Antimicrobial Activity Comparison Profile of *Piper betle* L., *Pluchea indica* L. and *Citrus aurantifolia* [Christm.] Swingle against Bacterial Isolates from Human Axillary Sweat with Odor Problem

***Nur Miftahurrohmah and Syarmalina**

Microbiology Laboratory, Faculty of Pharmacy, Pancasila University
Srengseng Sawah, Jagakarsa, South Jakarta, Indonesia, 12640

Abstract

The research to compare the antimicrobial activity profile of *Piper betle* L. leaf, *Pluchea indica* L. leaf, and *Citrus aurantifolia* [Christm.] Swingle fruit against bacteria isolated from axillary sweat of six probandus having axillary odor problem had been done. Eight pure bacterial isolates came from this process, and then identified with Gram stain and several biochemical reactions. *Staphylooccus aureus, S. epidermidis,* and *S. haemolyticus,* known as bacteria that usually present in a large amount at human axilla skin with odor problem, were used also in this research. The tested plants were prepared with procedures that usually used by the Indonesian people as traditional home medicine to face the axillary odor problem. Furthermore, fucidic acid and potassium alumunium phosphate were used as reference drugs. Antimicrobial activity of all of the plants and the reference drugs against 11 bacteria were studied using agar diffusion method. The results showed that liquid extracted from *Citrus aurantifolia* [Christm.] Swingle. fructus had the highest antimicrobial activity compared to the two other plants against bacteria isolated from axillary sweat with odor problem, which was equal with the antimicrobial activity of potassium alumunium phosphate 20% w/v solution. Antimicrobial activity of betel leaf and *beluntas* leaf were equal with the antimicrobial activity of 19.06 µg/mL of fusidic acid solution.

Keywords: betel leaf, *Piper betle* L., *beluntas* leaf, *Pluchea indica* L., lime fruit, *Citrus aurantifolia* [Christm.] Swingle, antimicrobial, axillary odor.

Abstrak

Telah dilakukan penelitian untuk membandingkan aktivitas antimikroba dari daun sirih (*Piper betle* L.), daun beluntas (*Pluchea indica* L.), dan buah jeruk nipis (*Citrus arantifolia* [Christm.] Swingle) terhadap bakteri yang diisolasi dari keringat ketiak enam sukarelawan yang memiliki masalah bau ketiak. Delapan isolat bakteri murni didapat dari proses ini dan kemudian diidentifikasi dengan pewarnaan Gram dan uji-uji biokimia. Bakteri *Staphylooccus aureus, S. epidermidis,* dan *S. haemolyticus* yang umumnya terdapat dalam jumlah banyak di kulit ketiak yang berbau juga digunakan dalam penelitian ini. Tanaman-tanaman uji disiapkan dengan cara yang biasa dilakukan oleh masyarakat Indonesia untuk mengatasi bau badan secara tradisional. Sebagai senyawa antimikroba standar, digunakan asam fusidat dan tawas (kalium aluminium fosfat) untuk dibandingkan aktivitasnya dengan tiga tanaman uji. Aktivitas antimikroba seluruh tanaman uji dan larutan senyawa antimikroba terhadap 11 bakteri diteliti dengan menggunakan metode difusi agar. Hasil uji menunjukkan bahwa air perasan jeruk nipis memiliki aktivitas antimikroba terbesar dibandingkan dengan dua tanaman uji lainnya terhadap bakteri yang diisolasi dari keringat ketiak yang berbau, yang sebanding dengan aktivitas antimikroba larutan tawas 20% b/v. Aktivitas antimikroba daun sirih dan daun beluntas sebanding dengan aktivitas antimikroba larutan asam fusidat 19,06 µg/mL.

Kata kunci: sirih, *Piper betle* L., beluntas, *Pluchea indica* L., jeruk nipis, *Citrus aurantifolia* [Christm.] Swingle, antimikroba, bau ketiak.

Introduction

Sweating is a normal physiologycal process in our body to regulate its temperature. Excessive sweating occurs when we exposed to the hot temperature, during heavy exercise or in the stress and anxiety condition. Sweat is an odorless fluid secreted by sweat/sudoriferous gland that distributed in our skin. There are two types of sweat gland: eccrine gland and apocrine gland. The eccrine glands distributed over most of our body and are responsible for the watery secretions responsible for regulating the body's temperature. These glands perform the important functions of thermoregulation and excretion. The

*Corresponding author. e-mail: fz28306@yahoo.com

eccrine glands are active since birth, whereas the apocrine glands become active after puberty. The apocrine glands develop in areas abundant in hair follicles, such as on scalp, armpits/axilla and groin (Tortora and Nicholas 1990).

Based on the pioneering work of Shelley *et al.* (1953), the typical strong axilla odor can only be released from apocrine secretions. The action of skin bacteria is needed to generate the odorifeous compounds from non-smelling molecules present in the sweat secreted (Shelley *et al.* 1953). In axilla skin, there are a dense population of bacteria that dominated by *Staphylococcus sp.* and *Corynebacterium sp.* (Natsch *et al.* 2003; Bradley 2004).

Axilla bad odor have become a social problem because the people who has this case would feel uncomfortable and also another people surround him/her. Most people try to counter the bad odor problem by using products such as deodorants, antiperspirants, or by using traditional home medicine. Some traditional home medicine that usually used by Indonesian people to overcome bad odor problem are lime fruit (*Citrus aurantiifolia* [Cristhm.] Swingle), betel leaf (*Piper betle* L.) and *beluntas* leaf (*Pluchea indica* L.) (Mursito 2000; Dalimartha 2006).

This reseach is aimed to compare the antimicrobial activity of lime fruit (*Citrus aurantiifolia* [Cristhm.] Swingle), betel leaf (*Piper betle* L.) and *beluntas* leaf (*Pluchea indica* L.) against axilla bacterial isolates, *Staphylococcus aureus, S. epidermidis* and *S. haemolyticus* which those of three are general normal flora found at human axilla skin and more abundant in people with axillary odor problem.

Experiment

Materials

Materials used in this research were Nutrient Agar (Oxoid), blood agar base, peptone broth, lactose broth, media and reagents for IMVIC test (Indol media, MRVP media, Simmon's citrate agar, Kovac's reagent, methyl red, Barrit's reagent), TSIA (triple sugar iron agar), LIA (lysine iron agar), carbohydrate fermentation media (glucose, lactose, maltose, saccharose, mannitol), and Gram staining reagents (Gentian violet, lugol iodine, carbol fuchsin, ethanol). Axilla sweats sample were taken from 6 male hard worker probandus with odor problems, using swab method. Reference microorganisms (*Staphylococcus aureus, S. epidermidis and S. haemolyticus*) were bought from Microbiology Laboratory, Faculty of Medicine, University of Indonesia, Jakarta. Reference antimicrobial agents were potassium aluminium phosphate pro analysis and fucidic acid (Fucithalmic®, contains 10 mg fucidic acid per 5 g ointment). Plants used are *Piper betle* L. leaf, *Pluchea indica* L. leaf and *Citrus aurantifolia* [Christm.] Swingle fructus. Laboratory equipments used in this research are common glass laboratory equipments, paper disc 6 mm in diameter, laminar air flow cabinet (Gelman Sciences Clean Bench), autoclave (New clave HL36Ae), incubator and oven (Memmert), waterbath (Memmert), vortex (Thermolyne Maximix Plus), light microscope (Olympus CX-21) and spectrophotometer UV-vis (Shimadzu UV 1601).

Methods

Bacterial isolation from axilla sweat

Axilla sweat sample were taken from 6 male hard worker probandus with odor problems, using sterile cotton swab that swabbed to each left and right axilla skin. The cotton swabs were then inoculated into peptone broth media and incubated for 48 h at 37°C. These bacterial cultures were streaked on Nutrient agar plates to get pure cultures. From this process, 8 bacterial pure cultures were obtained.

Bacterial identification

All of the bacterial pure cultures were identified using Gram staining and several biochemical tests including IMVIC test, carbohydrate fermentation tests and H_2S production test using TSIA and LIA media. Reference microorganisms (*S. aureus, S. epidermidis and S. haemolyticus*) were also identified using the same methods (Cappucino and Natalie 1983).

Preparation of test solutions from plants materials

Test solutions from each of the plants materials were prepared as traditionally used by Indonesian people to face odor problem. U_1 test solution was made from liquid extract of *jeruk nipis* (lime) fruit. U_2 was made from 10 mL of U_1 mixed with 2 mL of 10% w/v *kapur sirih* (whiting). U_3 test solution was made from 10 g of *sirih* (betel) leaves that crushed together with 10 mL of water and then filtered. U_4 test solution was made from 10 mL of U_3 mixed with 2 mL of 10% w/v whiting. U_5 test solution was made from *beluntas*

leaves that crushed together with 10 mL of water and then filtered. U_6 test solution was made from 10 mL of U_5 mixed with 2 mL of 10% w/v whiting (Mursito 2000; Dalimartha 2006).

Preparation of reference drug solutions

Potassium aluminium phosphate, a compound that usually used as antiperspirant agent and contained in many deodorant antiperspirant product was used as reference drug solution in 20% w/v concentration. Another reference drug is fusidic acid, a bacteriostatic agent. Fusidic acid concentration that used in this research was 19.06 □g/mL based on the result of bacterial (*S. aureus, S. epidermidis* and *S. haemolyticus*) susceptibility testing to this antibiotic.

Antimicrobial activity test of the plants and the reference drug solutions

To analyze the antimicrobial activity of the three plants and the two reference drug solutions, the agar diffusion method were used. The paper discs (6 mm in diameter) were saturated with each solutions and inoculated onto agar media in Petri dishes containing bacterial isolates to be tested (8 axilla sweat isolates, *S. aureus, S. epidermidis, S. haemolyticus*). All of the Petri dishes were then incubated at 37°C for 24 h. The inhibition zone formed around each of the paper discs were observed and recorded after the incubation period. All of the tests were done in triplicates

Results and Discussions

Human axilla is a skin region that supports a dense bacterial population, which is dominated by two genera: *Staphylococcus* and *Corynebacteria* (Bradley 2004). A strong correlation was found between a high population of *Corynebacteria* and a strong axillary odor formation (Leyden *et al. 1981;* Shehadeh and Kligman 1963). Based on the research done by Starkenmann *et al.* (2005), *S. Haemolyticus* is the most important bacteria in the production of strongest odoriferous volatile sulfur compound from its precursor, (s) – 3 – methyl – 3 – sulphanylhexane – 1 -ol (Stakenmann *et al.* 2005).

In this research, axilla sweat sample were taken from 6 male hard worker probandus with axillary odor problems, using sterile cotton swab that swabbed to each left and right their axilla skin. Eight pure isolates came from this process, they were 3KiLB2, 3KaLB1, 3KaLB2, 4KiLB1, 4KiLB2, 4KaLB1, 4KaLB2, 5KiLB1 and 6KiLB2. Each of the isolates were then identified by Gram staining and several biochemical tests. The results of Gram staining and biochemical tests of the isolates were shown in table 1 and 2.

Table 1 showed that all of 8 axilla bacterial isolates were Gram positive bacteria and bacilli in form except $3KaLB_2$ that has cocci shape. This finding suggested that the isolates were maybe member of *Staphylococcus* or *Corynebacterium* genera.

Table 1. Gram Staining Results and Cell Morphology of 8 Axilla Bacterial Isolates, *S. aureus, S. epidermidis* and *S. haemolyticus*

Bacterial isolates	Gram	Cell morphology
$3KiLB_2$	+	bacilli
$3KaLB_2$	+	cocci
$4KiLB_1$	+	bacilli
$4KiLB_2$	+	bacilli
$4KaLB_1$	+	bacilli
$4KaLB_2$	+	bacilli
$5KiLB_1$	+	bacilli
$6KiLB_2$	+	bacilli
S. aureus	+	cocci

Based on Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994), member of *Staphylococcus* genera are Gram positive cocci while *Corynebacterium* are Gram positive bacilli. Several biochemical tests result, as seen at table 2, showed that majority of the isolates were maybe belong to *C. striatum* due to its activity in producing acid from glucose, maltose and lactose, and also positive in methyl red test. *C. striatum* is one of bacteria that also contributed in producing volatile sulfur compound (Stakenmann *et al.* 2005; Holt *et al.* 1994). However, it must be studied further with more methods i.e. molecular method to confirm that the isolates were member of *Staphylococcus* or *Corynebacterium* genera.

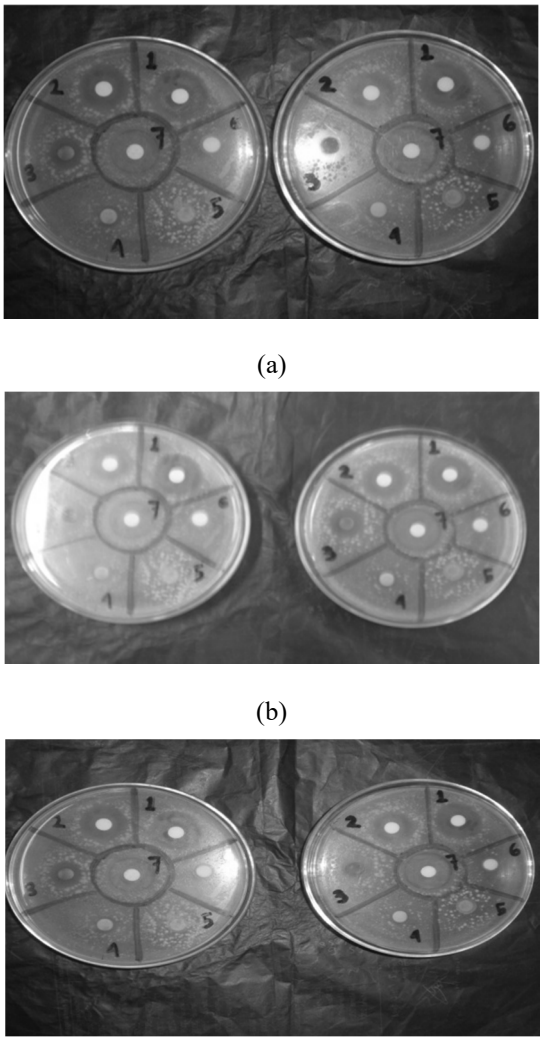
All of the isolates and three bacteria that usually found in axilla skin (*S. aureus, S. epidermidis and S. haemolyticus*) were then subjected to antimicrobial activity test using the three plants solutions and the

two reference drug solutions. The antimicrobial activity test results of all of the solutions were resumed in table 3 and figure 1.

To show H_2S production, the positive result showed by bubble formation in inverted Durham tube; IMVIC = Indole, Methyl red, Voges Proskauer and Simmon's Citrate test; In = Indole test, positive result showed by red coloration at the medium surface after the addition of Kovac's reagent due to the formation of indole; MR = Methyl red test, positive result showed by medium color change from yellow to red after the addition of methyl red indicator due to the formation of acid product; VP = Voges Proskauer test, positive result showed by the formation of red color after the addition of Barrit's reagent due to the formation of neutral product, SC = Simmon's citrate test, positive result showed by the medium color change from green to blue due to the use of citrate as carbon source by the bacteria; H_2S prod. = production of H_2S, positive result showed by black sedimentary of FeS in the stab area in TSIA and LIA medium due to the complex formation between H_2S gas produced by the bacteria and ferrosulfide that contains in the medium.

Diameter of paper disc: 6 mm; *n* = 3; $3KiLB_2$, $3KaLB_1$, $3KaLB_2$, $4KiLB_1$, $4KiLB_2$, $4KaLB_1$, $4KaLB_2$, $5KiLB_1$ and $6KiLB_2$ = codes for 8 axilla bacterial isolates; U_1: liquid extract of lime fruit; U_2: 10 mL of U_1 mixed with 2 mL of 10% w/v *kapur sirih* (whiting); U_3: 10 g of betel leaves, crushed with 10 mL of water and then filtered; U_4: 10 mL of U_3 mixed with 2 mL of 10% w/v whiting; U_5: 10 g of beluntas leaves crushed with 10 mL of water and then filtered; U_6: 10 mL of U_5 mixed with 2 mL of 10% w/v whiting; U_7: 20% w/v potassium aluminium phosphate; U_8: 19.06 µg/mL fusidic acid.

The use of some aromatic plants as traditional home medicine to face body odour problem could be a simple and good alternative solution beside the use of commercial/chemical deodorant - antiperspirant products. It is cheaper, easier and more safety than the use of chemical ingredients contained in such products. In Philipa Darbe, 2004, stated that paraben compound that usually contained in deodorant-antiperspirant product, could cause irritation on axilla skin in a long term application and may induce mamae cancer after a long period of time. However, these hypotheses still remain on debating among researchers (Kompas Cyber Media 2005)



(a)

(b)

(c)

Figure 1. The results of antimicrobial activity test of 7 test solutions against *S. haemolyticus* (a), *S. aureus* (b) and *S. epidermidis* (c).

Some plants that usually used as traditional home medicine by Indonesian people to overcome bad odor problem are lime fruit (*Citrus aurantiifolia* [Cristhm.] Swingle), betel leaf (*Piper betle* L.) and *beluntas* leaf (*Pluchea indica* L.) (Mursito 2000; Dalimartha 2006). *Sirih*/betel leaf has been used for a long time as antiseptic. It is also known contains active substances that can eliminate body odor. Betel known potent to eliminate body odor caused by mainly bacteria or fungi. Betel leaf contains until 4% of volatile oil (hydroxi cavicol, cavicol, cavibetol).

Beluntas leaves contain alkali which acts as an antiseptic and chemical content such as amino acids (leucine, isoleucine, tryptophan, threonine), fat,

calcium, phosphorus, iron, Vitamin A and C. To eliminate body odor, *beluntas* leaves commonly engulfed in raw or steamed first.

Lime fruit contains volatile oils such as limonen, citral, phelandren, thymol and linalool. It also contains flavonoids (poncirin, rhoipholyne, naringin), glycosi-des (hesperydine, isohesperidyne, aurantiamarine), citric acid, tryptophane, lysine, calsium, phosphorus, iron and vitamin (A, B_1, C). The use of lime fruit to overcome body odor problem usually mixed with a little amount of whiting then rubbed at armpit area (Mursito 2000; Dalimartha 2006).

According to the result of antimicrobial activity test as seen in Table 3, lime fruit showed the highest anti-microbial activity then the two other plants. This result maybe correlated with the high amount of volatile oil contained in the liquid extracted from the fruit.

The test solutions preparation procedure of all of the plants may also play role in the differences of antimicrobial activity. Betel leaf and *beluntas* leaf were mixed with a little amount of water to be used as the test solutions while the lime fruit was extracted directly without adding water. However, the result showed that all of the three plants were have antimicrobial activity against the 8 axilla bacterial isolates, *S. aureus, S. epidermidis and S. haemolyticus*.

Table 2. Biochemical characteristics of 8 bacterial isolates, *S. aureus, S. epidermidis* and *S. haemolyticus*

Bacterial isolates	Carbohydrate Fermentation										IMVIC Test				H_2S Prod.
	Glucose		Lactose		Maltose		Saccharose		Mannitol						
	A	G	A	G	A	G	A	G	A	G	In	MR	VP	SC	
$3KiLB_2$	+	-	+	-	+	-	+	-	+	-	-	+	-	-	-
$3KaLB_2$	+	-	+	-	+	+/-	+	+/-	+	+	-	+	-	-	-
$4KiLB_1$	+	+	+	+/-	+	+	+	+	+	+/-	-	+	-	-	-
$4KiLB_2$	+	-	+	-	+	+/-	+	+/-	+	-	-	+	-	-	-
$4KaLB_1$	+	+/-	+	-	+	+/-	+	+	+	-	-	+	-	-	-
$4KaLB_2$	+	+	+	+	+	+/-	+	+	+	+	-	+	-	-	-
$5KiLB_1$	+	+	+	+	+	+/-	+	+	+	+	-	+	-	-	-
$6KiLB_2$	+	-	+	-	+	-	+	-	+	-	-	+	-	-	-
S. aureus	+	-	+	-	+	-	+	-	+	-	-	+	-	-	-
S. epidermidis	+	-	+	-	+	-	+	-	+	-	-	+	-	-	-
S. haemolyticus	+	-	+	-	+	-	+	-	+	-	-	-	-	-	-

Table 3. Antimicrobial activity of test solutions against 8 axilla bacterial isolates, *S. aureus, S. epidermidis* and *S. haemolyticus* (all done in triplicates)

Bacterial isolates	Inhibition zone (mm)							
	U_1	U_2	U_3	U_4	U_5	U_6	U_7	U_8
$3KiLB_2$	21.33±0.6	19.0±1.0	10.33±0.6	8.0±0.0	9.67±0.6	7.67±0.6	21.67±0.6	10.0±0.0
$3KaLB_2$	21.67±0.6	19.33±0.6	9.67±0.6	7.33±0.6	9.67±0.6	7.33±0.6	21.67±0.6	10.33±0.6
$4KiLB_1$	21.33±0.6	19.33±0.6	10.67±0.6	8.0±0.0	10.33±0.6	7.33±0.0	22.0±0.0	10.33±0.6
$4KiLB_2$	22.0±0.0	19.67±0.6	10.0±0.0	7.67±0.6	9.67±0.6	7.67±0.6	22.0±0.0	10.0±0.0
$4KaLB_1$	21.67±0.6	19.33±0.6	9.67±0.0	7.67±0.6	9.67±0.6	7.67±0.6	21.67±0.6	10.33±0.0
$4KaLB_2$	21.0±1.0	19.0±1.0	10.33±0.6	7.33±0.6	10.0±0.0	7.67±0.6	22.0±0.0	10.33±0.6
$5KiLB_1$	20.67±0.6	19.0±0.0	10.0±0.0	7.33±0.6	9.67±0.6	7.33±0.6	21.33±0.6	9.67±0.6
$6KiLB_2$	21.0±0.0	19.67±0.6	9.67±0.6	7.0±0.0	9.67±0.6	7.0±0.0	21.67±0.6	9.67±0.6
S. aureus	21.33±0.6	19.67±0.6	10.67±0.6	7.67±0.6	10.67±0.6	7.33±0.6	22.0±0.0	10.67±0.6
S. epidermidis	21.67±0.6	19.33±0.6	10.0±0.0	7.67±0.6	10.0±0.0	7.67±0.6	21.67±0.6	10.0±0.0
S. haemolyticus	21.67±0.6	19.33±0.6	9.67±0.0	7.67±0.6	9.67±0.0	7.67±0.6	21.67±0.6	9.67±0.0

Diameter of paper disc: 6 mm; 1 = U_1: liquid extract of lime fruit; 2 = U_2: 10 mL of U_1 mixed with 2 mL of 10% w/v *kapur sirih* (whiting); 3= U_3: 10 g of betel leaves, crushed with 10 mL of water and then filtered; 4 = U_4: 10 mL of U_3 mixed with 2 mL of 10% w/v whiting; 5 = U_5: 10 g of beluntas leaves crushed with 10 mL of water and then filtered; 6 = U_6: 10 mL of U_5 mixed with 2 mL of 10% w/v whiting; 7 = U_7: 20% w/v potassium aluminium phosphate. $3KiLB_2$, $3KaLB_1$, $3KaLB_2$, $4KiLB_1$, $4KiLB_2$, $4KaLB_1$, $4KaLB_2$, $5KiLB_1$ and $6KiLB_2$ = codes for 8 axilla bacterial isolates; A = acid production, positive result showed by medium color change from red to yellow; G = gas

As the reference drugs, *tawas* (potassium aluminium phosphate) and fusidic acid were used. Potassium aluminium phosphate is a common ingredient in commercial deodorant-antiperspirant products with the average concentration between 17-20%. In this research, 20% w/v of potassium aluminium phosphate was used as reference. Fusidic acid is a bacteriostatic agent that can inhibit primarily Gram positive bacteria such as from *Corynebacterium* and *Staphylococcus* sp. In this study, 19.06 µg/mL of fusidic acid was used as another reference drugs. The fusidic acid concentration was choose based on the bacterial suceptibility test against *S. aureus, S. epidermidis and S. hemolyticus.* The results showed that the antimicrobial activity of the liquid extracted from lime fruit was equal with the antimicrobial activity of potassium aluminium phosphate 20% w/v solution, whereas the antimicrobial activity of betel leaf and *beluntas* leaf were equal with the antimicrobial activity of 19.06 µg/mL of fusidic acid solution.

Conclusion

From this research, it can be concluded that the liquid extracted from lime fruit (*Citrus aurantifolia* [Christm.] Swingle) had the highest antimicrobial activity compared to the two other plants (betel leaf / *Piper* betle L. and *beluntas* leaf / *Pluchea indica* L.) against 8 bacteria isolated from axillary sweat with odor problem, *Staphylococcus aureus, S. epidermidis* and *S. haemolyticus*. This activity was equal with the antimicrobial activity of potassium alumunium phosphate 20% w/v solution, while the antimicrobial activity of betel leaf and *beluntas* leaf were equal with the antimicrobial activity of 19.06 µg/mL of fusidic acid solution.

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References

Bradley D, 2004, Picking up the Sweat Scent, Chem. Biodiv., 1, 1022-1035, 1058-1072.

Cappucino JG, Natalie S, 1983, Microbiology: A Laboratory Manual, Addison Wesley Publishing Company, Canada, 19-47.

Dalimartha S, 2006, Atlas Tumbuhan Obat Indonesia, Trubus Agriwidya, Jakarta.

Holt JG, Krieg NR, Sneath PHA, Staley JT, Williams ST, 1994, Bergey's Manual of Determinative Bacteriology, 9th ed., William and Wilkins, Maryland.

Kompas Cyber Media, 2005, Kisah Antara Ketiak, Deodoran dan Kanker, diambil dari http://www.depkes.go.id, diakses tanggal 24 Maret 2007.

Leyden JJ, Kenneth JM, Erhard H, John NL, Albert MK, 1981, The Microbiology of the Human Axilla and Its Relationship to Axillary Odor, Journal of Investigative Bacteriology, 77, 413-416.

Mursito B, 2000, Tampil Percaya Diri dengan Ramuan Tradisional, Penebar Swadaya, Depok.

Natsch AH, Gfeller P, Gygax J, Schmid, Acuna G, 2003, A Spesific Bacterial Aminoacylase Cleaves Odorant Precursors Secreted in the Human Axilla, J. Biol. Chem. 278(8): 5718-5727.

Shehadeh NH, Kligman AM, 1963, The Effect of Topical Antibacterial Agents is the Bacterial Flora of the Axilla. J. Invest. Dermatol. 40: 61.

Shelley WB, Hurley HJ, Nicholas AC, 1953, Axillary Odor: Experimental Study of the Role of Bacteria Apocrine Sweat and Deodorants, Arch. Dermatol. Syph. 68: 430.

Starkenmann C, Niclass Y, Trocazz M, Clark AJ, 2005, Identification of The Precursors of (S)-3-methyl-3-sulfanylhexan-1-ol, The Sulfury Malodour of Human Axilla Sweat, Chem. Biodivers. 2(6), 705-716.

Tortora GJ, Nicholas PA, 1990, Principles of Anatomy and Physiology. Harper and Row Publishers., New York, 1990.