Bioactive Compound of *Syzygium zeylanicum* Leaves Asthe *Escherichia coli* and *Staphylococcus aureus* Antibacterial

Hamidah[1*], Salni[2], Nina Tanzerina[2]
[1*] Student of Environmental Management Pascasarjana SriwijayaUniversity
[2] Lecturer of Biology department FMIPA SriwijayaUniversity
[*]email: fennyhamidah@student.pps.unsri.ac.id
Received on 07th September 2017 and Accepted on 11th April 2017

ABSTRAK

Escherichia coli merupakan salah satu bakteri penyebab infeksi pada saluran pencernaan manusia yang menyebabkan penyakit diare, sedangkan *Staphylococcus aureus* merupakan salahsatu bakteri penyebab infeksi luka pada kulit seperti bisul dan jerawat. Penelitian ini menggunakan daun *Syzygium zeylanicum* karena memiliki potensi sebagai antibakteri karena mengandung senyawa aktif. Tujuan dari penelitian ini untuk mengetahui aktivitas antibakteri fraksi dan senyawa aktif daun *Syzygium zeylanicum* terhadap *E. coli* dan *S. aureus.* Penelitian dilaksanakan pada bulan November 2015 sampai dengan Januari 2016. Metode yang digunakan pada penelitian ini adalah ekstraksi secara maserasi, fraksinasi secara fraksinasi cair-cair, uji aktivitas antibakteri dan penentuan konsentrasi hambat minimum dengan metode difusi agar dan isolasi senyawa aktif dengan metode kromatorafi kolom. Bakteri uji yang digunakan adalah *E. coli* dan *S. aureus*. Hasil penelitian ini menunjukkan bahwa fraksi Metanol air aktif terhadap bakteri uji. Dari fraksi metanol air diperoleh 1 senyawa antibakteri, dari botol 1,3,5 yang diduga senyawa tannin dengan nilai *Rf*0,416. Konsentrasi Hambat Minimum dari fraksi Metanol air daun jambu air nasi-nasi adalah 1000 µg/mL untuk *E. coli* dan 500 µg/mL untuk *S. aureus.* Konsentrasi Hambat Minimum dari senyawa aktif terhadap *E. coli* dan *S.aureus* adalah pada konsentrasi 500 µg/mL. Fraksi dan senyawa aktif daun *Syzygium zeylanicum* memiliki aktivitas antibakteri terhadap *E. coli* dan *S.aureus* dengan senyawa aktif adalah tanin.

Kata kunci :Myrtaceae, Aktivitas antibakteri, *Syzygium zeylanicum*, tanin,

ABSTRACT

Escherichia coli is one of the bacteria that cause infections of the human digestive tract, such as diarrhea, while *Staphylococcus aureus* is one of the bacteria that cause infections of the skin injury such as boils and pimples. This study used*Syzygium zeylanicum leaves*because it has potential as an antibacterial because it contains active compounds. This study aimed was determined the antibacterial activity of the fraction and the active compound in *Syzygium zeylanicum* leaves against *E. coli* and *S. aureus*. Research conducted in November 2015 to January 2016. The method used in this research was extracted by maceration, fractionation by liquid fractionation, antibacterial activity test, and determination of minimum inhibitory concentration with the diffusion method and isolation of active compounds by column chromatography method. The bacteria used in this test are *E. coli* and *S. aureus*. The results of this research showed the water methanol active fraction against the bacteria that used in this test. The methanol water fraction had obtained one antibacterial compound in bottle 1,3,5 which shows the value of tannin Rf 0,416. The minimum inhibitory concentration of water methanol of water apple leaves is 1000 µg/mL for *E. coli* and 500 µg/mL for *S. aureus.* The minimum inhibitoryconcentration of the active compound to *E. coli* and *S.aureus* in 500 µg/mL.The fraction and the active compound of *Syzygium zeylanicum* leaves have an antibacterial activity with *E. coli* and *S. aureus* and the active compound is tannin.

Keywords: Myrtaceae, *Syzygium zeylanicum,* Anibacterial activity, tannin

INTRODUCTION

The infectious disease was caused by the bacterium *E. coli* and *S. aureus* can be cured with antibiotics. The commonly used antibiotic was sulfonamides, ampicillin, cephalosporins, chloramphenicol, tetracycline and aminoglycosides (Ganiswarna, 1995). Atreatment with antibiotics will bring the side effects if it used for a long time, Mulyadi and Sulistiyani (2013) because it causes bacteria to become resistant.

Syzygium zeylanicum plant or Betti's plant in Tebedak village area of OganIlir regency, this plant is untilized it's leaves to treat the wound caused by the scratch. Anoop (2014), *Syzygium zeylanicum leaves* had used for joint sore and the oils had obtained from the leaves of *Syzygium zeylanicum* and used as an arthritis drug medicine.The *Syzygium zeylanicum* reported as a stimulant, antimicrobial and antirheumatic.

The research can be done by using the proactive ingredient from natural antibacterial compounds contained in the plant. One of the plants with antibacterial potential is *Syzygium zeylanicum leaves.*Based on phytochemical screening Anoop (2014), wounds healing can be done by the presence of secondary metabolites in plants such as alkaloids, flavonoids, phenols, glycosides, plant sterols, terpenoids, saponins and tannins. Salni*et al.*,(2011) stated, in order to obtain proactive antibacterial compounds can be done by several stages of the extraction, fractionation and isolation.

MATERIAL AND METHODS

Thisstudy was conducted in November 2015-January 2016. Located in the Laboratory of Genetics and Biotechnology, Department of Biology, Faculty of Mathematics and Natural Sciences, University of Sriwijaya, Inderalaya.

Formulation of Simplisia

At first, *Syzygium zeylanicum* leaves with good morphological was choosed and thendried it withindirectly light. Then crushed the leaves using a blender until it powdered. Making NA medium *(NutrientAgar)* and NB*(Nutrient broth)*refers to Bridson (1998).

Extraction

The simplicia of *Syzygium zeylanicum* leaves was weighed as much as 250 grams, which was inserted into erlenmeyer and added with methanol to 1000 mL. The mixture of these solvent was stirred until homogen and covered with aluminum foil. These solvent was left for two days than it was filtered with filter paper. The extract was evaporated and was obtained until the viscous extract by *Rotary evaporator*. The extract was dried with thecondensed hair dryer to obtain a dryer extract (Mulyani*et al.,* 2013).

Fractionation

Fractionation was conducted by the FCC (Fractionation Liquids) with solvent n-hexane, ethyl acetate and methanol. The extract was dissolved into methanol and distilled water in the ratio 1:1 200 mL. Furthermore inserted into the separating funnel, and added 200 mL of N-hexane, then shaken gently and allowed to stand. After the visible layers separate solutions, the solution removed and separated fractions into a jam jar. Fractionation using a solvent ethylacetate followed by the way, and the same volume. This is done to obtain a liquid fraction of n-hexane, ethyl acetate fraction of liquid, and the fraction of the methanol-water is then evaporated with a Rotary evaporatorto obtain the thick extract fractions and dried with a hair dryer to obtain the dry fraction. All three fractions obtained tested the activity of the bacteria (Salni*et al.,* 2013).

Fraction Antibacterial Activity Test

The tested fraction was required 2000 µg/mL and was inserted in DMSO. The suspension *of E. coli* and *S. aureus* was added 0,1mL petri dish, then added 10 ml of medium NA*(*NutrientAgar*),*homogenized and left to freeze. Medium containing bacteria inserted 6 mm paper disc that has been dipped in control, N-hexane fraction, ethyl acetate fraction, methanol water by diffusion in order. Later in the incubation temperature of 37^0C for 24 hours.

The Concentration ofFraction Manufacture

The active fraction from the tested activity of antibacterial was weighed by using analytical scale. The highest concentration was made bydilution with solvent DMSO 0,5 mL by using a syringe inserted into the bottle vial that will be used. Then it was taken 0,5 mL of concentration that has made 4000 µg/mL and placed in the bottles containing DMSO concentration of 0,5 mL of the 2000 µg/mL. The same thing was get the next concentration to obtain a concentration of 62,5 µg/mL (Salni*etal.*,2011).

Bioautografi Test

Bioautografi testwas done by using thin layer chromatography. Fraction Active spotted 3 times on a plate of silica gel GF_{254} (KLT) with capillary pipette, and then developed with a mobile phase corresponding to the separation of the compounds contained infractions, in this study used a mobile phase of methanol water: ethyl acetate with a ratio of 9: 1. The chromatograms were traced into a petri dish containing bacterial. The chromatograms were left clinging on agar for ± 60 minutes so that the active compound diffuses into the agar medium, then removed carefully and incubated for 24 hour at 37°C.

Purification of Active Compounds

Active compound purification of the active fraction of gravity is done by column chromatography with an adsorbent of silica gel 60 F_{254} on a 1,7 cm diameter column. Fraction is dripped slowly on the top of the column with a mobile phase solvent elementaccording to the rate of election of 40-50 drops per minute, do illusion until exhausted component. Volume fraction was accommodated 10 mL in which the active isolates were obtained and were searched the minimum inhibitory concentration values in antibacterial test using the agar diffusion method.

Data Analysis

The data were obtained from the experiment that weretabulated in M.Excel, the difference obtained was analyzed using standard deviation (Standard deviation) and presented in descriptive analysis.

RESULTS AND DISCUSSION

Extraction *Syzygium zeylanicum* Leaves

This research was used the extraction of *Syzygium zeylanicum leaves*with 250 grams. From the extraction wasobtained in pasta around 51,7 grams, with the extraction percentage of 20,68%. Extraction was done by maceration method for extraction by maceration can dissolve the compound as well. Rahmawati*et al.*,(2013), maceration method chosen as a way of extraction because the process is simple and can produce extracts in large quantities. The results obtained by evaporation of the filtrate condensed extract. Condensed extract from the extraction of red guava fruit *(Psidium guajava* L.) is then weighed and the result of evaporation of 11,377 grams with a percentage of 45,49%.

Fractionation *Syzygium zeylanicum* Leaves

Based on the results of fractionation that was used a solvent according to the degree of polarity. N-hexane (non-polar), ethyl acetate (semi-polar) and methanol (polar) is known from 23 grams extract of *Syzygium zeylanicum* leaves, that was get each fraction as shown on Table 1.

Table 1The Fractionation Results of the *Syzygium zeylanicum* Extraction

No	Solvent	The Fraction (gram)	The Percentage (%)
1.	N-hexane	3,6	15,79
2.	Ethyl acetate	7,8	34,21
3.	Water methanol	11,4	50
	Total	22,8	100

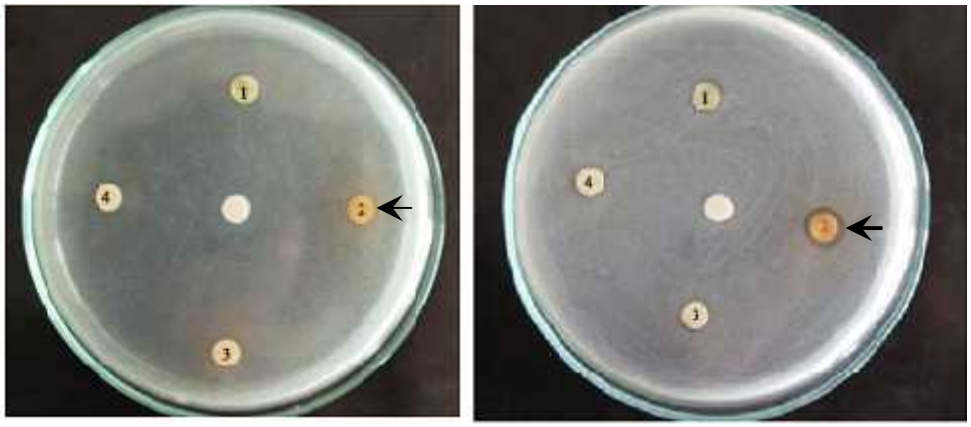
This result showed that the greatest fraction of *Syzygium zeylanicum* leaveswas contained in the methanol fraction with a weight of 11,4 grams and a percentage weight of 50%.The increase of methanol fraction was greater than the fraction of n-hexane and ethyl acetate fraction. In the fractionation process, the compounds in the extract will be bound with a suitable solvent to the level of polarity. In the opinion of Nurdin *et al.,* (2010) that the methanol solvent was capable to dissolve all components of both polarities, semi-polar and non-polar. Harbone (1987) states that the polar nature of the solvent, the greater the number of fractions obtained.

Activity Test Antibacterial Fraction Methanol *Syzygium zeylanicum* Leaves Against *Escherichia coli* and *Staphylococcus aureus*

Activity test fraction of n-hexane, ethyl acetate fraction and a methanol fraction of water from the *Syzygium zeylanicum* leaves is performed at a concentration of 2000 µg/mL.Fractions were tested for antibacterial activity to find the type of fraction that are active against *E. coli* and *S.aureus.* The results of the antibacterial testing of fractions (Table 2 and Figure 1).

Table 2.Results of antibacterial activity test methanol fraction of *Syzygium zeylanicum* leaveson the growth of *E. coli* and *S. aureus*

No.	**Fraction**	**Diameter Zone of Inhibition (mm ± standard deviation)**	
		Escherichia coli	***Staphylococcus aureus***
1	N-hexane,	0	6,4 ± 0,28
2	Ethyl acetate	6,5 ±0,07	6,3 ± 0,98
3	Water methanol	**7,0± 0,63**	**9,75 ±0,35**
4	Extract	0	6,85± 0,49



A **B**

Figure 1. Antibacterial Activity Test Results of Methanol fraction *Syzygium zeylanicum* leaves on Growth *E. coli*and *S. aureus*
Caption:1. N-hexane fraction, 2. Water Methanol fraction, 3. Ethyl acetate fraction, 4.Exract.
The figure of 1A shows*E.coli* and the figure of 1B shows *S.aureus*(showing inhibition zone)

Based on Table 2, it can be seen that the result of antibacterial activityfrom methanol fraction had the inhibition zone against *E. coli* of 7,05 mm and *S. aureus*of9,75 mm ferrous.Based on the diameter of formed methanol fraction was categorized to the medium of antibacterial. Davis and Stout (1971), the strength of the antibacterial activity is very strong in inhibitor zone of 20 mm, and theinhibition of 10-20 mm is strong. If the resistor has an inhibitor zone of 5-10 mm that was categorized to themedium antibacterial. The inhibitor zone of 5 mm or less was categorized weak antibacterial.

E. coli has an inhibitor zone of 7,05 mm whereas inhibition of *S. aureus* has inhibitory of 9,75 mm, it indicates that *S. aureus* more sensitive to antibacterial, that was the reason as *S.aureus* had big inhibitory zone, it can also be caused the differences in cell membrane structure. According to research Aini *et al.*, (2015), the structure of the cell membrane of gram positive bacteria have more peptidoglycan whereas Gram negative bacterial cells slightly peptidoglycan. The difference is what causes the cell membrane antibacterial easily penetrate the cell membrane of gram positive bacteria.

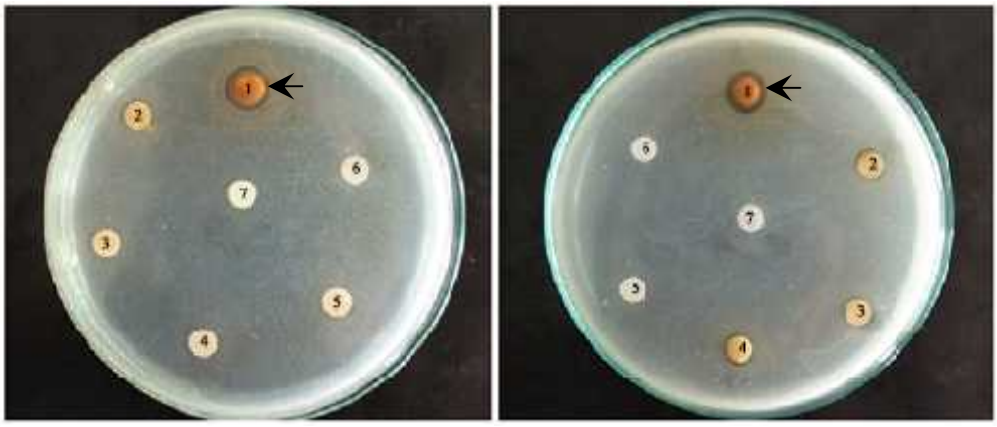
Determination of Minimum Inhibitory Concentration (MIC) Fraction Methanol water against *Escherichia coli* and *Staphylococcus aureus*

Based on the results of the activity test (Figure 2 and Table 3)Indicates the air methanol fraction is the most effective fraction of the n-hexane fraction and the ethyl acetate fraction. Antibacterial activity from open air methanol fraction to *E.coli* and *S.aureus* to obtain the result of minimum inhibitory concentration (MIC).

Based on Table 3 The of inhibition zone is at a concentration of 4000 µg/mL with a inhibitor zone of 10,35 mm on test bacteria *E. coli* and 12,0 mm on the test bacteria *S. aureus*, while the smallest of the inhibitory zone at a concentration of 500 µg/mL for *S.aureus* with a inhibitor zzone of 7,15 mm while the resistor 1000 µg/mL for *E. coli*with an inhibitor zone of 7,1 mm. The smaller the value of the concentration, it will be the smaller inhibition zone is formed. In accordance with the opinion of Salni *et al.*, (2013), the power fraction activity decreased along with the decrease in the value of concentration, so that the inhibitor zone is formed will be smaller.

Table 3 Minimum Inhibitory Concentration (MIC) Fraction Methanol WaterAgainst*E. coli* and *S. aureus*

No.	Bacteria	Fraction Concentration (µg /mL)	Average Diameter Inhibition (mm ± standard deviation)
1	*Escherichia coli*	4000	10,35 ± 0,21
		2000	7,25 ± 0,35
		1000	**7,1± 0,07**
		250	0
		125	0
		500	0
		62,5	0
2	*Staphylococcus aureus*	4000	12,0± 0,70
		2000	8,5 ±0,28
		1000	8,1 ± 0,14
		500	**7,15 ± 0,21**
		250	0
		125	0
		62,5	0



A **B**

Figure 2 Minimum Inhibitory Concentration (MIC) of water Methanol fraction *Syzygium zeylanicum* leaves on Growth *E. coli* and *S. aureus*

Caption: 1. The concentration of 4000 µg/mL, 2. 2000 µg/mL, 3. 1000 µg/mL 4. 500 µg/mL, 5. 250 µg/mL, 6. 125 µg/mL, 7. 62.5 µg/mL The figure of 2A shows*E.coli* and the figure of 2B shows *S.aureus*(showing inhibition zone)

On the Table 3 was shown the inhibitory concentrations of an *E. coli* on 1000 µg/mLthat was included as the weak inhibition. While in *S. aureus* have inhibitory of 250 µg/mL, it is included in the category that is strong enough. In the opinion of Holetz*etal*.,(2002) states that, the value of the minimum inhibitory concentration (MIC) of the active antibacterial compound has three categories of establishments which have a concentration of less than 100 µg/mL is very strong, if the concentration is between 100-500 µg/mL it does show that of concentration is strong enough, if the concentration is between 500-1000 µg/mL is weak and if the concentration of more than 1000 µg/mL means that the compounds have no antibacterial activity.

According to research Rosaidah and Mahita (2012), states that, with the use of guava leaf extract an average diameter of bacterial inhibition zone ranges from 6,5 mm-

11,5 mm. This shows that the greater the concentration used, the greater the inhibition zone diameters obtained, meaning that the antibacterial activity of guava leaf extract increases with increasing concentration of the extract. Jawetz *et al.*, (2005), the antibacterial activity is influenced by several factors such as the concentration of the extract, contains antibacterial compounds, extracts diffusion power, and type of bacteria is inhibited.

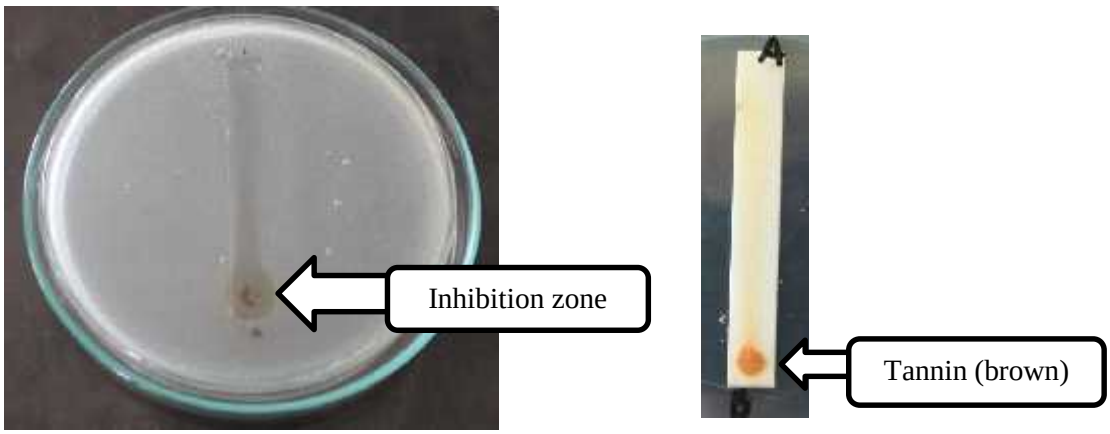
Bioautografi Test and Determination of Active Compound

Methanol fraction bioautografi water test using silica gel $Fplate_{254}$ to determine the class of the active compound with the appropriate eluent ratio 8:2 (ethyl acetate: methanol) as the mobile phase with the appearance of patches of H_2SO_4.Bioautografi test results (Table 4).

Table 4 Test Results Bioautografi water and methanol fraction group Active Compounds against Determination of *E. coli* and*S. aureus*

Active Fraction	Rf Values	Color Shaped	Group Compound
water Methanol	0.41	Brown	tannin

Caption: Comparison eluent 8: 2 (Ethyl acetate: Methanol)



(a)Bioautografi Test (b) the TLC plate

Figure 3 The Results of water and methanol Bioautografi Active Compounds Determination of *Syzygium zeylanicum* leaves toward bacterium *E. coli* and *S. aureus*

Results bioautografi test on the TLC plate visible brown color indicating tannin. According Harbone (1987) states that, tannin yellowish to brown. While the TLC plate is placed in a petri dish looks a clear zone formed in cultured bacteria due to the tannin. According Darwis*et al*., (2013), tannin is a complex phenolic compounds containing hydroxyl groups. Tannin are phenolic, then tannin have the same mechanism with a phenol compound in inhibiting the growth of bacteria.The mechanism of phenol compounds, According to Dwidjoseputro (1994), phenol compounds enter into bacterial cell pass through the cell membrane of bacteria and cytoplasmic membrane, in bacterial cell of phenol compounds cause the aggregation of protoplasmic proteins so that the circumstances metabolism become inactive and bacteria become obstructed.

According to research Rosaidah and Mahita (2012) found, tannin is the main component in the leaves and seeds because of the amount of tannin content more than the

other compounds. Based on the research that has been done by Widaty (2008) through phytochemical screening test guava leaf extract contains tannins 13.51%.

Purification and Antibacterial Activity Test Compounds Active Fraction

Purification of the active compound from the methanol fraction of water to the adsorbent column chromatography with silica gel 60 $F_{254.}$ eluent used as mobile phase was an eluent ratio of 9:1 (ethyl acetate: methanol water) at a rate of elution 40- 50 drops per minute. Fractions testing activity against *S. aureus* is a fraction with odd numbers. Tests using only one test bacteria, this is because the fraction of the test compound is active against *E. coli* together with *S.aureus*,so only have one bacterium alone. Testing is done only on the odd bottle due to the possibility of 61 bottles of compounds obtained from the purification has the same active compound in the adjacent bottles. Diameter of clear zone test results of the antibacterial activity of the methanol fraction of water purification with the odd bottle (Figure4).

Based on Figure 4 shown the fraction of the number of bottles of 1, 3 and 5 active against *S. aureus* with the formation of inhibition zone around the paper disc. In the bottles 7 to 51 did not show any inhibition zone. But there are also inhibitory zone on the bottle numbers 53, 57 and 51 and the number 55, 61 there is no inhibition zone, this is because it provides 100% methanol as mobile phase. According Tinambunan*et al.*,(2012), giving the extract solution with a concentration of 100% aimed to determine the lowest levels of sample extracts that deliver antibacterial activity against bacteria tested.

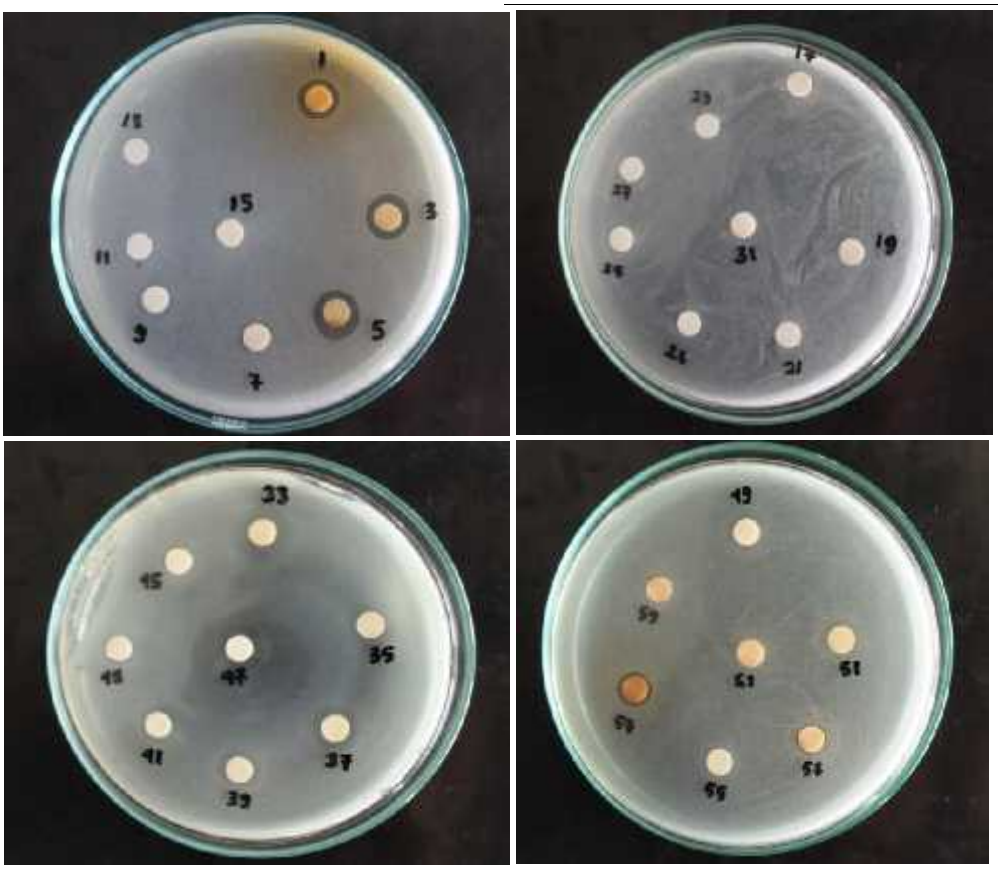


Figure 4
Caption: Test Results Activities antibacterial compound purification of Fraction Methanol water with a number of bottles of odd against *S. aureus*

Based on the resultsof active compound activity from the *Syzygium zeylanicum* leaves against bacterial test, the compounds can inhibit the growth of *S. aureus* which is a representative gram-positive bacteria. Kusmayati and Agustini (2007) that gram-positive bacteria tend to be more sensitive to antibacterial, because the structure of the cell membrane of gram-positive bacteria was simplebecause it contains low lipid that made it easier for antibacterial compound to enter the cell of gram-positive bacteria.

CONCLUSIONS

Active fractions of the water methanol extract from *Syzygium zeylanicum* leaves have antibacterial activity with a diameter of 7,0 mm, inhibition of *E. coli* and 9,75 mm for *S. aureus*.Minimum Inhibitory Concentration (MIC) of the methanol fraction of water is 1000 µg/mL for *E. coli* 500 µg/mL for *S. aureus*.The methanol compounds of fraction purified water from the *Syzygium zeylanicum* leaves istannin with*Rf*0,416. Minimum Inhibitory Concentration (MIC) of pure compound is 500 ug / ml to against *E. coli*and *S.aureus.*

REFERENCES

Aini, N, H., Saleh, C., and Erwin. 2015. UjiToksisitas Dan Aktivitas Anti bakteri Ekstrak Daun Merah Tanaman Pucuk Merah (*Syzygium myrtifolium* Walp.) Terhadap Bakteri *Staphylococcus aureus* dan *Escherichia coli*. *Jurnal Kimia Mulawarman.*1(13) :35-39.

Anoop, M, V., Bindu, A, R. 2014.Pharmacognostic and Physico-Chemical Studies on Leaves of *Syzygium zeylanicum* (L.) DC. *International Journal of Pharmacognosy and Phytochemical Research*. 6(4) :685-689.

Bridson, E, Y. 1998. *The Oxoid Manual*. Wade Road, Basingstoke, Hampshire. England.

Davis , W, W., and Stout, T, R. 1971. Disc Plate Method Of Microbiological Antibiotic Assay. *Journal Of Microbiology*. 22(4) :659-665.

Darwis, W., Romauli, S., and Kasrina. 2013. Uji Efektivitas Ekstrak Daun Iler-Iler (*Coleuss cutellarioides* (Linn) (Benth) Sebagai Antibakteri *Staphylococcus aureus*. *Konservasi Hayati.* 9(2) :55-59.

Dwidjoseputro. 2005. *Dasar-Dasar Mikrobiologi*. PT djambatan:Jakarta.

Ganiswarna, S, G. 1995. *Farmakologi dan Terapi Edisi 4*.UI-Fakultas Kedokteran. Jakarta.

Harbone, J, B. 1987. *Metode Fitokimia*. Diterjemahkan oleh Kosasih Padmawinata dan Iwang Sudiro.Penerbit ITB. Bandung.

Holetz, F, B., Pessini, G, L., Sanchez, D., Aparicio, G. Cortez, C, V. Nakamura., andFilho, B, P, D. 2002. Screening of Some Plants Used in The Brazillian Folk Medicine For The Treatment of Infections I. *Journal of BiolineInternational*.http://www.bopline-org.br/request02229.

Jawetz, E., Mellnick, J, L., Adellberg, E, A. 2005. *Mikrobiologi Kedoteran*. Edisi 20.EGC. Jakarta.

Kusmayati andAgustini, N. W. R. 2007.UjiAktivitasSenyawaAntibakteridariMikroalga (*Porphyridiumcruentum*).*Biodiversitas.* 8(1) :48-53.

Mulyani, Y., Bachtiar, E., and Untung, M, K, A., 2013. Peranan Senyawa Metabolit Sekunder Tumbuhan Mangrove Terhadap Infeksi Bakteri *Aeromonas hydrophila* pada Ikan Mas (*Cyprinuscarpio* L.). *Jurnal Akuatika*. 4(1) :1-9.

Nurdin, A M., Titin, S, F, M., and Zackiyah. 2010. Penentuan Pelarut Terbaik Dalam Mengekstraksi Senyawa Bioaktif Dari Kulit Batang *Artocarpus heterophyllus*. *Jurnal Sains dan Teknologi Kimia.* 2(1) :150-158.

Rahmawati, F., Dwiyanti, G., and Sholihin, H. 2013. Kajian Aktivitas Antioksi dan Produk Olahan Buah Jambu Biji Merah (*Psidium guajava* L.). *Jurnal Sains dan Teknologi*. 1(4) :1-8.

Rosaidah and Mahita, A, W. 2012. Potensi Ekstrak Daun Jambu Biji Sebagai Anti bacterial Untuk Menanggulangi Serangan Bakteri *Aeromonas hydrophila* Pada Ikan Gurame (*Osphronemus gourami lacepede*). *Jurnal Akuatika.*1(3) :19-27.

Salni, Aminasih, N., and Sriviona, R. 2013. Isolasi Senyawa Antijamur dari Rimpang Lengkuas Putih (*Alpinia galangal* (L.) Willd) dan Penentuan Konsentrasi Hambat Minimum Terhadap *Candida albicans*. *Prosiding Semirata FMIPA Universitas Lampung.*

Salni, Marisa, H., and Wedya, M, R. 2011. Isolasi Senyawa Antibakteri Dari Daun Jengkol (*Pithecolobium lobatum* Benth) dan Penentuan Nilai KHM. *Jurnal Penelitian Sains.* 14(1) :1-4.

Tinambunan, H., Melki., and Isnaini. 2012. Efektivitas Ekstrak Bakteri yang Berasosiasi Dengan Spons dan Karang Lunak Sebagai Antibakteri dari Perairan Pulai Tegal Lampung. *Maspari Journal.*4(2) :225-230.