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Identification of active compounds and anti-acne activity from extracts and fractions of surian (*Toona sinensis*) leaves planted in Sumedang, West Java, Indonesia

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Abstract. *Febriyani E, Falah S, Andrianto D, Lastini T. 2018. Identification of active compounds and anti-acne activity from extracts and fractions of surian (*Toona sinensis*) leaves planted in Sumedang, West Java, Indonesia. Biodiversitas 19: 1406-1412.* Surian or toon (*Toona sinensis* Merr) leave is one of the herbs containing many bioactive compounds as potential sources of anti-acne agents. The aim of this work is to identify and evaluate the anti-acne activity of Surian leaves extract and fractions against acne bacteria. Crude extracts of Surian leaves from various solvents (methanol, ethanol, and ethyl acetate) were screened for their antimicrobial activity using disc diffusion. The results showed that ethyl acetate extract demonstrated the highest inhibition against *Propionibacterium acne* (49.93%), *Staphylococcus epidermidis* (57.14%), *Staphylococcus aureus* (18.93%), and *Eschericia coli* (82.03%). The results of Brine Shrimp Lethality Test (BSLT) showed that the methanol extract had the highest cytotoxicity of LC_{50} at 29.76 µg/mL. The extract was then fractionated using column chromatography, resulted in 6 fractions. The fraction 3 showed the best antibacterial effect against *P. acne* (28.27%), *S. epidermidis* (7.38%) and *S. aureus* (10.38%). Minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) test were carried out using liquid dilution, whereas dimethyl sulfoxide (DMSO) and Clindamycin were used as a negative and positive control, respectively. The MIC and MBC test assays of fraction 3 varied for different bacteria *P. acne* bacteria (5000 and 5000 ppm), *S. aureus* (1250 and 10000 ppm), *E. coli* (2500 and 5000 ppm), while for *S. epidermidis* the fraction 3 was able to inhibit with MIC value of 5000 ppm. Based on microscopic observation using Scanning Electron Microscope (SEM), the fraction 3 at the dose of 5000 ppm could promote the cellular destruction of *P. acne*. Further experiment using Liquid Chromatography-Tendem Mass Spectrometry (LC-MS/MS) showed various compounds associated with the anti-acne activity of the fraction 3, such as pheophorbide a, pheophytin a and dihomo-γ-linolenic acid.

Keywords: Antibacterial, ethyl acetate extract, *Propionibacterium acne*, surian leaves

INTRODUCTION

Pimple (*acne vulgaris*) is regarded as the commonest skin disorder and constitutes a chronic inflammatory disease in pilosebaseus. It is caused by several factors such as excessive sebum production, keratolytic, as well as bacterial activity from *P. acne*, *S. aureus*, *S. epidermidis*, and *E. coli*. Without proper treatment, pimple could cause infection that promotes painful ulcer and suppuration. The use of chemicals such as Clindamycin antibiotic, benzoyl peroxide, and sulphur soap is commonly used for pimple treatment. However, topical and oral antibiotic for acne may cause resistance, while the use of chemical treatment also induces irritation, pruritus, and exfoliation. Therefore, acne treatments using natural agents from medicinal plants are considered to have beneficial impacts to health in term of safety and harmful side effects (DGPMD MoH 2006).

Health treatments using medicinal plants have been implemented for thousand years ago, from generation to generation across the globe. The numerous studies on medicinal plants have offered a great contribution to the development of traditional medicine. Like other countries such as China, Korea, and India, Indonesia is reported to be one of the greatest medicinal plant users due to its high biodiversity. The Indonesian tropical forest is rich in various medicinal plants. Surian (*Toona sinensis* Merr) is one of the most interesting plants for traditional medicine (MoH 2016).

Surian is classified as a member of Meliaceae and widely studied for its antioxidant, anticancer, pesticide, antifungal, and antibacterial properties. The surian is considered in our experiment since most parts of the plant (seed, bark, root, stalk, and leaf) are used for traditional medicine in some countries (Shu et al. 2008). Various studies on Surian plants have been developed for both industrial and pharmaceutical sectors. Suhatri et al. (2014) found that ethyl acetate fraction of Surian leaves at a dose of 50 mg/kg body weight showed a desirable protective effect on aortic endothelial cells of male quail for atherosclerosis disease. The ethanol extract of Surian leaves contained alkaloid, flavonoid, polyphenol, and terpenoid that could show antioxidant activity at IC_{50} of 4.8 ppm (Yuhernita and Juniarti 2011). Falah et al. (2015) reported that phytochemical compounds in Surian leaves extracts included alkaloid, triterpenoid, flavonoid, tannin, phenol, and steroid. The minimum inhibitory concentration

of methyl gallate isolated from Surian leaves against *E. coli* and *S. aureus* was 7.5 mg/mL (Ekaprasada et al. 2015).

The objective of this study was: (i) To evaluate the cytotoxicity of extracts and natural compounds using BSLT; (ii) To evaluate anti-acne activity of Surian leaves extracts and fractions against acne bacteria (*P. acne*, *S. epidermidis*, *S. aureus*, and *E. coli*), (iii) To observed the bacteria cellular destruction microscopically using SEM; (iv) To identify active compounds associated with anti-acne activity using LC-MS/MS.

MATERIALS AND METHODS

Extraction of surian leaves

Leaves of Surian plant were used in this study. The leaves were taken from plants (age >30 years) in Rancakalong, Regency of Sumedang, West Java, Indonesia. Surian leaves were washed using water and dried using the solar heat. The dried samples then were crushed using a blender and sieved (60 mesh) to obtain a leaf powder. Various solvents (ethanol, methanol, and ethyl acetate) were used to extract the leaf powder. The leaf powder was macerated using the solvent (1: 10 w/v) and shaken at 150 rpm for 24 h. The filtrate was then evaporated using rotary vacuum evaporator at 50 °C and 50 rpm.

Brine Shrimp Lethality Test (BSLT)

The cysts of *Artemia salina* were hatched in seawater (100 mL) for 48 h. The larvae (10 individuals) was transferred into a test tube and added with 1 mL sample solution with different concentrations (5–1000 ppm) at triplicates. After 24 h of incubation, the dead and living larvae were counted and analyzed for determination of LC_{50} using Probit analysis at a confidence level of 95%.

Fractionation using Column Chromatography

Twenty grams of silica gel G_{60} (230 mesh) (Merck) was transferred into the column and followed by constant agitation to remove bubbles. The mobile phase was a mixture of n-hexane : ethyl acetate (ratio from 50: 0 (v/v) to 0: 50 (v/v)), while static phase was silica gel. The Surian leaves extract (1 g) was injected into the column. The eluent was collected in 10-ml vial tube and followed by Thin Layer Chromatography (TLC) analysis. Fraction spots with similar Rf were collected and evaporated using rotary vacuum evaporator.

Determination of antibacterial activity using disc diffusion

The bacterial isolates were cultured in the sterilized medium. All glassware and medium are sterilized using an autoclave (Tomy Model Es 314; 121°C; 1 atm). The *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 bacteria were cultured in Nutrient Broth (NB) Sigma medium for 24 h, the *P.* acne ATCC 11827 was in Brain Heart Iinfusion (BHI) Broth Sigma medium for 72 h under anaerobic condition, and *S. epidermidis* (local bacterial obtain from Laboratory of Microbiology, Bogor Agricultural University) was in BHI medium for 24 h in aerobic condition. The Nutrient Agar (NA) Sigma and BHI Agar (15 mL, 45 ºC) were poured into a sterile petri dish. The bacterial suspension (20 µL) was dropped over the media and spread using spreader glass. Paper disc (previously exposed to 20 µL sample at a concentration of 10000 ppm were prepared by dissolving in dimethyl sulfoxide (DMSO 20%)) was planted in the media. DMSO 20% was used as the negative control while Clindamycin 20 ppm was used as the positive control. Antibacterial activity was determined by measuring a clear zone of inhibition around the paper disc.

Determination of MIC and MBC

The MIC and MBC determination followed a method of Batubara et al. (2009) and modification of Arung et al. (2017). Each well of a sterile 96-well plate contained sterile liquid medium (95 µL), sample (100 µL, serial concentration diluted in dimethyl sulfoxide DMSO 20%, with the final concentration ranging from 78.125 ppm to 10000 ppm) or control (100 µL) and 5 µL 10^{-2} CFU/mL of bacterial suspensions. The microtiter plate was incubated at 37ºC for 24 hours (for *E. coli*, *S. aureus*, and *S. epidermidis*) and *P. acne* was incubated for 72 hours under anaerobic condition. Extract concentration at which there was no bacterial growth was determined as MIC. The 10 µL of each medium with no detectable bacterial growth was inoculated in 100 µL fresh medium. The concentration at which there was no bacterial growth after the second inoculation was determined as the MBC. DMSO 20% was used as the negative control while Clindamycin was used as the positive control. This experiment was performed at triplicates.

Microscopic analysis using SEM

The best result of MIC experiment was further observed using Scanning Electron Microscope (SEM) Type JSM 5000 to evaluate destruction of bacterial cells. The bacterial cells were fixed in 2.5% glutaraldehyde on 0.1 M phosphate-buffered saline (PBS) for 4 hours. The samples were washed four times with PBS, followed by twice rinses with distilled water, then dehydrated in ethanol solutions of 60%, 70%, 80%, 90% and three times in 100%, for 10 min each. Afterward, the samples were dried in a freeze-dryer for a period of 48 h and subsequently warmed at room temperature overnight. The samples were coated using Pt and Au to protect sample during scanning.

Identification of bioactive compounds using LC-MS/MS

Sample solution (5µL) at concentration of a1000 µg/mL was injected to LC-MS/MS and run at 0.2 mL/min. The LC-MS/MS instrument used in this study was LC System Ultra Performance Liquid Chromatography and Mass Spectrophotometry Electrospray Ionization. The analysis was performed for 23 min at 50°C.

Data analysis

Determination of LC_{50} in BSLT used Probit analysis using Statistical Package for Social Science (SPSS) 21 at confidence level of 95%. The antibacterial activity for each

microorganism was analyzed using one-way analysis of variance (ANOVA). P value < 0.05 was considered as significant. Minitab 16.0 was employed for statistical analysis.

RESULTS AND DISCUSSION

Cytotoxicity test

The Probit analysis showed that methanol extract of Surian leaves demonstrated cytotoxicity at LC_{50} of 29.76±1.21 mg/mL, while LC_{50} of ethanol 96% and ethyl acetate extract was 45.41±13.96 mg/mL and 571.32±12.95 mg/mL, respectively. The lower LC_{50} indicates, the higher cytotoxicity meaning that the samples have higher bioactive compounds.

Antibacterial properties

Crude extracts of surian leaves

Ethyl acetate extract of Surian leaves demonstrated the highest antibacterial activity (Table 1) inhibiting the growth of *P.acne* (49.93±0.01%), *S.epidermidis* (57.14±0.10%), *S.aureus* (18.93±0.34%), and *E.coli* (82.03±0.00%). On the contrary, the lowest antibacterial activity was attributed to methanol inhibiting the growth of *P.acne* (20.99±0.00%) and *E.coli* (66.93±0.23%), while ethanol extract is the lowest antibacterial activity for *S.epidermidis* (37.30±0.12%) and *S.aureus* (10.29±0.30%). The crude extract of Surian leaves had a significant ($p<0.05$) effect to antibacterial activity.

Fraction of surian leaves

The strongest antibacterial activity was showed by the ethyl acetate extract of Surian leaves compared to methanol and ethanol extracts (Table 1). The extract was then fractionated using column chromatography with n-hexane: ethyl acetate as mobile phase and silica gel G_{60} Merck as a static phase. The separation technique was based on the distribution of components within the static phase and mobile phase due to the different physical properties of the component. The separation mechanism using column chromatography with n-hexane as the mobile phase, a polar Surian leaves active compounds will be attracted to the stationary phase (silica gel) by hydrogen bonding. As a result, polar compounds will move very slowly, while non-polar compounds will move quickly out of the column because it was bound by the phase of motion that was non-polar. Furthermore, the separation was followed by a more polar solvent to separate and push the polar compounds out of the column. After separation, 39 eluates were obtained and collected in the vial tubes. They were then analyzed using TLC and observed under UV light of 254 nm. The eluates were grouped according to retention time (Rf), resulting in 6 fractions (Table 2).

The highest antibacterial activity of Surian leaves fractions inhibiting the growth of *P. acne* was fraction 3 (28.27±0.02%), fraction 4 (10.33±0.01%) and fraction 5 (2.13±0.01%). Meanwhile, the strongest antibacterial activity against *S. epidermidis* was attributed to fraction 3 (2.70±0.01%), 5 (12.46±0.01%), and 6 (13.17±0.01%). Furthermore, *S. aureus* was strongly inhibited by fraction 2 (9.01±0.01%), 3 (10.38±0.01%) and 6 (7.61±0.00%), while no inhibition activity was found for *E. coli* (Table 2). The fractions had a significant ($p<0.05$) effect to the antibacterial activity of *P. acne*, *S. epidermidis*, and *S. aureus*. Next, fraction with the strongest antibacterial activity (fraction 3, 5, and 6) was tested for determination of MIC and MBC using liquid dilution method.

Tabel 1. Antibacterial activity of Surian leaves extracts based on disc diffusion experiment

Extract (10000 ppm)	Antibacterial activity (%)*			
	P. acne	*S. epidermidis*	*S. aureus*	*E. coli*
Methanol	20.99±0.00^{d}	41.59±0.10^{b}	10.86±0.16^{c}	66.93±0.23^{c}
Ethanol	30.00±0.00^{c}	37.30±0.12^{bc}	10.29±0.30^{c}	81.38±0.18^{b}
Ethyl acetate	49.93±0.01^{b}	57.14±0.10^{b}	18.93±0.34^{b}	82.03±0.00^{b}
Clindamycin(+)	100±0.00^{a}	100±1.39^{a}	100±0.66^{a}	100±0.00^{a}
DMSO 20% (-)	0.00±0.00^{e}	0.00±0.00^{c}	0.00±0.00^{d}	0.00±0.00^{d}

Note : *The values of antibacterial activity represented by mean ± standard error from 3 replications; Different superscript alphabetic letters were significantly different at ($p< 0.05$) by Tukey test

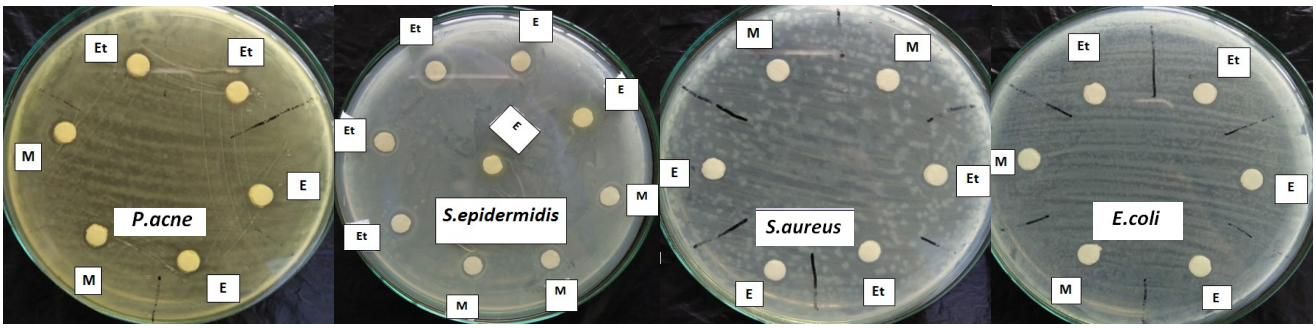

Figure 1. Antibacterial activity extracts on bacteria *P. acne*, *S. epidermidis*, *S. aureus*, and *E. coli*. Surian leaf extract; E: Ethanol; M: Methanol; Et: Ethyl acetate

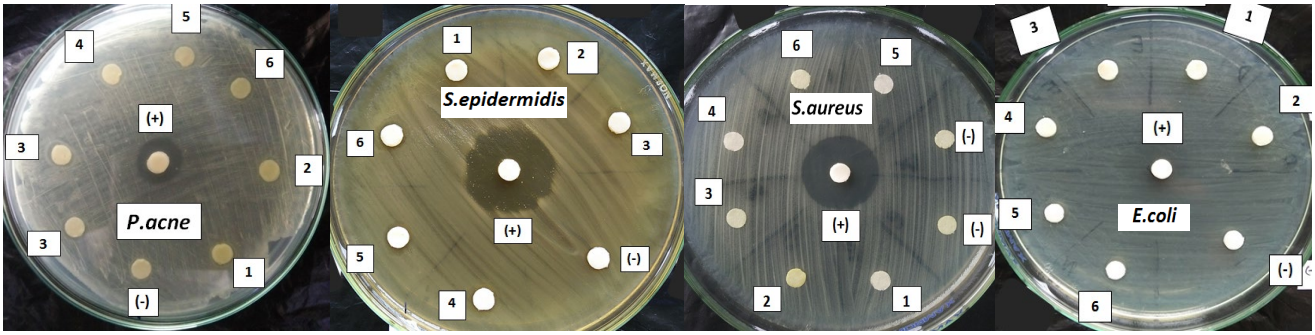

Figure 2. Antibacterial activity of Surian leaves fractions on bacteria *P. acne*, *S. epidermidis*, *S. aureus*, and *E. coli*. Number on petri dish shows the fractions, 1. Fraction 1; 2. Fraction 2; 3. Fraction 3; 4. Fraction 4; 5. Fraction 5; 6. Fraction 6.

Table 2. Antibacterial activity of 6 fractions obtained from column chromatography

Fraction (10000 ppm)	Antibacterial activity (%)*			
	P.acne	*S.epidermidis*	*S.aureus*	*E.coli*
1	0.00±0.00^{e}	0.00±0.00^{b}	0.00±0.00^{e}	0.00±0.00
2	0.00±0.00^{e}	0.00±0.00^{b}	9.01±0.01^{b}	0.00±0.00
3	28.27±0.02^{b}	7.38±0.01^{b}	10.38±0.01^{b}	0.00±0.00
4	10.33±0.01^{c}	2.70±0.01^{b}	4.32±0.00^{cd}	0.00±0.00
5	2.13±0.01^{d}	12.46±0.01^{b}	3.45±0.01^{cd}	0.00±0.00
6	0.00±0.00^{e}	13.17±0.01^{b}	7.61±0.00^{bc}	0.00±0.00
Clindamycin(+)	100±0.00^{a}	100±1.39^{a}	100±0.66^{a}	100±0.00
DMSO 20% (-)	0.00±0.00^{e}	0.00±0.00^{b}	0.00±0.00^{d}	0.00±0.00

Note : *The values of antibacterial activity represented by mean ± standard error from 3 replications; Different superscript alphabetic letters was significantly different at ($p < 0.05$) by Tukey test

Tabel 3. MIC and MBC of the selected fractions

Fraction	Tested bacteria							
	P. acne		*. epidermidis*		*S. aureus*		*E. coli*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
3	5000	5000	5000	-	1250	10000	2500	5000
5	1250	-	5000	-	625	2500	2500	-
6	1250	-	25000	-	625	-	625	-

Note : n= Triplicates

MIC and MBC of the fractionated extracts

Fraction 3, 5, and 6 of Surian leaves demonstrated the highest antibacterial activity. These fractions were then used for determination of MIC and MBC. As shown in Table 3, MIC of fraction 3 reached 5000 ppm for *P. acne* and *S. epidermidis*, 1250 ppm for *S. aureus*, and 2500 ppm for *E. coli*. In addition, MBC of fraction 3 reached 5000 ppm for *P. acne* and *E. coli* and reached 10000 ppm for *S. aureus*. Based on that result, fraction 3 was selected for further testing.

Microscopic observation

The effects of fraction 3 on the cellular morphology of *P. acne* were observed using SEM. The results demonstrated that the destruction of cell wall appeared as a consequence of the treatment. Changes in cell walls of lytic bacteria suggested that the cell degradation was caused by active compounds present in Surian leaves, which demonstrated the similar antibacterial effect compared to positive control (Figure 3).

Identification of the bioactive compound

Figure 2 exhibited the chromatogram of LCMS/MS experiment. There were 14 compounds identified in the fraction 3 according to the database in Chemspider (Table 4). We found that (2S,4S,5S,7S)-5-Amino-N-[2,2-dimethyl-3- (1H-1,2,3-triazol-1-yl) propyl]-4-hydroxy-2isopropyl-7-[4-methoxy-3- (3methoxypropoxy) benzyl]-8 methyl nonanamide was regarded as the most abundant compound. Furthermore, the chromatogram showed that 3 compounds might be responsible for antibacterial activity against acne bacteria, i.e., pheophorbide a, pheophytin a, and dihomo-γ-linolenic acid (Table 4).

Discussion

Cytotoxicity could be classified into 4 groups: non-cytotoxic (LC_{50} > 1000 ppm), low cytotoxicity (LC_{50} 500 > 1000 ppm), moderate cytotoxicity (LC_{50} 100 > 500 ppm), and high cytotoxicity (LC_{50} 0 < 100 ppm) (Mentor et al. 2014). Based on LC_{50}, the highest and the lowest cytotoxicity in this experiment was attributed to the methanol extract and ethyl acetate extract, respectively. Yuhernita and Juniarti (2011) found that methanol extract of Surian leaf contained alkaloid, flavonoid, polyphenol, and terpenoid. Falah et al. (2015) reported that phytochemical component of Surian leaves included alkaloid, triterpenoid, flavonoid, tannin, phenol, and steroid. In this study, BSLT experiment was performed to evaluate bioactivity of an extract, including anti microorganism. The difference in Surian leaves extracts cytoxicity may result from types of solvents and content of secondary metabolites.

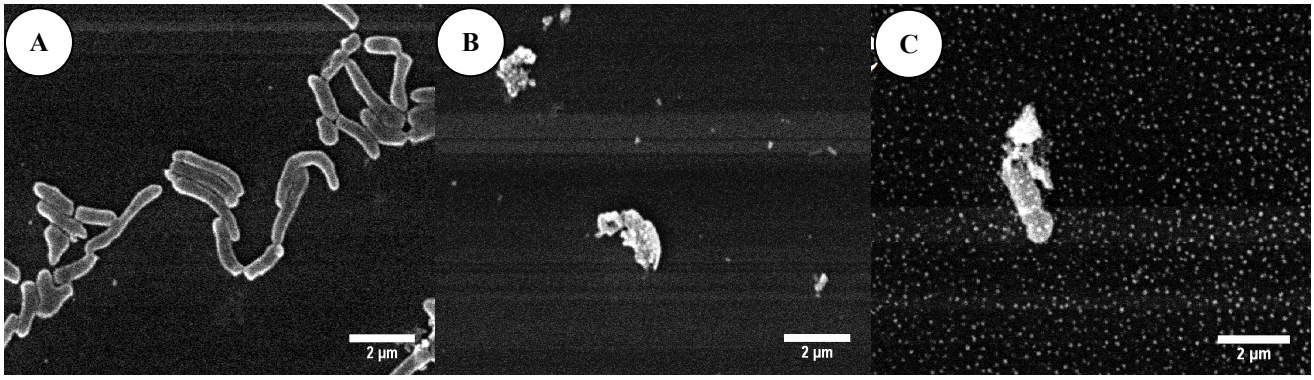

Figure 3. Morphology of *P. acne* cells observed under SEM at the magnificence of 10000×. A. Negative control (DMSO 20%), B. Positive control (Clindamicyn), C. Fraction 3 (5000 ppm)

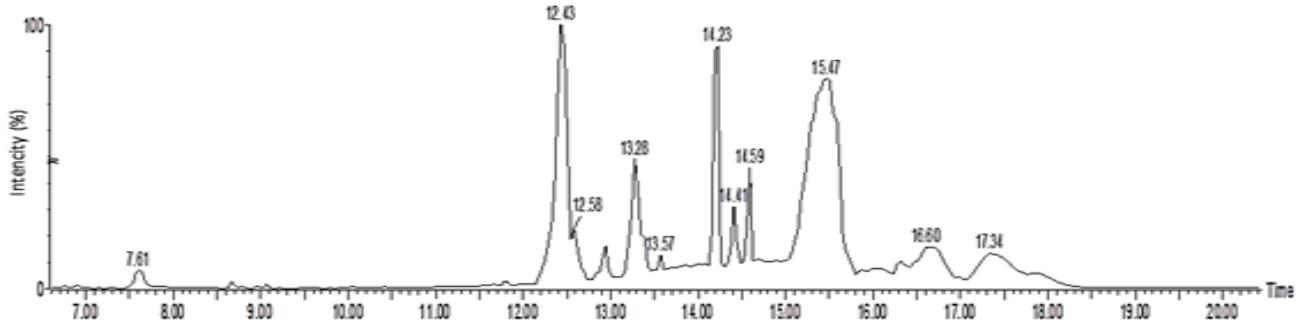

Figure 4. Chromatogram of LCMS/MS fraction 3

Table 4. Bioactive compounds of LC-MS/MS fraction 3

Retention time (min)	Molecular weight (*m/s*)	IUPAC name	Intencity (%)
12.43	590.43	(2S,4S,5S,7S)-5-Amino-N-[2,2-dimethyl-3- (1H-1,2,3-triazol-1 yl)propyl]-4-hydroxy-2 isopropyl-7-[4-methoxy-3- (3-methoxypropoxy) benzyl]-8 methyl nonanamide	22.57
	546.40	(1-[Dodecyl (2-hydroxyethyl) amino]-3-{[(3aR,5R, 5aS,8aS,8bR) 2,2,7,7-tetramethyl tetrahydro-3aH-bis [1,3]dioxolo[4,5-b: 4',5'-d] pyran-5-yl]methoxy}-2-propanol)	
	502.37	5- (7-Carboxyheptyl)-2-hexyl-3-cyclohexene-1-carboxylic acid - 2,2',2" nitrilotriethanol (1: 1)	
	458.35	(Ethyl (3S)-3- (dodecylamino)-3-[(3aR,5R,6S, 6aR)-6-methoxy-2,2-dimethyltetrahydrofuro[2,3-d][1,3]dioxol -5 yl]propanoate	
13.28	632.44	(2S)-3-{[6-Deoxy-6- (octanoylamino)-β-D-gluco pyranosyl]oxy}-1,2-propanediyl di octanoate	6.41
13.57	609.26	N- (6-{[3- (5-Methyl-7-oxo-3-phenyl-7H-furo[3,2-g] chromen-6-yl)propanoyl]amino} hexanoyl)-L-phenylalanine	6,41
	588.41	((3R,4S,5R,6R,7R,9S,12S,13S,14R)-14-Ethyl 4,13-dihydroxy-7-methoxy-3,5,9,11,13 pentamethyl-2-oxo-12- (2-oxoethyl)oxacyclo tetradecan-6-yl3,4,6-trideoxy-3 (dimethyllami no)-β-D-xylo-hexopyranoside	
14.23	593.28	Pheophorbide a	14.10
14.59	535.27	2- (4-benzhydrylpiperazin-1-yl)-7- (3,4-dimethoxy phenyl)-7,8-dihydroquinazolin-5 (6H)-one)	14.10
16.60	593.27	Scortechinone F	12.91
	533.25	1-{5-[2- (Benzyloxy)phenyl]-4-[4- (4-morpholinyl) phenyl]-1-phenyl-4,5-dihydro-1H-1,2,4-triazol-3-yl} ethanone	
	522.59	Tri-n-dodecylamine	
	871.57	Pheophytin a	
	307.26	Dihomo-γ-linolenic acid	

Ethyl acetate extract of Surian leaves demonstrated the highest antibacterial activity (Table 1). Ethyl acetate is semi-polar, volatile solvent and enables to dissolve semi polar compounds in cell wall such as aglycone flavonoid. It is also a non-toxic and non-hygroscopic solvent. In addition, ethyl acetate could bind antibacterial-promoting compounds such as flavonoid polyhydroxy and phenol (Wardhani and Sulistyani 2012). Phytochemical compound in ethyl acetate extracts of surian leaves were alkaloid, tannin, phenolic, steroid, saponin, and terpenoid (Falah et al. 2015; Riva'i 2017). The presence of flavonoid, phenolic, and alkaloid in the ethyl acetate extract of Surian leaves was associated with antibacterial activity against acne bacteria. Based on the result from Table 1 and Table 2, there was a decrease in antibacterial activity between the crude extract and the fractionation yield. The component of the compounds can actually give the maximum effect if it is in a complex condition along with components of other compounds (Virganita et al. 2009).

The MIC and MBC results of each fraction could vary, depending on the type of bacteria and antibacterial compounds. Fraction 3 enables to show bacteriostatic and bactericidal effects against *P. acne*, *S. aureus*, and *E. coli*, but it only showed a bacteriostatic activity against *S. epidermidis* (Table 3). Fatisa (2013) reported that the susceptibility of Gram-positive and Gram-negative bacteria to antibacterial agents might be dissimilar due to a different composition of their cell wall structures such as peptidoglycan, lipid, and crosslinking, which could remarkably affect penetration, binding, and activity of the antimicrobial agent. *P. acne*, *S. aureus*, *S. epidermidis* are regarded as Gram-positive bacteria, and their cell walls are composed of polysaccharide and protein with antigen properties and less lipid content (1-4%). Gram-positive bacteria tend to be more sensitive to antibacterial compounds. This is because the structure of Gram-positive cell wall is simpler, making it easier for antibacterial compounds to enter the cells (Kusmiyati and Agustini 2007). Meanwhile, *E. coli* is classified as Gram-negative bacteria with high lipid content on their cell walls (11-22%). In addition, their cell walls are composed of three layers: lipoprotein, phospholipid (outer membrane) and lipopolysaccharide. The presence of outer membrane phospholipid is capable of reducing the penetration of antibacterial compounds into cells (Fitri and Bustam 2010)

Microscopic observation showed that the addition of Clindamycin as positive control affected deleterious changes in bacterial cells such as rough surface, lysis, and destructed of the cells (Figure 1). Compared to negative control, the cells were unaltered, basil cells with the smooth surface, and no lysis. Treatment of fraction 3 at a dose of 5000 ppm could retard the growth of *P. acne*, which might be linked with the presence of antimicrobial agents. The growth inhibition was revealed by the similar cellular changes observed in positive control (Clindamycin). The fraction 3 enables to promote the cell membrane lysis. Mechanism of the antibacterial compound could be observed from cell leakage, leading to cell destruction. This destruction occurred after the cell membrane exposed to the antibacterial compounds. The interaction of antibacterial compounds could promote alteration or degradation of the cells, contributing to the mechanism of bacterial inactivation. At less lethal concentration, the antibacterial agents may cause cellular injury and promote changes and destruction of the cells, which in turn destroy their metabolic activity. Also, the antibacterial agents could promote more severe injury on the cells, leading to cell death. As control positive, Clindamycin could demonstrate bacteriostatic and bactericidal activity. It enables to suppress protein synthesis through forming a linkage to the 50S ribosomal subunit, thus reducing mRNA translation. At this situation, the bacteria are unable to synthesis protein; thus, their growth is stopped and then died (Frankel 1975).

The bioactive compounds in fraction 3 of Surian leaves responsible for antibacterial activity against acne bacteria were pheophorbide a, pheophytin a and dihomo-γ-linolenic acid (Table 4). Kraatz et al. (2014) reported that pheophorbide a could inhibit the growth of *S. aureus* and *E. coli* that are resistant to erythromycin and showed MIC of 50 µg/mL. Gomes et al. (2015) also found that antibacterial activity of pheophytin, isolated from *Sidastrum micranthum*, was capable to suppress the growth of *S. aureus*, *S. epidermidis*, and *E. coli* with MIC of 75 µg/mL. Additionally, dihomo-γ-linolenic acid (DGLA) showed MIC of 1024 mg/L and MBC of 2048 mg/L and enabled to reduce the growth of *P. acne* and *S. aureus* under in vitro experiment (Desbois and Lawlor 2013). In conclusion, this research showed that fraction 3 has various compounds associated with anti-acne activity such us pheophorbide a, pheophytin a, dihomo-γ-linolenic acid, and some other compounds like (2S,4S,5S,7S)-5-Amino-N-[2,2-dimethyl-3- (1H-1,2,3-triazol-1yl)propyl]-4-hydroxy-2-isopropyl-7-[4-methoxy-3- (3-methoxypropoxy) benzyl]-8 methyl nonanamide which may also be responsible for antibacterial activity against acne bacteria.

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