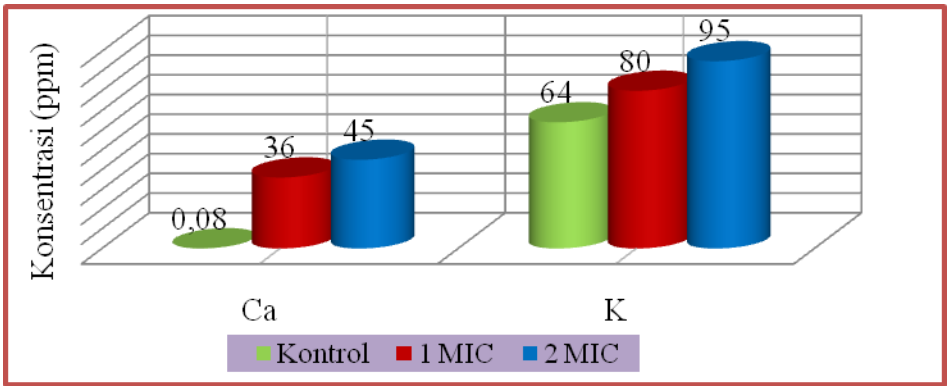

Figure 4. Results of measurements of metal ion concentrations of Ca2+ and K+ of the 1st hour fraction in the supernatant of S.epidermidis culture with concentrations of 1 MIC and 2 MIC

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.

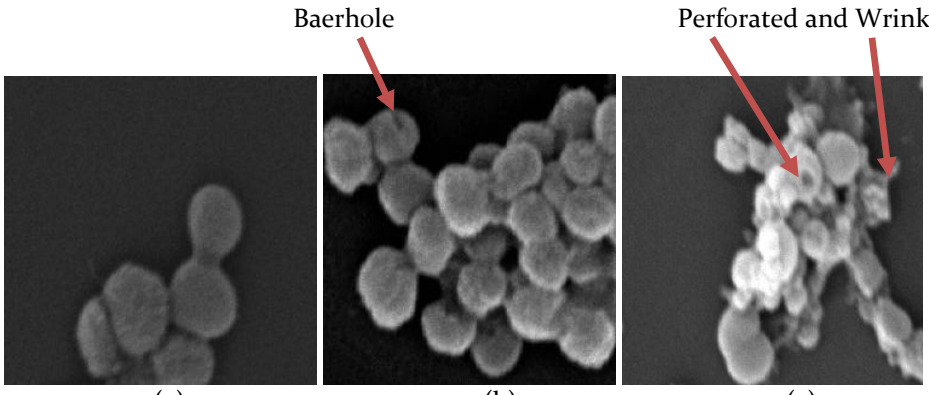

Figure 5.S.epidermidis cell morphology. (a) control, (b) S.epidermidis cells after 1 MIC treatment, (c) S.epidermidis cells after 2 MIC treatments (magnification 10,000x). The arrow indicates a leak.

2. 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 antibacterial inhibition of 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+.

REFERENCES

Agusta, A. 2000. Essential Oils of Indonesian Tropical Plants. ITB Publisher. Bandung.

Al Farisi, S. 2009. Mechanism of Antibacterial Inhibition of Betel Leaf Essential Oil (Piper Bettle Linn; Piperaceae) Against Staphylococcus Epidermidis. Essay. Faculty of Medicine and Health Sciences UIN Jakarta.

Bilbiana, L., and Hastowo, S. 1992. Microbiology. Jakarta : Rajalawi Press.

Brooks, GF, Butel, JS, and Morse, SA, 2005. Jawetz, Melnick & Adelberg's Medical Microbiology. translated by Eddy M, Kuntaman, Eddy BW, Ni Made M, Setio H, and Lindawati A. Edition 1. Volume 1. Salemba Medika. Jakarta.

Carson, CF, Brian, JM, Riley, TV, 2002. Mechanism of Action of Melaleuca alternifolia (Tea Tree) Oil on Staphylococcus aureus Determined by Time Kill, Lyses, Leakage, and Salt Tolerance Assays and Electron Microscopy. Antimicrobial Agents and Chemotherapy 6 : 1914-1920.

Chambers, HF. 2007. Goodman and Gilman Therapeutic Pharmacology,

translated by Cucu ., Ella, E., Winny, RS., Amalia, H., July, M. Edition

10. Volume 2. EGC Medical Book Publishers. Jakarta.

Cowan, MM 1999. Plant products asd antimicrobial agents. J. Microbiology Reviews 612 (4) : 564 – 582.

Cox, SD, Mann, CM, Markham, JL, Bell, HC, Gustafson, JE, Warmington,

JR and Wyllie, SG. 2000. The Mode of Antibacterial Action of The Essential Oil of Melaleuca Alternifolia (Tea Tree Oil). J of Applied Microbiology 88 : 170-175.

Didik, G., and Mulyani, S. 2004. Natural Medicine Science (Pharmacognosy) Volume I. Independent spreaders. Jakarta

Fathillah, AR, Yusoff M, Rahim ZHA. The Effect of Psidium guajava and Piper betle extracts on the Morphology of Dental Plaque Bacteria. Mr. J Med Sci 2009;25(6):928-933.

Harapani, M., Agusta, A., and Rahayu, DC. 1996. Chemical components of essential oils from two kinds of betel (yellow and green leaves). Proceedings of the Symposium on Medicinal and Aromatic Plants. Biology Research and Development Center-LIPI. Bogor. 58-63.

Hermawan, A. 2007 . Effect of betel leaf extract (piper betle l.) on the growth of staphylococcus aureus and escherichia coli using the disk diffusion method. Essay. UAIR Faculty of Veterinary Medicine.

Hertiani, T., and Purwantini, A. 2002. Distillation results of betel leaf ethanol extract (Piper betle L) from several areas in Yogyakarta and antifungal activity against Candida albicans. Indonesian Pharmacy Magazine 13 (4): 193-199.

Jawetz, E., Melnick, JL, Adelberg, EA 1995. Medical Microbiology, Medical Microbiology 20th Edition. Translation: Edi Nugroho and RF Maulany. EGC, Jakarta

Mann, CM and Markham, JL 1998. A new method for determining the minimum inhibitory concentration of essential oils. Journal of Applied Microbiology 84. 538-544.

Miksusanti. 2009. Activity and mechanism of Temu Kunci (Kaempheria pandurata Roxb) essential oil and its incorporation into sago starch as an antibacterial edible film. Dissertation. IPB

Nalina, T., and Rahim, ZHA 2007. The Crude Aqueos Extract of Piper betle L. and its Antibacterial Effect Toward Streptococcus mutans. American Journal of Biotechnology and Biochemistry 3(1). 2007. 10-15.

Novalny, D. 2006. Effect of chopped leaf size and distillation time on the yield and characteristics of betel (Piper betle Linn.) oil. Essay. IPB

Parwata, IOAM, Rita, WS., Yoga, R. 2009. Isolation and free anti-radical testing of essential oils in betel leaves (Piper betle Linn.) Ultra violet-visible spectroscopy. Journal of chemistry 3 (1), January 2009: 7-13

Pratiwi, ST 2008. Pharmaceutical Microbiology. Jakarta: Erlangga

Pelczar, MJ, and ECS, Chan. 1998. Fundamentals of Microbiology. Volume 1. UI Press. Jakarta.

Pudjiastuti, 1996. Review of Medicinal Plant Research Results at Various Institutes.

Volume 111. Ministry of Health. Jakarta..

Rachmat, M., Hartati, MS, and Wahyuono, S., 2000. Antibacterial activity and mouthwash preparations containing essential oils of betel leaves (Piper betle Linn.) and analysis of their essential oil composition. Indonesian Pharmacy Magazine Vol.II, No.4, 2000 , 235-240.

Rodriguez-Tudela JL, Barchiesi, F., Bille, J., Chryssanthou, E., Cuence, M., Denning, D., Donelly, JP, Dupont, B., Fegeler, W., Moore, C., Richardson , M., Verwiej, PE 2002. Method for The Determination of Minimum Inhibitory Concentration (MIC) by Broth Dilution of Fermentative Yeasts. EUCAST. E. Dis 7.1

Sastroamidjojo S., 1997. Original Indonesian Medicines. People's Diane. Jakarta

Setiabudi, R., and Gan, VHS 1995. Pharmacology and Therapy, 4th Edition. FKUI Pharmacology section. Jakarta. 571-583

Skocibusic, M., Bezic, N., Dunkic, A. 2006. Phytochemicals composition and antimicrobial activities of essential oils from satureja subspicata vis growing in Croatia. Food Chem 96: 20 – 28.

Sudarsono., Pudjoarinto A., Gunawan D., Wahyuono S., Donatus IA., Dradjad., Wibowo S., and Ngatidjan. 1996. Medicinal Plants. PPOT UGM. Yogyakarta.

Suliantari. 2009. Antibacterial activity and mechanism of inhibition of green betel extract (Piper betle Linn) against food pathogenic bacteria. Dissertation. IPB

Sulianti, SB., and Chairul. 2002. Comparison of the chemical components of the essential oil of wild betel (Piper ornatum) originating from South Sulawesi and the island of Seram with common betel (Piper betle). Biology News Vol:3, No.3, 2002.

Sumarnie., Priyono., Praptiwi. Identification of chemical compounds and antibacterial activity of extract of piper sp. Papuan origin. Field of Botany. Center for Biology-LIPI CSC Cibinong Jabar. Journal of environmental technology Vol : 10, No.3, 2009 : 271 – 276

Suppakul, P., Sanla-Ead, N., Phoopuritham, P. 2006. Antimicrobial and Antioxidant Activities of Betel Oil. Kasetsart J. (Nat. Sci) 40 (Suppl. 2006: 91-100.

Syahrurachman, Agus, et al. 1994. Textbook of Medical Microbiology Revised Edition. Script Formation. Jakarta.

Taguri, T., Tanaka, T., and Kounol. 2006. Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. Biology pharmacy bull 29 (11) : 2226 – 2235

Tengah, IGP, 2005, Study of: Inventory, Determination, and Methods of Using Medicinal Plants in "Lontar Usada" in Bali, Indonesian Ministry of Health. Jakarta.

Warsa, UC, Karsinah., Lucky HM, Suharto., and Aidilfiet C. 1993. Textbook of Medical Microbiology. Jakarta. 18-22, 47-48, 103-112.

Y, Jamal., and Agusta, A. 2001. Chemical components of essential oils of three Piperaceae. Technical reports, project inventory and characterization of biological resources.194-201