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Improving the Safety of Blood Products (Packaged Red Blood Cell) through Anti-Bacterial Activity of Novel Synthesized Chalcone Against Isolated *S. epidermidis* and *S. aureus*

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ABSTRACT Chalcone was a compound with several activities and one of them was antibacterial activity. Bacterial contamination in blood product was still a problem in Indonesia. Various studies to improve the safety of blood product by inhibiting the bacterial growth in blood product was developed. Chalcone, is one of the natural compounds that has ability to inhibit the bacterial growth, so it can be possible to use that compound for anti-bacterial use in the future. This study aim is to evaluate the anti-bacterial activity of novel synthesis chalcone (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one against *S. epidermidis* and *S. aureus* isolated from packed red cell blood product. A novel chalcone was synthesized by Claisen-Schmidt Methods while anti-bacterial activity test was done by Agar Diffusion Methods. Chalcone that has been synthesized then characterized by Fourier Transform Infra-Red (FTIR) Methods and Gas Chromatography – Mass Spectrometry (GC-MS). Chalcone was synthesized through a condensation reaction between an aldehyde (benzaldehyde) and ketone (acetophenone) with NaOH as alkaline catalyst. The results from sedimentary analysis using FTIR showed an absorption of the C=C group in the typical medium of chalcone compounds, at the wave number of 1516.96 cm^{-1}. The results of GC-MS characterization resulted a GC chromatogram with one peak with time of retention (tR) 19.68 minutes and relative purity 100%. MS mass spectrum shows that the molecular ion (M^+) of this compound was detected at 224 which equivalents to the molecular weight of the chalcone compound. Anti-bacterial test showed that the synthetic chalcone compound with 5% concentration had an inhibitory ability of 56.02% on *S. epidermidis* and 54.10% on *S. aureus*, while 2.5% chalcone concentration had an inhibitory ability of 29.17% on *S. epidermidis* and 50.45% on *S. aureus*, and 1.25% chalcone concentration had inhibitory ability 0% on *S. epidermidis* and 37.27% on *S. aureus*. A novel synthesized chalcone one this study has good inhibitory activity against *S. epidermidis* and *S. aureus*. The community could access the packaged red blood cell blood products that free from bacterial contamination.

INDEX TERMS anti-bacterial activity, blood products, novel chalcone synthesis.

I. INTRODUCTION

Contamination of bacteria on blood products was still a problem in transfusion medicine [1]. Packed Red Cells (PRC) was blood products with highest bacterial contamination. The largest number of bacteria isolated from PRC were *Staphylococcus epidermidis* (*S. epidermidis*) and *Staphylococcus aureus* (*S. aureus*) [2]. Contaminated blood products could become the cause of morbidity and mortality when it given to the recipients [3].

Chalcone was composed by aromatic ketone and enone in an open chain form [4]. Chalcone also described as benzyl acetophenone with two fragrant rings (rings A and B) that connected by three aliphatic carbon series [5]. Chalcone

belongs to natural biocides with α, β-unsaturated ketone chain on its structure. Chalcone was had various biological activities such as antimicrobial, anti-inflammatory, anticancer and antiviral [6].

Various studies have described that chalcone has antibacterial activity against *S. aureus*. Newly synthesized chalcone of (E)-1-(2-hydroxyphenyl)-3-(2,4-dimethoxy-3-methylphenyl)prop-2-en-1-one has ability to work synergistically modulate the effect of Norfloxacin against *S. aureus* [7]. Chalcones inhibit the growth of *S. aureus* by inhibiting the sortase A (srtA) gene [8]. The chalcone compound named (E)-3-(4-(dimethylamino)phenyl)-1-phenylprop-2-en-1-one has inhibitory activity against several blood products contaminant such as *S. aureus* [9].

Activity of chalcone against *S. epidermidis* was less-researched. *S. epidermidis* was found as contaminant on 4 of 100 samples of PRC in Iraq [10]. *S. epidermidis* was frequently found on human skin [11]. Touching the disinfected site while collecting blood was one of the factors that promote contamination of bacteria on blood products [12].

Antimicrobial activity of chalcone must be investigated. This study aim was to evaluate the anti-bacterial activity of novel synthesis chalcone (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (FIGURE 1) against *S. epidermidis* and *S. aureus* isolated from packed red cell blood product, by measuring the inhibitory zone of novel synthesized chalcone on *S. epidermidis* and *S. aureus*.

The difference between this research and other studied were from the solvent. On this research, we use methanol as solvent, and the synthesis results was checked on gram positive bacteria only.

II. METHODDHOLOGY

The methods on this research was using Claisen Schmidt condensation methods that has done by Attardee [13]. The research of chalcone synthesis that has done by Asiri [14] shown that chalcone derivatives from pyrazoline can inhibit the gram positive and negative bacteria. Chalcone has wide spectrum anti-bacterial activity [15].

Chalcone synthesis was done by Claisen-Schmidt condensation methods while antibacterial activity testing was done by agar-diffusion methods. This research methods have received ethical clearance with number: KE/1039/09/2019 that has issued by Medical Ethics Committee of Universitas Gadjah Mada.

A. CHALCONE SYNTHESIS PROCEDURES

Chalcone synthesis refers to [13] with several modification. A piece of natrium hydroxide base was dissolved at 5 mL methanol. Add 5 mmol 4-hydroxyacteophenone drop by drop to the 5 mL methanol. The mixture was stirred for 2 minutes, then add 5 mmol benzaldehyde drop by drop to the 5 mL methanol. The mixture was stirred continuously at room temperature for 24 hours. The reaction was monitored by thin layer chromatography. Ice of aquadest was added when the reaction was finished then add 2 M HCl drop by drop until the sediment was formed. The sediment was filtered by filter

The materials on this research were having Pro-analytic quality from Sigma Aldrich: 4-hydroxyacetophenone, benzaldehyde, natrium hydroxide, hydrochloric acid, methanol, aquadest, nutrient agar (NA), brain-heart infusion (BHI), alcohol 70%, vancomycin (Merck Pro Analysis), dimethyl sulfoxide (Merck Pro Analysis), bacterial isolate of *Staphylococcus epidermidis* and *Staphylococcus aureus*. The tools to synthesis and characterization of novel synthesized chalcone was: round glassware, beaker glass, Erlenmeyer, glass funnel, magnetic stirrer, dilution glass, glass stirrer, digital scale, filter paper, pH meter, KLT plate, fourier transform infrared spectrophotometer (FTIR, Shimadzu Prestige 21), gas chromatography-mass spectrometry (GC-MS, AGILENT GC type 5973 Shimadzu QP 2010S), autoclave, incubator 5% CO_2 and Laminar Air Flow.

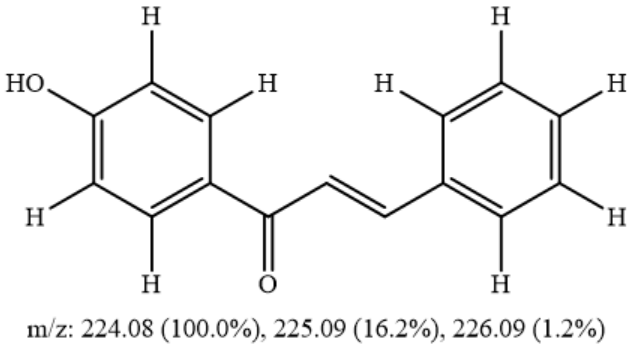

FIGURE 1. **(E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one**

paper. The reaction product was dried and weighed to calculate the product yields, then the product was characterized by FTIR spectrophotometer and GC-MS.

B. ANTI-BACTERIAL ACTIVITY TEST PROCEDURES

Chalcone was dissolved to dimethyl sulfoxide (DMSO) with variation of concentration 5.0%; 2.5%; and 1.25%. The negative control on this study was DMSO while the positive control was vancomycin 10.0%. Chalcone (25 µL) was injected to bacterial agar well. Bacterial inhibition zone was observed after 24 hours of incubation period and the inhibition zone was measured using a caliper. The inhibitory activity of chalcone against bacteria was expressed by the appropriate inhibitory power in the formula Eq. (1):

$$inhibitory\ ability = \frac{diameter\ of\ chalcone}{diameter\ of\ positive\ control} x100\% \quad (1)$$

There were several categories of anti-bacterial activity test. The antimicrobial activity obtained from the formula were categorized: strong if the percentage of inhibitory ability was ≥70%; quite strong if the percentage of inhibitory ability was in the range of 50-70% and weak if the percentage of inhibitory activity was <50% [16].

III. RESULT

The result of this research will be described on the following points. There are several points such as chalcone synthesis reaction, chalcone characterization by FTIR spectrophotometer and GC-MS, and chalcone antibacterial activity.

A. CHALCONE SYNTHESIS

Chalcone compounds are synthesized through a condensation reaction between aldehydes (benzaldehyde) and ketones (acetophenone) using a basic catalyst, namely NaOH. Afigurell materials dissolved in methanol solvent. The condensation reaction took place for 24 hours at room temperature with constant stirring and continuously monitored using thin layer chromatography to determine the maximum formation of reaction products. After 24 hours of stirring, the mixture was then added with distilled water and given drop by drop 2M HCl solution until a product precipitate was formed. The precipitate formed was filtered through filter paper and dried in a desiccator flask. The dry reaction product was then weighed to calculate the yield of the resulting reaction product: the yield obtained was 83.93% (0.94/1.12 gram). The prediction of chalcone synthesis forming was shown on fig. 2.

B. CHALCONE CHARACTERIZATION

The resulting precipitate was then characterized using FTIR and GC-MS. The results of the FTIR analysis (FIGURE 3) showed several functional group absorptions including the absorption of the carbonyl group (C=O) of ketones in a conjugated position with aromatic rings and olefins appearing at wave number 1620.21 cm^{-1}. Another absorption is the absorption of the C=C group of typical medium chalcone compounds at a wave number of 1516.96 cm^{-1}.

The compound characterization was continued using GC-MS and resulted in a GC chromatogram with 1 (one) peak with a retention time (tR) of 19.68 minutes and a relative purity of 100% (FIGURE 4).

The MS mass spectrum showed that the molecular ion (M+) of this compound was detected at 224 which was equivalent to the molecular weight of the chalcone compound (FIGURE 5), this result further confirmed that the target compound had been formed.

Based on the mass spectra obtained, it is estimated that the fragmentation pattern that occurs is as follows: the first stage is the release of C_6H_5 radicals to produce m/z 148 fragments, then the second stage is the release of C_2H_2 radicals and produces m/z 121 fragments which are the base peaks (FIGURE 6).

C. ANTIBACTERIAL ACTIVITY TESTING

The chalcone compounds that have been successfully synthesized and characterized were then tested for antibacterial activity using the agar diffusion method. Positive control used 10% vancomycin antibacterial compound. The test bacteria used consisted of Gram-positive bacteria, namely Staphylococcus epidermidis and Staphylococcus aureus. Exposure of chalcone compounds to the test bacteria was carried out for 24 hours in an incubator. After the exposure/incubation period is complete, the inhibition zone/clear zone produced by the chalcone compound is measured against the test bacteria using a caliper. The resulting clear zone data and the calculation results of the inhibition ability of chalcone compounds against test bacteria are presented in TABLE 1.

IV. DISCUSSION

The chalcone compound of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one was synthesized by Claisen-Schmidt condensation reaction. Claisen-Schmidt has been used for ~140 years to synthesized important compounds, and one of them was chalcone. One of the base catalysts that used on Claisen-Schmidt condensation reaction was NaOH. The hydroxy acetophenone would attach to carbonyl function on benzaldehyde to form a chalcone compound [17]. Claisen-Schmidt condensation reaction with NaOH resulting an excellent yields of α,α'-*bis*(substituted-benzylidene)cyclo-alkanones (96-98%) [18]. The dry reaction product on this research then weighed to calculate the yield of the resulting reaction product: the yield obtained was 83.93% (0.94/1.12 gram).

The absorption of the carbonyl group (C=O) of ketones in a conjugated position with aromatic rings and olefins appearing at wave number 1620.21 cm^{-1}. Another absorption is the absorption of the C=C group of typical medium chalcone compounds at a wave number of 1516.96 cm^{-1}. The typical C=C absorption of chalcone compounds is in the range (1500-1590cm^{-1}). The absorption that most confirms that the chalcone target compound has been formed is the absorption of the trans C-H bending vibration at a wave number of 986.77 cm^{-1} and the cis-C-H vibration at 898.21 cm^{-1}. These two cis-trans absorptions are a sign of the success of the condensation reaction between acetophenone and benzaldehyde [19]. From the FTIR analysis it can be strong indication that chalcone compound of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one has successfully synthesized.

The compound characterization was continued using GC-MS and resulted in a GC chromatogram with 1 (one) peak with a retention time (tR) of 19.68 minutes and a relative purity of 100% (FIGURE 4). The MS mass spectrum showed that the molecular ion (M+) of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one compound was detected at 224 which was equivalent to the molecular weight of the chalcone compound [20], this result further confirmed that the target compound had been formed. Based on the mass spectra obtained, it is estimated that the fragmentation pattern [21] that occurs is as follows: the first stage is the release of C_6H_5 radicals to produce m/z 148 fragments, then the second stage is the release of C_2H_2 radicals and produces m/z 121 fragments which are the base peaks. Mass spectral information of this synthesized compound indicate that it has been formed the target chalcone compound (E)-1-(4

hydroxyphenyl) -3-phenylprop-2-en-1-one with high level purity. The (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one resulted from the synthesis process above then being

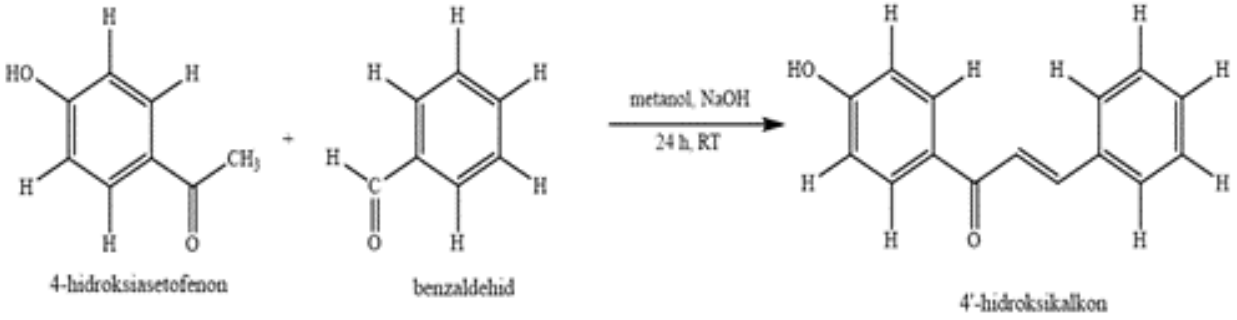

FIGURE 2. Chalcone forming reaction of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one by Claisen-Schmidt methods

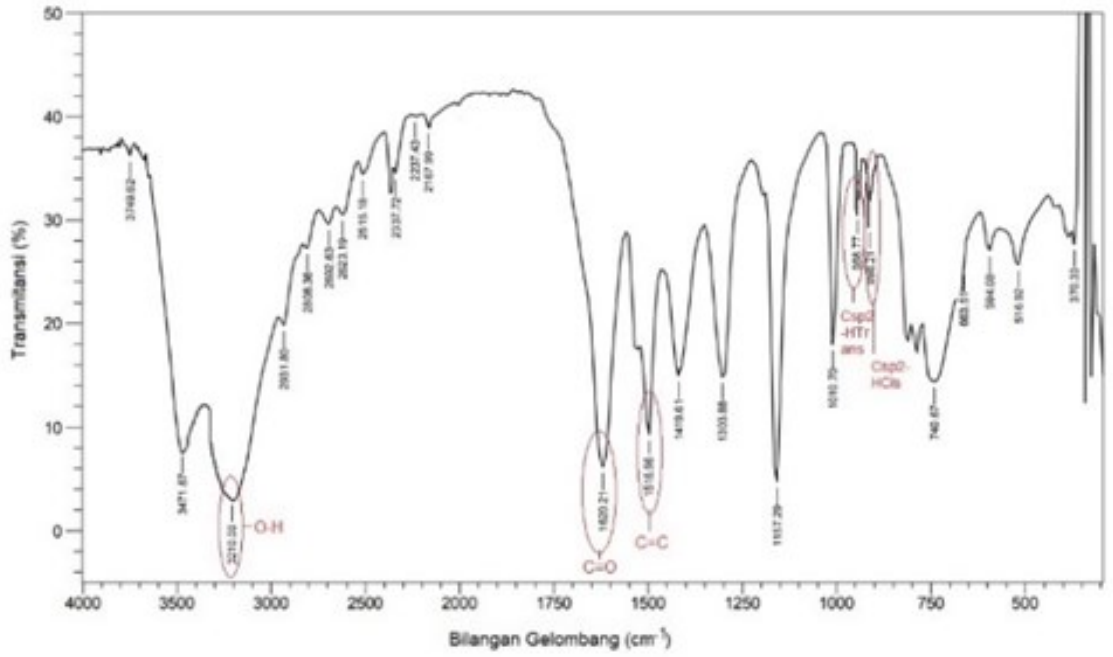

FIGURE 3. Fourier Transform Infra-Red (FTIR) Spectra result of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one compound

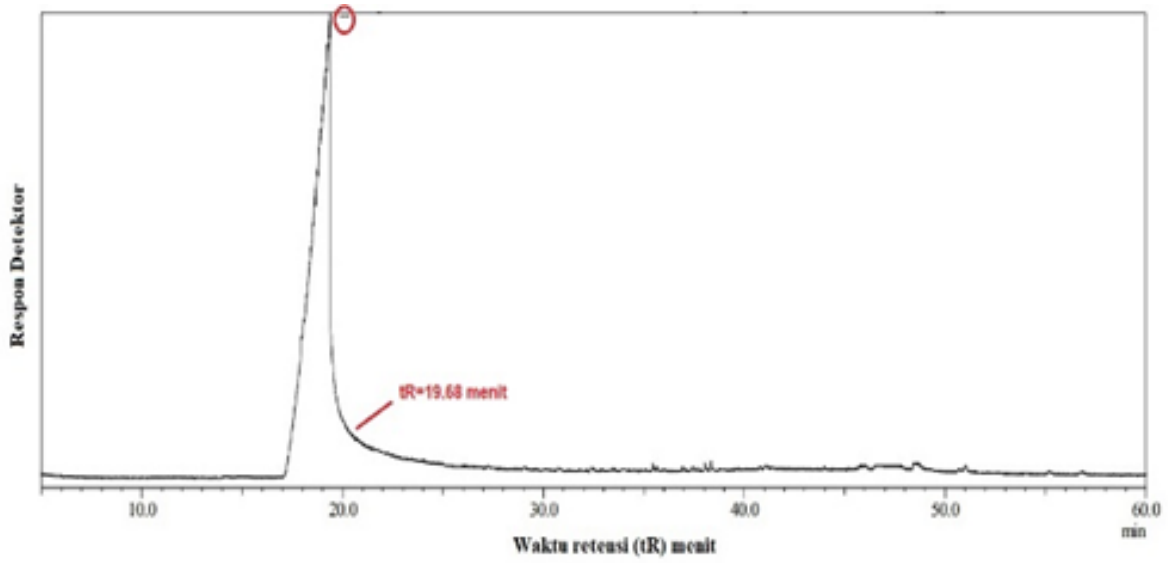

FIGURE 4. GC chromatogram of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one compound

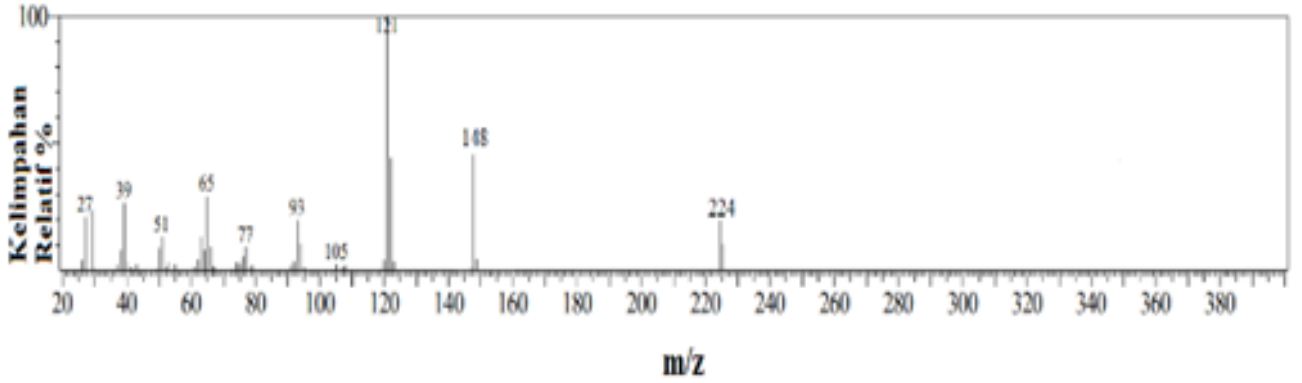

FIGURE 5. Mass spectra of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one compound

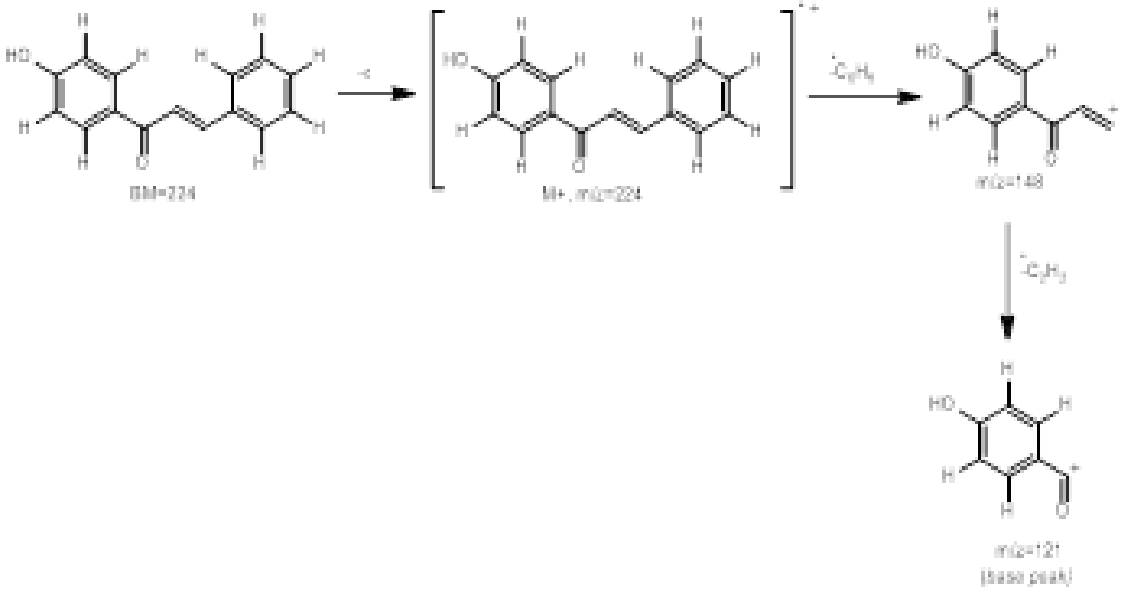

FIGURE 6. Fragmentation pattern of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one compound

TABLE I
Chalcone Inhibitory Activity Against Gram-Postive Bacteria

Chalcone Concentration	*Staphylococcus epidermidis*		*Staphylococcus aureus*	
	Clear zone (mm)	Inhibitory ability (%)	Clear zone (mm)	Inhibitory ability (%)
5.0%	12.1	56.02	11.9	54.10
2.5%	6.3	29.17	11.1	50.45
1.25%	0	0	8.2	37.27
Positive control	21.6	100.0	22.0	100.0
Negative control	0	0	0	0

Description:
Positive control : vancomycin
Negative control : dimethyl sulfoxide (DMSO)

tested of its antibacterial activity by agar diffusion methods. This method was chosen because it was an easy method, effective, efficient on consumable using and give an accurate result. The bacterial strain that used on this test was *Staphylococcus epidermidis* and *Staphylococcus aureus* that isolated from Packed Red Cells (PRC).

Chalcone compound of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one dissolved to dimethyl sulfoxide (DMSO) with concentration variation were 5.0%; 2.5%; and 1.25%. DMSO was chosen as solvent because it was not had antibacterial activity, so it could be negative control on this research. Chalcone compounds were made on three concentration variations, namely: 5.0%, 2.5% and 1.25% dissolved in dimethyl sulfoxide (DMSO) which also acted as a negative control, because it has been shown to have no inhibitory activity against bacteria [22]. The positive control

on this research was vancomycin 10.0%. Chalcone (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one (25 µL) was injected to bacterial agar well. Bacterial inhibition zone was observed after 24 hours of incubation period and the inhibition zone was measured using a caliper.

A compound can be classified to be good potential antibacterial compound if it can perform an inhibition zone > 6 mm and classified to be very good potential antibacterial compound if the inhibition zone > 20 mm [23]. The inhibition zone of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one could be seen on TABLE 1.

Chalcone compound (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one have active tendency to inhibit the growth of *S. aureus* while on its lowest concentration (1.25%), the diameter of inhibition zone was 8.2 mm (37.37%), on 2.5% concentration, the diameter of inhibition zone was 11.1 mm (50.45%) and on highest concentration (5.0%), the diameter of inhibition zone was 11.9 mm (54.10%).

On *S. epidermidis*, the lowest concentration of chalcone (1.25%) was not shown inhibitory activity. On 2.5% concentration, the inhibitory zone was 6.3 mm (29.17%) and on the highest concentration, the inhibitory zone was 12.1 mm (56.02%). There were two possibilities that (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one has effectively worked on specific bacteria while another possibility was the concentration of that compound need to be gained. Antibacterial activity of chloro-chalcone with 5 concentrations (20, 30, 40, 50, and 60 ppm) against gram positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*). The inhibitory diameter of chloro-chalcone increased as the concentration increased both at gram positive and gram-negative bacteria [17].

Positive control on this research was vancomycin 10.0%. Vancomycin was widely used antibiotics against gram-positive bacteria [24]. It chose because the structure of gram-positive bacteria consists of thick layer of peptidoglycan. Vancomycin binds the peptidoglycan precursors on gram-positive bacteria and it would inhibit the growth and division of gram-positive bacteria [25].

On *S. epidermidis*, the lowest concentration of (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one was not seen any inhibitory activity. It can happen because *S. epidermidis* having the quorum sensing (QS) system that make each cell of *S. epidermidis* communicate to increase the population density and coordinate gene expression to control cell at high numbers [26]. There were 61 genes on *S. epidermidis* that lead divergent clones to cause active infection [27], so it can be more infectious. Chalcone has good antimicrobial activity against MRSA because it has a methoxy group at 4' position [28]. Molecular docking analysis of chalcone compounds has capability to bind with NorA and MepA binding site [29]. Another research of novel chalcone thiazole hybrids shown that the scaffold can be used for developing a new class of antibiotics [30]. The chalcone (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one was less effective against *S. epidermidis* because those bacteria having a good survival ability against the change on its environment condition through various way of defense

The limitations on this research were the chalcone synthesis result was only tested on gram positive bacteria only. For further studies, the compounds should test on gram negative bacteria and with the different type of antibiotics as positive control.

V. CONCLUSIONS

The chalcone compound (E)-1-(4-hydroxyphenyl)-3-phenylprop-2-en-1-one has successfully synthesized and it was resulting 83.93% product yields. The synthesized compound has good inhibitory ability against *S. epidermidis* and *S. aureus*. The evaluation results shown that synthesized chalcone has 50.45% effectivity on 2.5% concentration on *S. aureus* and on *S. epidermidis* has 56.02% effectivity.

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