The Anti Microbial Activities of The Extracts of Red Fruit*(Pandanus conoideus Lam*) Pre-dried by *Détente Instantanée Contrôlée*(DIC)

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Abstract

Red fruit (*Pandanus conoideus* Lam.) is an indigenous plant from Papua Province, Indonesia. Local communities believed that fruit of *P. conoideus* Lam. can treat several degenerative diseases such as cancer, arteriosclerosis, rheumatoid arthritis, and stroke. DIC is a high-steam pressure treatment, is also categorized as a HTST (High Temperature Short Time) process. In this study, the antimicrobial activity of the etanolic and the hexane extract of red fruit pre-treated and untreated by DIC, and the red fruit oil are observed. The red fruit oil and all the ethanolic extract obtained from the red fruit powder pre-treated and untreated by DIC do not show an inhibitory activity toward *Eschericia coli* and *Staphyloccoccus aureus*. Also, the hexane extract from DIC pre-treatment does not show an inhibitory activity toward *Eschericia coli* and *Staphyloccoccus aureus*, whereas that of the untreated shows an inhibitory activity, but is not as potent as amoxicillin.

Keywords: *Dètente Instantanée Côntrolée*, pre-drying, Red Fruit (*Pandanus conoideus)*, Antimicrobial.

1. Introduction

Indonesia is a country that has a biodiversity that can be processed into various of drugs. One of nutritious plant in Indonesia is red fruits (Subroto, 2005). Red fruit (*Pandanus conoideus* Lam) is an indigenous plant from Papua Province, Indonesia and Papua New Guinea. For local communities, it is believed that the fruit of *P.conoideus* can treat several degenerative diseases such as cancer, arteriosclerosis, rheumatoid arthritis, and stroke (Budi and Paimin, 2004).

Red fruits (*Pandanus conoideus* Lam.) have approximately 46% moisture content. This condition makes the red fruits can not be preserved more than 5 days, especially in the room temperature. In the room temperature (25-30 °C), red fruits become easily damaged. More over, in the cold temperature (10°C), red fruits can be preserved for 7-10 days.

The damage can cause the degradation of the beneficial content which is needed as medicine/supplement. This condition makes there are only two ways to prevent the damage, directly processes into the red fruit oil or preserved by drying method (Lisangan, 2005).

One of the pre-drying treatment method is DIC (*Détente Instantanée Contrôlée*) technology

which applies instant pressure-drop to modify the texture of the material and intensify functional behaviour. DIC treatment usually starts by creating a vacuum condition, followed by injecting steam to the material. This treatment is also categorized as a HTST (High Temperature Short Time) process. (Allaf *et al.*, 2009).

It is expected that after the DIC pre-drying treatment, the red fruit extract can be preserved for a long time and also the yield of the beneficial compound in the extract is expected will be higher than that of without DIC pre-drying treatment. This condition is expected can increase the medical activities of the red fruit extract compared to the one without DIC pre-drying treatment.

As we know, In Indonesia, infectious disease still be the top ten of the major disease. Antibiotics are among the most commonly prescribed drugs used in human medicine. The misuse of antibiotics is the single most important factor leading to antibiotic resistance around the world.

Antibiotic resistance is a serious threat to public health in Europe, because it leads to increase healthcare costs, extra length of stay in the hospital, treatment failures, and sometimes death (European Centre for Disease Prevention and Control, 2012). Many scientists have developed the use of medicinal plant to reduce the phenomenon of the antibiotic resistance in the world.

Phenolic compound possesses a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic, antimicrobial as well as the ability to modify the gene expression (Marinova *et al.*, 2005). *Lactobacillus spp.* and *S. aureus* (Gram-positive) appeared more susceptible to the action of phenolic acids than gram negative bacteria such as *E. coli* and *P. aeruginosa* (Karaca, 2011).

The antimicrobial activity of red fruit oil as an antimicrobial has been proven in previous studies. Red fruit oil products (*Pandanus conoideus* Lam) can inhibit the growth of *Streptococcus mutans bacteria* at levels of 100% v / v (Aisyah & Suhartanti, 2012). A research result obtained from Tan (2008), **PCO**, Pandanus Cocos Oil, the mixture of red fruit oil and coconut oil, does not have anti microbial activity against S*taphylococcus aureus* and *Escherichia coli*. And a result from Subroto (2005), **PCO** has anti microbial activity against S*taphylococcus aureus* and *Escherichia coli.* These two uncertain research results above is the proof that a further study about the antimicrobial activity of red fruit oil and also virgin coconut oil is needed

to be observed. Besides, the anti microbial activity of the red fruit extract has never been studied before. Thus, it is important to study the antimicrobial activity of the red fruit extract and also of the red fruit oil.

There are five samples that are tested in this research. The first sample is red fruit of optimum DIC pre-treatment (2.5 bar, 4 cycles,15 seconds) followed by optimum extraction condition (60% ethanol, 30°C, 1 hour) (Indarto, Mogi, Prasetia, Setiadi, 2013). The second sample is red fruit dried by conventional drying followed by optimum extraction condition (60% ethanol, 30°C, 1 hour) (Prasetia, Setiadi, 2013). The third sample is red fruit of optimum DIC pre-treatment (1.5 bar, 2 cycles, 15 seconds) followed by optimum extraction condition (hexane, 45°C, 1.5 hours) (Tan, Wahyuni, 2013). The fourth sample is red fruit dried by conventional drying followed by optimum extraction condition (hexane, 45°C, 1.5 hours) (Wahyuni, 2013). And the fifth sample is red fruit oil. The antimicrobial activity of red fruit oil is also analyzed because red fruit oil is considered as the representative of the juice (the red part of the red fruit) which the antimicrobial activity of the red fruit juice is important to be known.

Bacteria which are used are *Escherichia coli* and *Staphylococcus aureus* bacteria because these two bacteria represent gram positive bacteria (*Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli*) respectively. And also, these two bacteria are commonly used for determination of antimicrobial activity.

One of the antimicrobial susceptibility testing methods is the disk diffusion method which is based on the determination of an inhibition zone proportional to the bacterial susceptibility to the antimicrobial present in the disk. The advantages of this method are low cost, ease in modifying test antimicrobial disks when required, can be used as a screening test against large numbers of isolates, can identify a subset of isolates for further testing by other methods, such as determination of MIC (Bauman, 2011).

In this study, the effect of pre-drying treatment by DIC on the antibacterial activity of the red fruit extract and red fruit oil will be explored using the disk diffusion method.

2. Materials and Method

2.1. Rejuvenation of *Escherichia coli* and *Staphylococcus aureus* Bacteria in the Nutrient Agar Slant Media

Each of the pure culture of the *Escherichia coli* and *Staphylococcus aureus* bacteria is taken with sterile inoculating loop and it is streaked in the Nutrient Agar slant media. The Nutrient Agar slant media which is cultivated with *Escherichia coli* and *Staphylococcus aureus* bacteria is put into an incubator at 37^{o}C for 24 hours until showing a growth then it is continued with identification.

2.2. Preparation Suspension Bacteria

The culture of *Escherichia coli* and *Staphylococcus aureus* bacteria which has already been rejuvenated is added to 5 mL NaCl 0,9% solution and it is homogenized. Then the bacteria suspension is taken and it is diluted with NaCl 0,9% solution until the *optical density* (OD) of 0,6 is obtained in the 580 nm wave lenght of the spectrofotometry.

2.3 Preparation of the Testing Solution

Red fruit extract is weighed and diluted in the appropriate solvent in the following concentration below and the antimicrobial activity is tested in the *Escherichia coli* and *Staphylococcus aureus* bacteria for orientation:

a. 50.000 ppm : 50.0 mg extract is diluted in ethanol 96% p.a to make1.0 g solution.

b.750.000 ppm: 75.0 mg extract is diluted in ethanol 96% p.a to make 1.0 g solution.

c. 100.000 ppm: 100.0 mg extract is diluted in ethanol 96% p.a to make 1.0 g solution.

d. 125.000 ppm: 125.0 mg extract is diluted in ethanol 96% p.a to make 1.0 g solution.

Then, the Testing solution is centrifuged at 3000 rpm for 5 minutes.

2.3.1. Preparation of the Amoxicillin Comparator Solution

Accurately weighed amoxicillin powder (50,0 mg) is diluted in sterile *"aquabidestilata"* to make 50,0 mL, thus 1000 ppm amoxicillin solution is obtained. Then, 0,2 mL of1000 ppm amoxicillin solution is pipetted and added to sterile *"aquabidestilata"* to make 10,0 mL solution. Thus, 20 ppm amoxicillin solution is obtained. Then, the Amoxicillin Comparator Solution is centrifuged at 3000 rpm for 5 minutes.

2.3.2. Preparation of the Control Solution

Control solution is divided into 2 solution:

1.0 g ethanol 96% p.a solution

ethanol 96% p.a with cab-O-Sil: Cab-O-Sil is accurately weighed according to the sample which is described on part 3.3.2.1 (p. 42-43) based on the percentage Cab-O-Sil in the bulk:

Optimum condition of DIC and optimum extraction of Flavonoid and Total Phenol: 70.38%

ii. Conventional drying and optimum extraction of Flavonoid and Total Phenol: 76.11%
Optimum condition of DIC and optimum extraction of Tocopherol: 45.60%

iv. Conventional drying and optimum extraction of Tocopherol: 46.85%

Then, the Control Solution is centrifuged at 3000 rpm for 5 minutes.

3. Results and Discussion

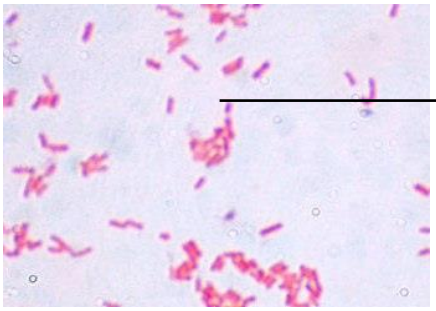
The powder of 40/50 mesh of red fruit is used for the extraction. For the red fruit with optimum condition of DIC pre-treatment based on flavonoid and total phenol content (2.5 bars, 15 seconds, and 4 cycles), the optimum extraction condition is kinetic maceration at 120 rpm, 30°C for 1 hour using 60% ethanol (1:10) (Prasetia, Setiadi, 2013). And for the red fruit which is directly dried by conventional drying, it was extracted with the same condition as well. The advantage of using the kinetic maceration is to achieve the equilibrium concentration faster in the help of the stirring motor and also this method is best suitable for use in case of the thermolabile drugs (Tiwari et al, 2011).

3.1. Antibacterial Activity Tes.

3.1.1. Qualitative identification of *Escherichia coli* and *Staphylococcus aureus* Bacteria

Qualitative identification of *Escherichia coli* and *Staphylococcus aureus* bacteria is aimed to make sure that the bacteria which are used is *Escherichia coli* and *Staphylococcus aureus* bacteria.

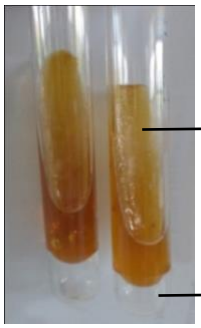
The result of Qualitative identification of *Escherichia coli* bacteria can be seen in **fig. 10 – 14** and **Table 1.**



→ Gram negative, red color, cocobacil form of cell

Fig. 10. The Gram Stain Reaction of *Escherichia coli* Bacteria (1000 times magnifying)

The cell wall of *Escherichia coli* consists of thinner peptidoglycan which has larger pores that fail to retain the crystal violet-iodine complex (gram I and II). The alcohol (Gram III) dissolves the outer layer, penetrates the cell, and washes away the crystal violet and may absorb the counter stain/safranin (Gram IV) which color is red. *Escherichia coli* is gram negative bacteria and has cocobacil form of cells (Tortora et al., 2012).



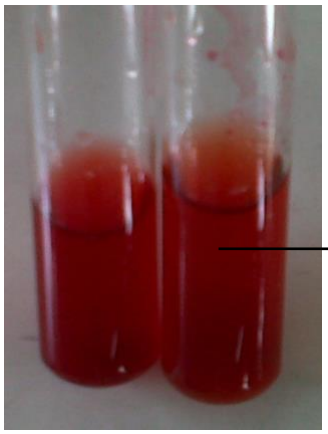
Positive results:
The color of the butt part and the slant part of the agar turn to yellow

Positive result:
The gas is produced in the fermentation process is showed by a lifting of the butt part from the base of the test tube

Before testing **After Testing**

Fig. 11. The Result of Lactose and Glucose Fermentation Testing of *Escherichia coli* bacteria

Escherichia coli is facultative anaerob bacteria that can ferment carbohydrates. *Escherichia coli* can use lactose and glucose as the substrate for continuing fermentative activities of an acid reaction in both slant and butt (both slant and butt turn to yellow). And substrates undergo anaerobic dissimilation and produce an organic acid that may be accompanied by gases such as hydrogen or carbon dioxide, shown by a butt part which is lifted up from the base of test tube (Cappuccino and Sherman, 2005). So, this test is positive, shows that *Escherichia coli* can ferment lactose and glucose.



MR Testing positive result: the color of the medium turns red

Before testing **After Testing**

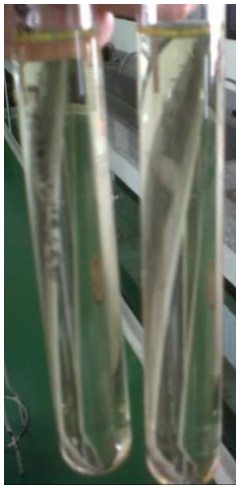
VP Testing
Negative result : no color change of the medium

After Testing

Fig. 12. The Result of MRVP (Methyl Red-Voges Proskauer Broth) Testing of *Escherichia coli* bacteria

Escherichia coli ferments glucose with the production of organic acid during the early incubation period. The low acidic pH(4) is stabilized and maintained at the end of incubation. The methyl red indicator will turn red in the pH range of 4 (Cappuccino and Sherman, 2005). So, the MR test is positive, shows that *Escherichia coli* ferments glucose into organic acid.

Escherichia coli does not produce non acidic or neutral end products, such as acetylmethyl carbinol from the organic acids that result from glucose metabolism (Cappuccino and Sherman, 2005). So, the VP test is negative.

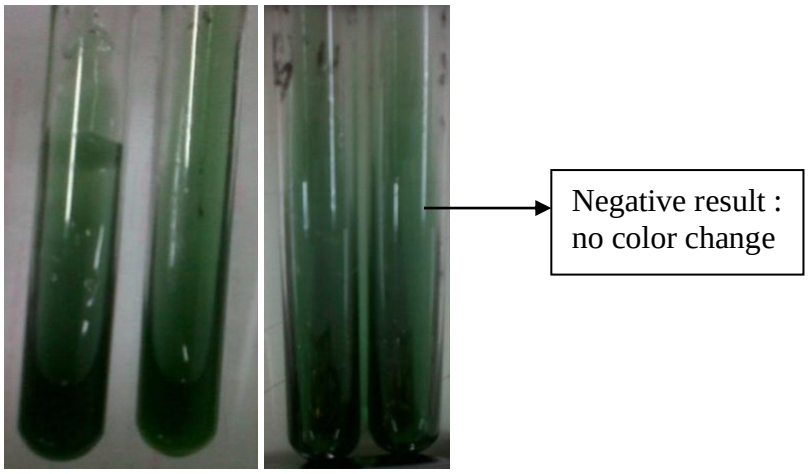


positive result : a red ring appears in the surface of the culture

Before testing **After Testing**

Fig. 13 The result of Indole testing of *Escherichia coli* bacteria

Escherichia coli can hydrolyze tryptophan in the SCA agar into indole. The presence of indole is detectable by adding Covac's reagent, which produces a cherry red reagent layer (Cappuccino and Sherman, 2005). So, this test is positive.



Before testing **After Testing**

Fig. 14 The result of citrate testing of *Escherichia coli* bacteria

Escherichia coli can not use citrate as a carbon source for their energy because the absence of citrate permease enzyme that facilitates the transport of citrate in the cell. The medium remain green indicates that the test is negative (Cappuccino and Sherman, 2005).

Table 1. The result of Qualitative Identification of *Escherichia coli* bacteria

No.	Kind of testing	Reference	Examination Result	Result
1.	Gram stain, color, form and structure of the cell	Gram negative, red color, cocobasil form of cell	Gram negative, red color, cocobasil form of cell	+
2.	Lactose and Glucose Fermentation Testing	+: The color of butt and slant part of agar turn to yellow and gas is produced	The color of butt and slant part of agar turn to yellow and gas is produced	+
3.	Methyl Red-Voges Proskauer Testing	Methyl Red + : Red Color Voges Proskauer - : Yellow color	Methyl Red : Red Color Voges Proskauer : Yellow color	+
4.	Indol Testing	+ : Red Ring	Red Ring	+
5.	Citrate Testing	- : Green color	Green color	+

Note:
Positive Result (+) shows the similarity with the reference (Merck, 2005)

The result of Qualitative identification of *Staphylococcus aureus* bacteria can be seen in **fig.15-17** and **Table 2.**

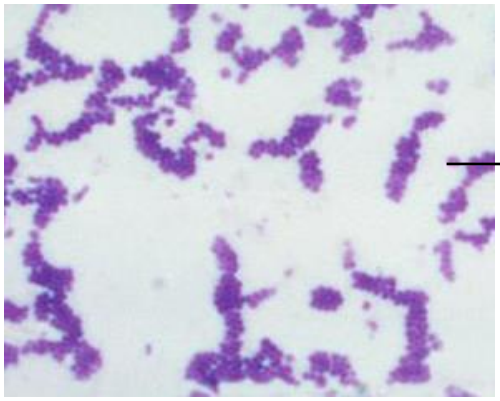


Fig. 15. The Gram stain reaction of *Staphylococcus aureus* bacteria (1000 times magnifying)

The cell wall of *Staphylococcus aureus* consists of thick peptidoglycan which is responsible for the more stringent retention of the CV-Iodine complex (gram I and II), as the pores are made smaller due to the dehydrating effect of the alcohol (gram III). Thus, the tightly bound primary stain (gram I) complex is difficult to remove, and the cells remain purple (Cappuccino and Sherman, 2005). *Staphylococcus aureus* is gram positive bacteria and has coccus form of cell (Tortora et al., 2012).

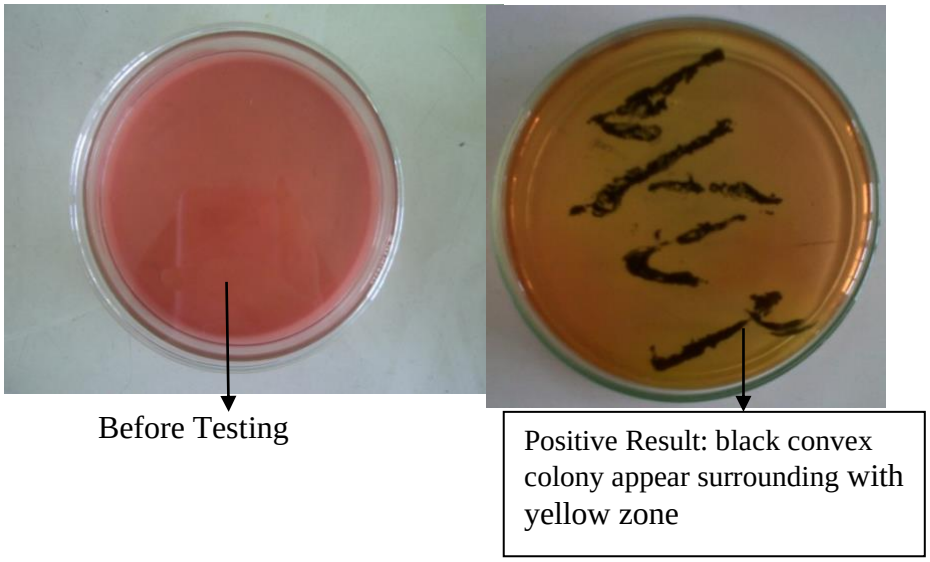
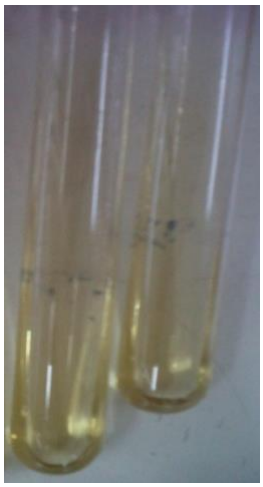


Fig. 16. The Result of Manitol and Telurit Degradation Testing of *Staphylococcus aureus* bacteria

Staphylococcus aureus can reduce tellurit become free tellurium shown by black convex colony appear surrounding with yellow zone (Cappuccino and Sherman, 2005).



Positive Result:
Bubbling Reaction(Gas produced)

Before Testing

Fig. 17. The Result of Catalase Testing *Staphylococcus aureus* bacteria

Staphylococcus aureus capable of producing catalase that can be determined by adding the substrate H_2O_2. Catalase rapidly degrades hydrogen peroxide into water and free oxygen which is indicated by the presence of bubble (Cappuccino and Sherman, 2005). So, this test is positive.

Table 2. The result of Qualitative Identification of *Staphylococcus aureus* bacteria

No.	Kind of testing	Reference	Examination Result	Result
1.	Gram stain, color, form and structure of the cell	Gram positive (+), purple color, a group of Coccus cell form	Gram positive (+), purple color, a group of Coccus cell form	+
2.	Manitol and Telurit Degradation Testing	+: black convex colony appear surrounding with yellow zone	black convex colony appear surrounding with yellow zone	+
3.	Catalase Testing	+: Bubbling reaction (Gas Production)	Bubbling reaction(Gas Production)	+

Note:
Positive Result (+) shows the similarity with the reference (Merck, 2005)

3.1.2. Determination of Antimicrobial Activity of The Red Fruit Extract And Red Fruit Oil

Bacteria is incubated for 20 hours at 37^{o}C because in 20 hour, the bacteria is in exponential phase, a phase where they multiply and synthesize surface proteins and essential proteins for growth, cell division and adhesion (Harris, 2002). So, 20 hours is the right time for determination of anti microbial activity. These two bacteria are mesophiles bacteria, which they

grow best in temperature ranging from 20ºC to about 40ºC, though they can survive at higher and lower temperature (Bauman, 2011). Temperature of 37ºC is chosen because normal body temperature is approximately 37ºC.

Bacteria suspension is made by adding NaCl 0.9 % isotonic solution because if hypertonic solution is added, the cell will loose water and the cell can die from crenation, or shriveling of its cytoplasm. And if hypotonic solution is added, the cell will gain water from its environment and swells to the limit of its cell wall until they burst (Bauman, 2011). Bacteria suspension that is made is measured at λ 580 nm until optical density of 0.6 is obtained. λ 580 nm is chosen because in λ 580 nm, bacteria, yeast, and molds can be examined well. Optical density 0.6 is obtained from the absorbance based on 0.5-1 McFarland scale.

Disk diffusion method is used because this method is straight forward to perform, reproducible, and does not require expensive equipment (Bauman, 2011). Antibiotic Medium I is used because this medium contained nutrition that provides bacteria to grow, also the control, comparator and testing solution can diffuse well and it is used for antibiotic assay testing (Atlas, 2004).

The red fruit extracts and the red fruit oil are diluted in the appropriate solvent (The solvent must dilute the extract well and does not have antimicrobial activity). After orientation to search for appropriate solvent, DMSO 30% in demineralized water is used as solvent for the ethanolic extracts, because DMSO 30% in demineralized water does not have an inhibition zone diameter and can dilute the extract well, marked by the diffusion of the extract in the medium (brown color of the extract diffuse to the medium). And acetone is used as the solvent for the hexane extracts and the red fruit oil, because acetone does not have an inhibition zone diameter and can dilute the extract well, marked by the orange color of the solution of hexane extract pre-treated by DIC.

There are two control solutions for each extract. For the ethanolic extracts, the control solutions are the mixture of DMSO 30% in demineralized water; and cab-o-sil which is diluted in the DMSO 30% in demineralized water based on the percentage of cab-o-sil in the bulk. For the hexane extract, the control solutions are acetone; and cab-o-sil which is diluted in the acetone based on the percentage of cab-o-sil in the bulk. And for the red fruit oil, acetone is used for the control solutions. Control solution is used to know whether there is no effect of inhibition zone diameter of the solvents and cab-o-sil. Amoxicillin is used as comparator solution because

Escherichia coli and *Staphylococcus aureus* is susceptible to amoxicillin (Frank & Tacconelli, 2012).

3. 3 Antimicrobial Activity Of The Red Fruit Extract And Red Fruit Oil Against *Staphyloccoccus aureus* and *Escherichia coli*

3.3.1 Antimicrobial Activity of Red Fruit Oil

The result of antimicrobial activity of the Red Fruit Oil against *Staphyloccoccus aureus* can be seen in **table 3, appendix 5 and 6 (p. 88 n p. 104).**

Table 3. The Result of Inhibition Zone Diameter of The Red Fruit Oil against *Staphyloccoccus aureus*

R	Various concentration of oil (ppm)						
	Inhibition Zone Diameter (cm)						Comparator (amoxicillin)
1	Pure Oil	46,738.3	190,731	297,644	361,972	745,480	20.04 ppm
	0	0	0	0	0	0	1.62
2	Pure Oil	52,106.3	176,148	300,480	351,299	746,532	20.8 ppm
	0	0	0	0	0	0	1.76
3	Pure Oil	51,259.35	200,902.9	332,356.5	391,905	713,159.1	20.72 ppm
	0	0	0	0	0	0	1.86

Control Solution: Acetone (used as solvent) does not give an inhibition zone diamete
From the result above, it can be seen that pure red fruit oil and the red fruit oil at the concentrations of 46,738.3 ppm – 746,532 ppm do not show an inhibitory activity toward *Staphyloccoccus aureus*.

Table.4. The Result of Inhibition Zone Diameter of The Red Fruit Oil against *Escherichia coli*

R	Various concentration of oil (ppm)						
	Inhibition Zone Diameter (cm)						Comparator (amoxicillin)
1	Pure Oil	46,738.3	190,731	297,644	361,972	745,480	20.04 ppm
	0	0	0	0	0	0	1.64
2	Pure Oil	52,106.3	176,148	300,480	351,299	746,532	20.8 ppm
	0	0	0	0	0	0	1.60
3	Pure Oil	51,259.35	200,902.9	332,356.5	391,905	713,159.1	20.72 ppm
	0	0	0	0	0	0	1.89

Control Solution: Acetone (used as solvent) does not give an inhibition zone diameter
The picture can be observed in Appendix 6 (p.109).

From the result above, it can be seen that pure red fruit oil and the red fruit oil at the concentrations of 46,738.3 ppm – 746,532 ppm do not show an inhibitory activity toward *Escherichia coli.*

3.3.2. Antimicrobial Activity of Ethanolic Extract of Red Fruit with DIC Pre-Treatment.

The result of antimicrobial activity of ethanolic extract of red fruit with DIC pre-treatment against *Staphyloccoccus aureus* can be seen in **table 5** and **appendix 4 and 5.**

Table 5. The Result of Inhibition Zone Diameter of the Ethanolic Extract of the Red Fruit with DIC Pre-Treatment against *Staphyloccoccus aureus*

R	Various concentration of extract (ppm)					
	Inhibition Zone Diameter (cm)					Comparator (amoxicillin)
1	13,690.5	15,285.7	17,565	23,015.7	27,717.2	20.04 ppm
	0	0	0	0	0	1.87
2	13,104.7	15,598.7	18,355.8	23,765.5	27,276	20.8 ppm
	0	0	0	0	0	1.57
3	13,508.72	13,614.33	20,528.35	23,262.92	25,807.22	20.72 ppm
	0	0	0	0	0	1.86

Control Solution: DMSO 30% in demineralization water (used as solvent) and Cab O-Sil diluted in DMSO 30% do not give an inhibition zone diameter

From the result above, it can be seen that ethanolic extracts of the Red Fruit with DIC Pre-Treatment at the concentrations of 13,104.7 ppm – 27,717.2 ppm do not show an inhibitory activity toward *Staphyloccoccus aureus.*

The result of antimicrobial activity of the Ethanolic extract of the red fruit with DIC pre-treatment against *Escherichia coli* can be seen in **table 6** and **appendix 5 (p.88) and 6 (p 105).**

Table 6 The Result of Inhibition Zone Diameter of the Ethanolic Extract of the Red Fruit with DIC Pre-Treatment against *Escherichia coli*

R	Various concentration of extract (ppm)					
	Inhibition Zone Diameter (cm)					Comparator (amoxicillin)
1	13,690.5	15,285.7	17,565	25,918.1	29,034.9	20.04 ppm
	0	0	0	0	0	1.46
2	12,611.1	15,598.7	18,355.8	23,765.5	27,276	20.8 ppm
	0	0	0	0	0	1.37
3	13,508.72	13,614.33	20,528.35	23,262.92	25,807.22	20.72 ppm
	0	0	0	0	0	1.49

Control Solution: DMSO 30% in demineralization water (used as solvent) and Cab O-Sil diluted in DMSO 30% do not give an inhibition zone diameter

From the result above, it can be seen that the ethanolic extracts of the Red Fruit with DIC Pre-Treatment at the concentrations of 12,611.1 ppm – 29,034.9 ppm do not show an inhibitory activity toward *Escherichia coli.*

3.3.3. Antimicrobial Activity of the Ethanolic Extract of the Red Fruit without DIC Pre-Treatment

The result of antimicrobial activity of the Ethanolic extract of the red fruit without DIC pre-treatment against *Staphyloccoccus aureus* can be seen in **table 7** and **appendix 5 (p.88) and 6 (p.106) .**

Table 7. The Result of Inhibition Zone Diameter of the Ethanolic Extract of the Red Fruit without DIC Pre-Treatment against *Staphyloccoccus aureus*

R	Various concentration of extract (ppm)					
	Inhibition Zone Diameter (cm)					Comparator (amoxicillin)
1	11,021.9	12,521.1	14,718.1	15,525.9	22,629.9	20.4 ppm
	0	0	0	0	0	1.87
2	11,402.1	15,431.1	17,003.3	19,291.7	23,445.2	20.4 ppm
	0	0	0	0	0	1.82
3	11,561.9	14,701.1	15,778.7	19,683.8	22,236.3	20.4 ppm
	0	0	0	0	0	1.79

Control Solution: DMSO 30% in demineralization water (used as solvent) and Cab O-Sil diluted in DMSO 30% do not give an inhibition zone diameter

From the result above, it can be seen that the ethanolic extracts of the Red Fruit without DIC Pre-Treatment at the concentrations of 11,021.9 ppm – 23,445.2 ppm do not show an inhibitory activity toward *Staphyloccoccus aureus.*

The result of antimicrobial activity of the Ethanolic extract of the red fruit without DIC pre-treatment against *Escherichia coli* can be seen in **table 8** and **appendix 5 and 6 (p.107).**

Table 8. The Result of Inhibition Zone Diameter of the Ethanolic Extract of the Red Fruit without DIC Pre-Treatment against *Escherichia coli*

R	Various concentration of extract (ppm)					
	Inhibition Zone Diameter (cm)					Comparator (amoxicillin)
1	15,621.11	17,513.04	20,165.58	24,643.12	28,860.78	20.72 ppm
	0	0	0	0	0	1.83
2	12,064.8	14,860.1	18,328	22,180.8	24,043	20.8 ppm
	0	0	0	0	0	1.78

3	9,180.2	14,064.1	18,004.2	22,044.1	27,720.6	20.4 ppm
	0	0	0	0	0	1.78

Control Solution: DMSO 30% in demineralization water (used as solvent) and Cab O-Sil diluted in DMSO 30% do not give an inhibition zone diameter

From the result above, it can be seen that the ethanolic extracts of the Red Fruit without DIC Pre-Treatment at the concentrations of 9,180.2 ppm – 28,860.78 ppm do not show an inhibitory activity toward *Escherichia coli.*

3.3.4. Antimicrobial Activity of the Hexane Extract of Red Fruit with DIC Pre-Treatment

The result of antimicrobial activity of the hexane extract of the red fruit with DIC pre-treatment against *Staphyloccoccus aureus* can be seen in **table 9** and **appendix 5 (p.88) and 6 (p.107).**

Table 9. The Result of Inhibition Zone Diameter of the Hexane Extract of the Red Fruit with DIC Pre-Treatment against *Staphyloccoccus aureus*

R	Various concentration of extract (ppm)					
	Inhibition Zone Diameter (cm)s					Comparator (amoxicillin)
1	21,384.1	24,186.9	31,486.2	40,185.8	**65,988.2**	20.04 ppm
	0	0	0	0	0	1.85
2	22,456.1	24,312.2	31,660.4	40,671.6	61,300.3	20.8 ppm
	0	0	0	0	0	1.97
3	21,606.84	28,074.22	32,477.92	39,065.9	58,212.77	20.72 ppm
	0	0	0	0	0	1.76

Control Solution: Acetone (used as solvent) and Cab O-Sil diluted in Acetone do not give an inhibition zone diameter

From the result above, it can be seen that the Hexane extracts of the Red Fruit with DIC Pre-Treatment at the concentrations of 21,384.1 ppm – 65,988.2 ppm do not show an inhibitory activity toward *Staphyloccoccus aureus.*

The result of antimicrobial activity of the hexane extract of the red fruit with DIC pre-treatment against *Escherichia coli* can be seen in **table 10** and **appendix 5 (p.88) and 6 (p.108).**

Table 10. The Result of Inhibition Zone Diameter of the Hexane Extract of the Red Fruit with DIC Pre-Treatment against *Escherichia coli*

R	Various concentration of extract (ppm)					
	Inhibition Zone Diameter (cm)					Comparator (amoxicillin)
1	21,384.1	24,186.9	34,525.2	42,295.7	62,476.1	20.04 ppm
	0	0	0	0	0	1.76
2	22,456.1	24,312.2	31,660.4	40,671.6	58,287.9	20.8 ppm
	0	0	0	0	0	1.60
3	21,606.84	28,074.22	32,477.92	39,065.9	58,212.77	20.72 ppm
	0	0	0	0	0	1.76

Control Solution: Acetone (used as solvent) and Cab O-Sil diluted in Acetone do not give an inhibition zone diameter

From the result above, it can be seen that the Hexane extracts of the Red Fruit with DIC Pre-Treatment at the concentrations of 21,384.1 ppm – 62,476.1 ppm do not show an inhibitory activity toward *Escherichia coli.*

3.3.5. Antimicrobial Activity of the Hexane Extract of Red Fruit without DIC Pre-Treatment

The result of antimicrobial activity of the hexane extract of the red fruit without DIC pre-treatment against *Staphyloccoccus aureus* can be seen in **table 11** and **appendix 5 (p.88) and 6 (p. 108).**

Table 11. The Result of Inhibition Zone Diameter of the Hexane Extract of the Red Fruit without DIC Pre-Treatment against *Staphyloccoccus aureus*

R	Various concentration of extract (ppm)					
	Inhibition Zone Diameter (cm)					Comparator (amoxicillin)
1	23,096.4	26,943.2	34,401.3	38,340.2	57,386.1	20.04 ppm
	1	1.06	1.11	1.14	1.24	2.13
2	22,841.4	26,644	33,664.3	40,101.1	58,242.8	20.04 ppm
	1.05	1.10	1.17	1.25	1.53	2.09
3	23,246.5	27,753.3	31,216.8	37,910.7	58,322.3	20.04 ppm
	0.99	1.02	1.07	1.09	1.22	2.25

Control Solution: Acetone (used as solvent) and Cab O-Sil diluted in Acetone do not give an inhibition zone diameter

From the result above, it can be seen that the Hexane extracts of the Red Fruit without DIC Pre-Treatment at the concentrations of 22,841.4 ppm – 58,322.3 ppm show an inhibitory activity toward *Staphyloccoccus aureus.*

From the first replication, it is obtained that the regression equation is y=0.000007x+0.866 with r_{value} is 0.9844. From the second replication, it is obtained that the regression equation is y=0.00001x+0.733 with r_{value} is 0.9950. From the third replication, it is obtained that the regression equation is y=0.000006x+0.851 with r_{value} is 0.9930. According to regression equation of each replication, it is obtained that the $r_{value} > r_{table}$ (α=0.05; n=5; r_{table}=0.878). It can be seen that the inhibition zone diameter is increasing with the increase of extract concentration.

From the average of intercept and slope of the three replication, the regression equation for hexane extract of red fruit without DIC pretreatment against *Staphyloccoccus aureus* is y=0.0000077x+0.8167, With an intercept (a=0.8167) dan slope (b=0.0000077). The example of calculation can be seen in **appendix 7 (p.114).**

The result of antimicrobial activity of the hexane extract of the red fruit without DIC pre-treatment against *Escherichia coli* can be seen in **table 12**, **appendix 5 (p.88) and 6 (p.113).**

Table 12. The Result of Inhibition Zone Diameter of the Hexane Extract of the Red Fruit without DIC Pre-Treatment against *Escherichia coli*

R	Various concentration of extract (ppm)					
	Inhibition Zone Diameter (cm)					Comparator (amoxicillin)
1	42,041.7	43,426.2	46,887	48,455.5	56,904.7	20.4 ppm
	1.12	1.15	1.19	1.20	1.28	1.72
2	42,014.9	45,767.2	48,574.5	53,189	58,432.6	20.72 ppm
	1.09	1.13	1.19	1.25	1.28	1.83
3	39,339.1	44,374.9	49,977.2	54,464.2	57,334.7	20.04 ppm
	1.05	1.10	1.12	1.19	1.21	1.80

Control Solution: Acetone (used as solvent) and Cab O-Sil diluted in Acetone do not give an inhibitions zone diameter

From the result above, it can be seen that the Hexane extracts of the Red Fruit without DIC Pre-Treatment at the concentrations of 39,339.1 ppm – 58,432.6 ppm show an inhibitory activity toward *Escherichia coli.*

From the first replication, it is obtained that the regression equation is y=0.00001x+0.687 with r_{value} is 0.9869. From the second replication, it is obtained that the regression equation is y=0.00001x+0.59 with r_{value} is 0.9859. From the third replication, it is obtained that the

regression equation is y=0.000009x+0.711 with r_{value} is 0.9829. According to regression equation of each replication, it is obtained that the $r_{value} > r_{table}$ (α=0.05; n=5; r_{table}=0.878). It can be seen that the inhibition zone diameter is increasing with the increase of extract concentration.

From the average of intercept and slope of the three replication, the regression equation for the hexane extract of the red fruit without DIC pre-treatment against *eschericia coli* is y= 0.00000977x+0.6627 With an intercept (a=0.6627) dan slope (b=0.00000977). The example of calculation can be seen in **appendix 7 (p.114).**

F.1.3.6. Comparison of Antimicrobial Activity of Red Fruit Extract and Red Fruit Oil Against *Eschericia coli* and *Staphylococcus aureus*

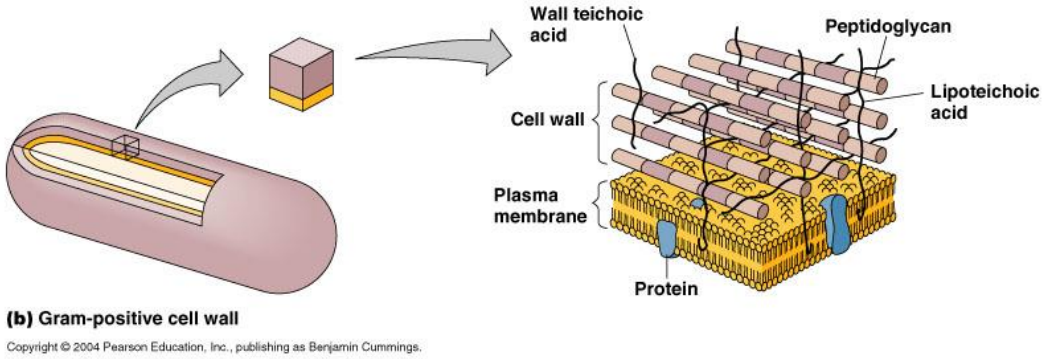


Fig. 18. Cell Wall and Cytoplasmic Membrane of Gram Positive Bacteria

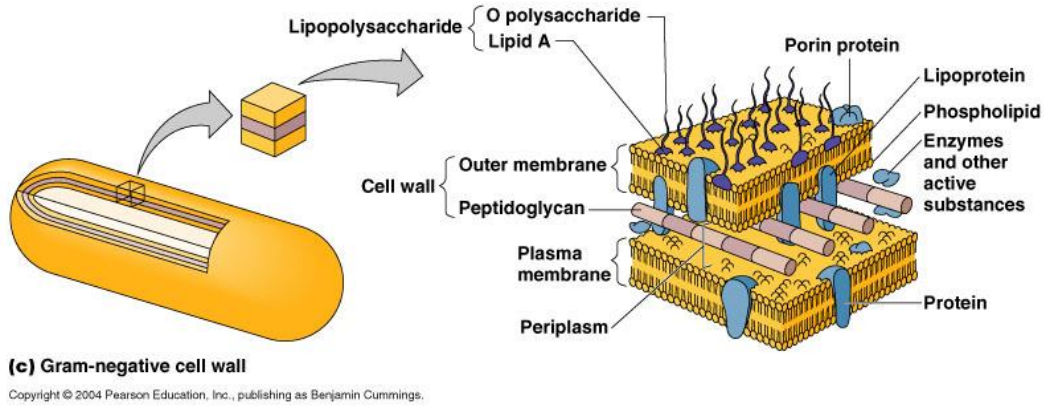


Fig.19. Cell Wall and Cytoplasmic Membrane of Gram Negative Bacteria

The red fruit oil does not show an inhibitory activity toward *Eschericia coli* and *Staphyloccoccus aureus* because the composition of red fruit oil is dominated with fat (75.28%) (Subroto,2005) which means that the red fruit oil consists of hydrophobic components in general. More over, in gram negative bacteria (*Eschericia coli*), most of hydrophobic components can not penetrate beyond the outer surface of polisaccharides and are kept out of the cell **(fig 19)** (Kane

& Kandel, 2001)**.** And the cell wall of gram positive bacteria (*Staphyloccoccus aureus),* consists of many layers of peptidoglycan, forming a thick, rigid structure **(fig 18).** Peptidoglycan consists of a repeating disaccharide attached by polypeptides, therefore it contains more polar substances. So, the chemicals in the red fruit oil can not diffuse through the bacterial cell wall and can not inhibit the bacterial growth.

A research result obtained from Tan (2008), **PCO**, Pandanus Cocos Oil, The mixture of red fruit oil and coconut oil, does not show an inhibitory activity toward S*taphylococcus aureus* and *Escherichia coli*. And a result from Subroto, (2005), **PCO** shows an inhibitory activity toward S*taphylococcus aureus* and *Escherichia coli.* These two uncertain research results above is the proof that a further study about the antimicrobial activity of red fruit oil and also virgin coconut oil is needed to be observed.

The examples of active substances that contained in the ethanolic extracts are total phenol and flavonoid. The examples of phenols which is toxic to microorganism are catechin and pyrogallol that are found in vegetable tannins. Tannins are also toxic to fungi, bacteria and viruses and inhibit their growth. Gallic acid, tannic acid, and elagic acid are also found have antibacterial, antifungal and antioxidant properties (Colak, Yapici, & Yapici, 2010).

But, in this research, the ethanolic extracts of the red fruit both of pre-treated and untreated by DIC do not show an inhibitory activity toward *Eschericia coli* and *Staphyloccoccus aureus.* This can happen because probably due to the high percentage of cab-o-sil in the ethanolic extract (±75%) which probably cause an amount of active substances in etanolic extract can not dissolve completely because the extract is bound to a high percentage of cab-o-sil. So, for the further study, the percentage of cab-o-sil in bulk is needed to be reduced. And the other method, for example, freeze drying can be considered to preserve the extract, besides the use of cab-o-sil. In this study, a polar solvent (ethanol 60%) and a non polar solvent (hexane) are used as the solvent for extraction. A further study is needed to search for the solvent for extraction. Probably, a semi polar solvent can be used for extraction in order to dilute not only polar, but also non polar active substances.

Hexane extract of the red fruit without DIC pre-treatment shows an inhibitory activity toward *Eschericia coli* and *Staphylococcus aureus* because hexane extract contains more non polar (lipophilic / hydrophobic) substances. One of the active substance that is contained in the hexane extract is tocopherol.

The envelope of gram negative bacteria (*Eschericia coli*) consists of outer membrane which is bilayer. The inner layer is composed of phospholipid, and the outer layer is composed of mainly Lipopolysaccharide **(LPS).** Highly lipophilic compounds such as steroids penetrate relatively easily through the outer membrane of several bacteria (Sikkema et al, 1995). So, active substances in the hexane extract of the red fruit without DIC pre-treatment can diffuse through cell wall of gram negative bacteria into the cytoplasmic membrane and give antimicrobial effect.

Besides, the cell wall of gram positive bacteria (*Staphylococcus aureus*) also contains teichoic acids, which consists primarily of an alcohol (such as glycerol or ribitol) and phosphate. There are two classes of teichoic acids: lipoteichoic acid and wall teichoic acid (Tortora,2012). Lipoteichoic acid **(LTA),** a heat-stable component of the cell membrane and the wall of most gram-positive bacteria, has structure similarity to **LPS** (Grunfeld et all, 2014). So, **LTA** is a hydrophobic component; and the active substances in the hexane extract of the red fruit without DIC pre-treatment can diffuse through the cell wall of gram positive bacteria into the cytoplasmic membrane and gives antimicrobial effect.

Also, the cytoplasmic membrane has a low permeability for polar and charged molecules. Non polar compounds can easily penetrate the lipid bilayer. The transfer of such molecules across the membrane, therefore, is most probably a diffusion process. The accumulation of lipophilic compounds into lipid bilayers may cause toxicity problems. Solutes that interact with the membrane will cause different perturbations of the bilayer depending on their preferential site of accumulation (Sikkema et al, 1995).

From the **table 9-12 (p. 48-50)**, it can be seen that the hexane extract of red fruit without DIC pre-treatment shows an inhibitory activity toward *Eschericia coli and Staphylococcus aureus*, but hexane extracts of red fruit with DIC pre-treatment does not. It can be probably because DIC pre-treatment followed by injection of saturated steam 100–144 °C at a fixed pressure level and maintained for a predetermined time (Allaf, 2009). This exposure to very high temperature steam may degrade some anti microbial active substances in the red fruit extract which need a further study to isolate all component in the red fruit extract that may be degrade during DIC process.

To compare the sensitivity of antimicrobial activity of hexane extract of red fruit without DIC pre-treatment between *Eschericia coli and Staphyloccoccus aureus,* the comparation of slope in each replication is used because in the correlation analyses, slope measures the strength

of relationship between X(Concentration) and Y(diameter) and slope is the characteristic of that two variables. So, slope can be used as the standard of comparison.

Table 13. Slope Comparison of the Regression of Antimicrobial Activity of Hexane Extract of Red Fruit without DIC Pre-Treatment Against *Eschericia coli and Staphyloccoccus aureus* Analyzed using Two-Sample T-Test Statistical Method

Two-sample T for ec vs sa

	N	Mean	StDev	SE Mean
ec	3	0.000009667	0.000000577	0.00000033
sa	3	0.00000767	0.00000208	0.0000012

Difference = mu (ec) - mu (sa)
Estimate for difference: 0.000002
95% CI for difference: (-0.000001, 0.000005)
T-Test of difference = 0; T-Value = 1.60; P-Value = 0.184; DF = 4
Both use Pooled StDev = 0.0000

From **table 13**, it can be seen that $t_{value} < t_{table}$ (two sided, α=0,05, DF=4, t_{table}=2.776) and $p_{value} >$ 0.05. It means that the inhibitory activity of the hexane extract without DIC pre-treatment toward *Eschericia coli* and *Staphyloccoccus aureus* is not significantly difference.

Based on **table 11 (p. 49)** and **table 12 (p. 50),** the hexane extracts of red fruit without DIC pre-treatment at the concentrations of 22,841.4 ppm – 58,322.3 ppm with the inhibition zone diameter of 9.9 mm-15.3 mm against *Staphyloccoccus aureus* and at the concentrations of 39,339.1 ppm – 58,432.6 ppm with the inhibition zone diameter of 10.5 mm-12.8 mm against *Eschericia coli* can not be considered as potent as amoxicillin with the inhibition zone diameter of 17.2 mm-22.5 mm.

The antimicrobial activity of all the ethanolic and hexane extracts and the red fruit oil are not as potent as amoxicillin. This may be due to the kinetic maceration extraction method which can not extract all the active substances optimally. A further study is needed to search for another method of extraction, for example kinetic re-maceration (repeatedly kinetic maceration).

MIC is the smallest amount of the drug that will inhibit growth and reproduction of the pathogen. While, MBC is the amount of drug required to kill the microbe rather than just the amount to inhibit it, as the MIC does (Bauman, 2011). For hexane extract of red fruit without DIC pre-treatment, the further research about MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration), for example using broth and agar dilution method, should be determined as the continuation of disk diffusion method.

Conclusion

Based on the experiment, the red fruit oil and all the ethanolic extracts obtained from the red fruit powder pre-treated and untreated by DIC do not show an inhibitory activity toward *Eschericia coli* and *Staphyloccoccus aureus*. Also, the hexane extract from DIC pre-treatment does not show an inhibitory activity toward *Eschericia coli* and *Staphyloccoccus aureus*, whereas that of the untreated shows an inhibitory activity, but is not as potent as amoxicillin.

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17.00		Thi Kieu	products by liquid chromatography-tandem mass spectrometry
17.00-17.10	PPCP-12	Aliza Alias	Evaluation of Immunological and Virological Response of First Line Antiretroviral Therapy (ART) for Non-Experience Patient in Malaysia
KARATON I			
16.20-16.30	PPCP-13	Nguyen Duc Tuan	Development, validation, and application for simultaneous assay of amlodipine, atorvastatin, ortho- and para-hydroxy atorvastatin as metabolites in human plasma by liquid chromatography- tandem mass spectrometry
16.30-16.40	PPCP-14	Mizaton Hazizul Hasan	Myrmecodia platyrea methanol tuber extract ameliorates hyperglycaemia in STZ-induced diabetic rats
16.40-16.50	PPCP-15	Purwantinin gsih	Effect of Eurycoma longifolia Roots Extract on Phase II Liver Drug Metabolism; Glutathion-S-Transferase (GST) Activity
16.50-17.00	PPCP-16	Tasya Oesricha Pakki	Validity and Reliability Test of Blood Glucose Self-Monitoring Devices and The Impact Study of Sample Collection Location to the Blood Glucose Measurement Results
17.00-17.10	PPCP-17	Putu Rika Veryanti	Effectiveness of Bronchodilator and Corticosteroid Treatment in Patients with Chronic Obstructive Pulmonary Disease (COPD)

PARALLEL SESSIONS III DAY 2 (09.00 – 09.40)

PEMANDENGAN I			
09.00-09.10	PESC-21	Khaled Alakhali	Evaluation of Quality of Life in Diabetic Patients of Aseer Region, Kingdom of Saudi Arabia.
09.10-09.20	PESC-22	Elis Cholisah	Patterns of Adherence Towards Oral Hypoglycemic Agents (OHA) and Their Effects On Major Clinical Outcomes Among Type 2 Diabetes Mellitus (T2DM) Patients In Bandung, Indonesia
09.20-09.30	PESC-23	Quynh Thi Huong Bui	Impact of a pharmacist-led educational intervention on quality of life among patients with asthma
09.30-09.40	PESC-24	Quyen Thi Ngoc Phan	An Assessment of Cough Medicine Dispensing Practices to Children Under 2 Years Old in Pharmacies in Ho Chi Minh City By Using Simulated-Patient Method
PEMANDENGAN II			
09.00-09.10	HMNP-15	Bui Minh	Antioxidant and cytotoxic constituents from Alangium salviifolium (L.f.) Wang., Alangiaceae
09.10-09.20	HMNP-16	Khusnul Fadhilah	Fruit residue as source of natural bioactive compound : Bioactive compound from Lansium domesticum Corr. fruit peel and its cytotoxicity against T47D cell line
09.20-09.30	HMNP-17	Yuliet	Improved Insulin Sensitivity by Ethanolic Extract of Hibiscus surattensis L. Leaves and Its Fraction in High Fat and Fructose Diet Rats
09.30-09.40	HMNP-18	Dian Natasya Rahardjo	The Antimicrobial Activities of The Extracts of Red Fruit (Pandanus conoideus Lam.) Pre-dried by Détente Instantanée Contrôlée (DIC)
PEMANDENGAN III			
09.00-09.10	HMNP-11	Rony Syahputra	Determination of Total Cardiac Glycoside on Vernonia amygdalina Leaves
09.10-09.20	HMNP-12	Odilia Dea Christina	The Effect Of Basil Leaf (Ocimum sanctum L.) Extract On Expression Of p53 And Bcl-2 In T47D Breast Cancer Cells
09.20-09.30	HMNP-13	Fajar Fakri	Acute Toxicity of Keladi Tikus (Thyphonium flagelliforme (Lodd.) Blume) Ethanol Extract on Zebrafish (Danio rerio) Embryo in vivo
09.30-09.40	HMNP-14	Mazlina Mohd Said	Assessment of Curcuma xanthorrhiza as an alternative treatment for hyperhidrosis
PEMANDENGAN IV			
09.00-09.10	PPCP-18	Vithyah Nadaraja	Vancomycin Dosing Requirements in Obese and Non-Obese Adult Patients in Tengku Ampuan Rahimah Hospital, Malaysia
09.10-	PPCP-19	Andri	Analysis of Filgrastim Primary Prophylaxis on Breast Cancer