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Antibacterial Activity of 4-hydroxychalcone Against Bacterial Contaminant of Packed Red Cells

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Abstract

The bacterial contamination of blood products is still become a serious problem in consideration, thus it can cause the risk of blood transfusion, namely bacterial sepsis. Packed red cells is blood product and potentially contain bacterial contaminant. The source of bacterial contamination can come from the skin disinfection process in less aseptic during blood collection, donor bacterimia, and blood processing. In response, currently there have been many antibacterial compounds developments purposely to reduce the risk of bacterial contamination of blood products. This study aimed to observe the activity of 4-hydroxychalcone compounds in contaminant bacteria isolated from packed red cells blood products. Bacterial isolates isolated from the packed red cells were Staphylococcus aureus based on cell morphology, biochemistry and colony shape. The antibacterial activity of 4-hydroxychalchone compound against Staphylococcus aureus isolates used the diffusion method. The results showed, there were antibacterial activity of 4-hydroxychalchone at a concentration of 2.5% 1.25% and 0.625% against packed red cells contaminant bacteria based upon the clear zone formed. In conclusion, the bacteria isolate was obtained from the PRC was gram positive bacteria, *Staphylococcus aureus. The 4-hydroxychalcone had successfully synthesized and has antibacterial activity against S. aureus isolated from PRC, with the biggest inhibition power was 71,36% at 5,0% of chalcone concentration.*

Introduction

Packed Red Cells (PRC) are the most requested product for transfusion in worldwide, since PRC are used for changing acute blood lose more than 30%, in order to restoring oxygen delivery all over the body(García-Roa et al., 2017). With the aim to provide blood transfusion as therapeutic, blood product including PRC must be free from transfusion transmitted disease and microbial contaminants (Zaaijer, 2017). The contamination of microbes is caused by unhygienic blood drawing, donor bacteremia, unsterile blood processing (Astuti & Maharani, 2014; Hassall et al., 2009; Kusumaningrum & Sepvianti, 2020).

In some previous studies reported that Thrombocyte Concentrates (TC) are the most susceptible to bacterial contaminations due to the storage conditions that support bacterial growth (Astuti & Maharani,

2014; Kusumaningrum & Sepvianti, 2020; Tjiptoprajitno et al., 2012). However, in some reports also informed that another blood product, including PRC also has high possibility to bacterial contamination and can lead to bacterial sepsis(Delaney et al., 2016; Sepvianti, Wiwit; Kusumaningrum, 2022). Previous study reported that *Yersinia enterocolitica* associated with some cases of clinical sepsis from contaminated PRC and triggering severe sepsis, although *Y. enterocolitica* is an entero-pathogen and generally responsible for diarrhea and abdominal pain (Guinet et al., 2011).

Several works had been done to reduce the bacterial contamination, including skin disinfection properly, blood drawing aseptically, and sterile blood product processing. However, another technique is indeed needed to avoid bacterial contamination in PRC, including developing potential antibacterial agent. Various chemical compound are reported could be antibacterial agent. In some previous study showed that chalcone compound has antibacterial activity against positive and negative gram bacteria (Kumar et al., 2020; Sepvianti et al., 2021; Sepvianti & Kusumaningrum, 2021). Derivate chalcone with hydroxyl group in its ring had antibacterial activity against bacteria due to hydrophobicity towards the bacteria cell wall (Rosa et al., 2019).

In this study, the identification bacterial contaminants will be conducted from PRC which is the potential blood product to bacterial contamination. PRC sample will be obtained from quality control results that had positively bacterial contaminated tested from Indonesia Red Cross using BACT/ALERT. Bacterial contaminant identification will be conducted by its phenotypic character based on cell shape, cell characteristics, colony shape, colony color, and biochemistry test. The obtained bacterial isolate will be used for antibacterial testing with chalcone that successfully synthesized in this research, which is chalcone derivate 4-hydroxychalcone that has hydroxyl group in para-position number 4.

Methods

Bacteria Identification and Antibacterial activity materials and tools

A set of sterile laboratory glassware, stirrer, digital scale, autoclave, incubator and laminar air flow were needed as tools. Bacterial isolate obtained from PRC that had been evaluated using BACT/ALERY in the aerobic and anaerobic bottle. Vancomycin antibiotic was used as positive control and DMSO was used as negative control.

Chalcone compound synthesis materials and tools

The chemical materials had Pro-analysis quality from Sigma-Aldrich: acetophenone, 4-hydroxybenzaldehyde, sodium hydroxide, chloride acid, methanol, and aquadest. Tools for synthesis and characterization of chalcone used: set of laboratory glassware, stirrer, digital scale, pH meter, TLC plate, Fourier transform infrared spectrophotometer (FTIR, Shimadzu Prestige 21) and gas chromatography-mass spectrophotometer (GC-MS, AGILENT GC type 5973 Shimadzu QP 2010S).

Phenotypic Characterization of Bacterial Contaminants

The PRC was evaluated using BACT/ALERT to detect the bacterial contaminants inside the product. The positive result showed when there was color change after the process ended. The bacterial contaminants isolate can be obtained from aerobic and anaerobic bottle culture provided by BACT/ALERT. The positive result from bottle culture then inoculated to the blood agar media and incubated at the 37°C for 24 h. The purification was needed to get the single isolate bacteria contaminant from specific colony that growth in the blood agar media. The isolate bacteria contaminant then characterized by its phenotype character such as: cell morphology, gram-staining, and colony morphology.

Chalcone Compound Synthesis

The chalcone compound synthesis was refer to (Attarde et al., 2014) procedure with several modifications. A piece of sodium hydroxide base was dissolved to the 5 mL of methanol, then 5 mmol of acetophenone were added drop by drop in the 5 ml of methanol. The mixture then stirred for 2 minutes before 5 mmol of 4-hydroxybenzaldehyde were added drop by drop to the 5 mL of methanol. The mixture stirred continuously at the room temperature for 24 hours, and the reaction was monitored by Thin Layer Chromatography. After the reaction stopped, the mixture was added with cold aquadest and it was added with HCl 2M solution drop by drop until it formed sediment. The sediment formed was filtered by filter paper. The reaction product was dried and scaled for measuring the reaction result. The reaction product was characterized using FTIR spectrophotometer and GC-MS.

Antibacterial Activity Test

The chalcone compound was dissolved in dimethyl sulfoxide (DMSO) with concentration variant from 10,0%; 5,0%; 2,5%; and 1,25%. DMSO used as negative control, whereas vancomycin 10% used as positive control. The bacterial isolate was poured to the media surface. The exact 25 ml of chalcone was injected into each wells of test media. The clear zone examination produced by the bacterial growth against chalcone and antibiotic was observed after 24 h. The inhibition activity of chalcone toward the bacteria specified by the inhibition power using the following formula:

$$\text{Inhibition power (\%)} = \frac{Chalcone\ clear\ zone\ diameter}{Control\ clear\ zone\ diameter\ (+)} \text{x}100\% \quad (1)$$

The antibacterial activity that obtained was categorized: strong when the inhibition power is ≥ 70%, adequate when the inhibition power is 50-70%, poor when the inhibition power is <50% (Ikhtiarudin et al., 2020).

Results and Discussion

The bacterial contaminant characterization and identification were evaluated to understand the bacteria species that had the ability for contaminating Packed Red Cell. In this study, the bacterial contaminant source obtained from PRC sample quality control result in Indonesia Red Cross. The PRC sample were tested for bacterial contamination using BACT/ALERT in anaerobic and aerobic condition. From the evaluation, two bacterial isolates (PN1 and PN2) were obtained from anaerobic BACT/ALERT culture. Both isolates were characterized by their phenotypic characters and the results were presented at Table 1.

Table 1. Phenotypic character of bacterial contaminant from PRC

Phenotypic Character	**Bacterial Isolate PN1**
Cell shape	Staphylococci
Gram	Positive
Colony surface	Circular
Colony shape	Opaque
Colony color	Yellow pigment
Motility	Non-motile
Hemolysis	Beta-hemolysis
Spore formation	No
Oxygen consumption	Anaerobic facultative
Catalase test	Yes
Oxidase test	No
Coagulase test	Yes
Mannitol test	Yes
Mannose test	No
Identification result	*Staphylococcus aureus*

In this study, one isolate had successfully isolated from the positive result of BACT/ALERT anaerobic culture that showed there was contaminant microbes inside. The identification result (Table 1) based on the phenotypic character showed that the PRC had contaminated by bacteria such as *Staphylococcus aureus*. It can be seen in the table that the phenotypic characters were positive gram bacteria with staphylococci cell shape, with the colony morphology circular surface, opaque shape, and yellow pigment color. The cell was non-motile, and has the beta-hemolysis type characteristics. It does not formed spore and anaerobic facultative for the oxygen consumption. The biochemistry test showed that the isolate has positive results for catalase, coagulase, and mannitol test, whereas the oxidase and mannose test has negative results.

Comparing to the previous study, bacterial contamination also occurred in Packed Red Cells, and various species can be contaminant candidate. The bacterial contaminant mostly occurred from the skin flora since the unhygienic skin disinfection such as *Staphylococcus hominis, Staphylococcus epidermidis* and *Propionibacterium acnes* (Astuti & Maharani, 2014; Hassall et al., 2009; Kusumaningrum & Sepvianti, 2020; Tjiptoprajitno et al., 2012). In this study, the identification results showed that *Staphylococcus aureus* also has the susceptibility to contaminate the PRC, although it was not skin flora species. However, the study from (Farzad et al., 2016) showed that *Staphylococcus aureus* as anaerobic positive gram bacteria also

occurred as blood product contaminants. Referring to (Depcik-Smith et al., 2001; McDonald, 2006) the organisms most frequently isolated from PRC are commensal or skin commensal from blood drawing site or from undetected donor bacteremia. (Hassall et al., 2009)also reported that gram positive bacteria are isolated soon after the donation, blood bank environment and blood bank technicians.

The chalcone compound synthesized through the condensation reaction between aldehyde (benzaldehyde) and ketone (acetophenone) using NaOH as base catalyst. All the liquid materials were dissolved in the methanol solution. The condensation reaction occurred for 24h at room temperature and stirred continuously. The reaction also evaluated using thin layer chromatography to study the forming of product reaction optimally. After 24 h of mixing, the cold distilled water were added and HCl 2M were added drop by drop until the sediment of product was formed. The formed sediment was filtered using the filter paper and was dried in the desiccator. The dried product was scaled to measure the yield result of the product. The result yield was 83,93% (0,94/1,12 gram). The prediction of chalcone forming reaction through the condensation reaction was presented at the Figure 1.

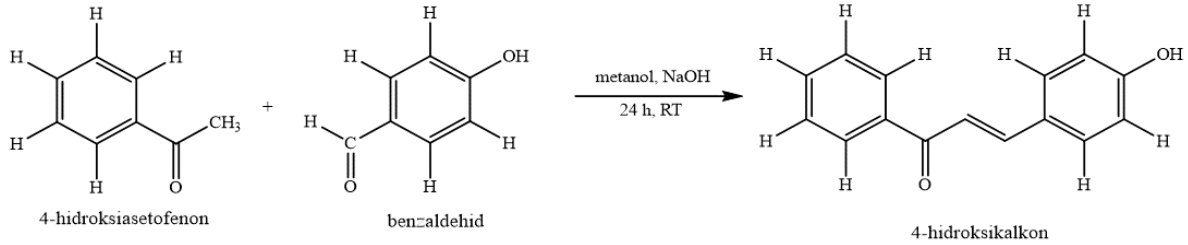

Figure 1. Chalcone compound forming reaction

The sediment result was characterized using FTIR and GC-MS. The FTIR analysis result (Figure 2) showed that the absorption of stretching vibration of -C-H trans and wavenumber 918,12 cm^{-1} and vibration of -C-H cis at 864,11 cm^{-1}. This absorption was a marker for chalcone forming, since this adsorption occurred caused by the double bond from acetophenone and benzaldehyde condensation reaction. This reaction released water (H_2O) as simple molecule. Hydroxy group of this chalcone occurred at 3018,24 cm^{-1} area, as a marker for chalcone formed by hydroxyl substituted synthesis. The ketone group at the conjugated position with aromatic ring and olefin occurred at wavenumber 1674,21 cm^{-1}.

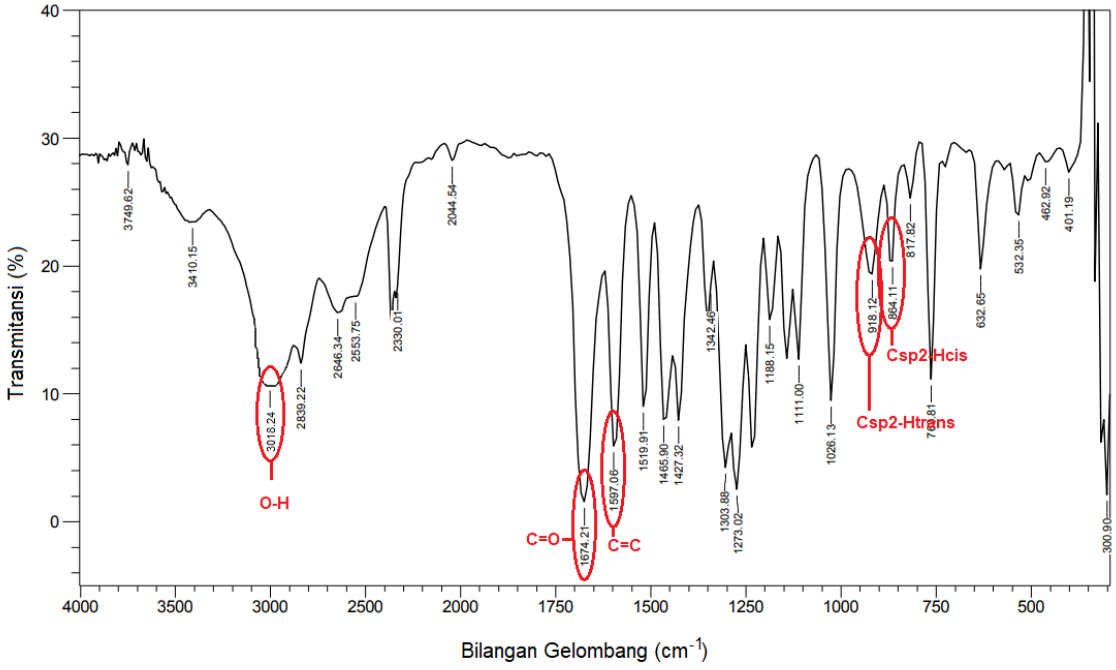

Figure 2. FTIR Spectra of 4-hydroxychalcone

The compound characterization using GC-MS had results as shown at the Figure 3, Figure 4 and Figure 5. In the Figure 3, the GC chromatogram had 1 (one) peak with retention time (tR) 19,30 minute and

obtained 100% of relative purity. The MS mass spectrum showed the molecular ion (M^+) and it was detected 244 equivalent with the molecular weight of chalcone (Figure 4). This result proved that the target compound had been formed already. Based on the obtained mass spectrum, the fragmentation pattern happened as follow: releasing OH radical and producing fragment m/z 208 as the first phase, in the second phase, the C_7H_7 radical released and produced fragment m/z 121 and in the last phase, CO_2 radical released and formed fragment m/z 78. This fragment also a base peak (Figure 5).

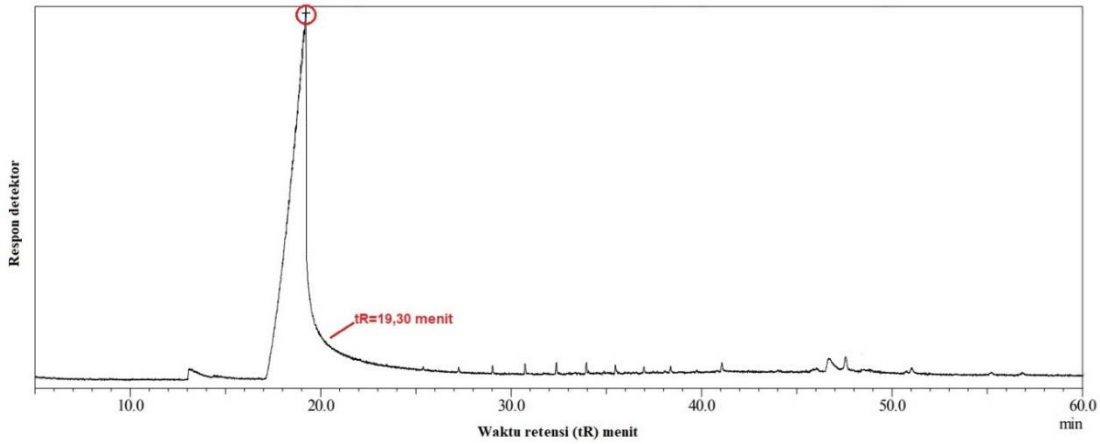

Figure 3. GC chromatogram of 4-hydroxychalcone

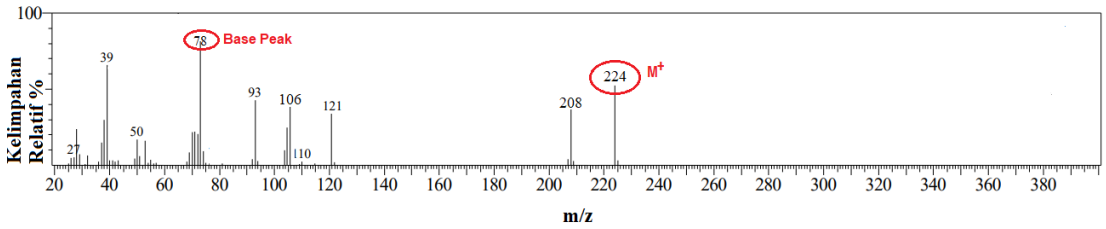

Figure 4. Mass spectra of 4-hydroxychalcone

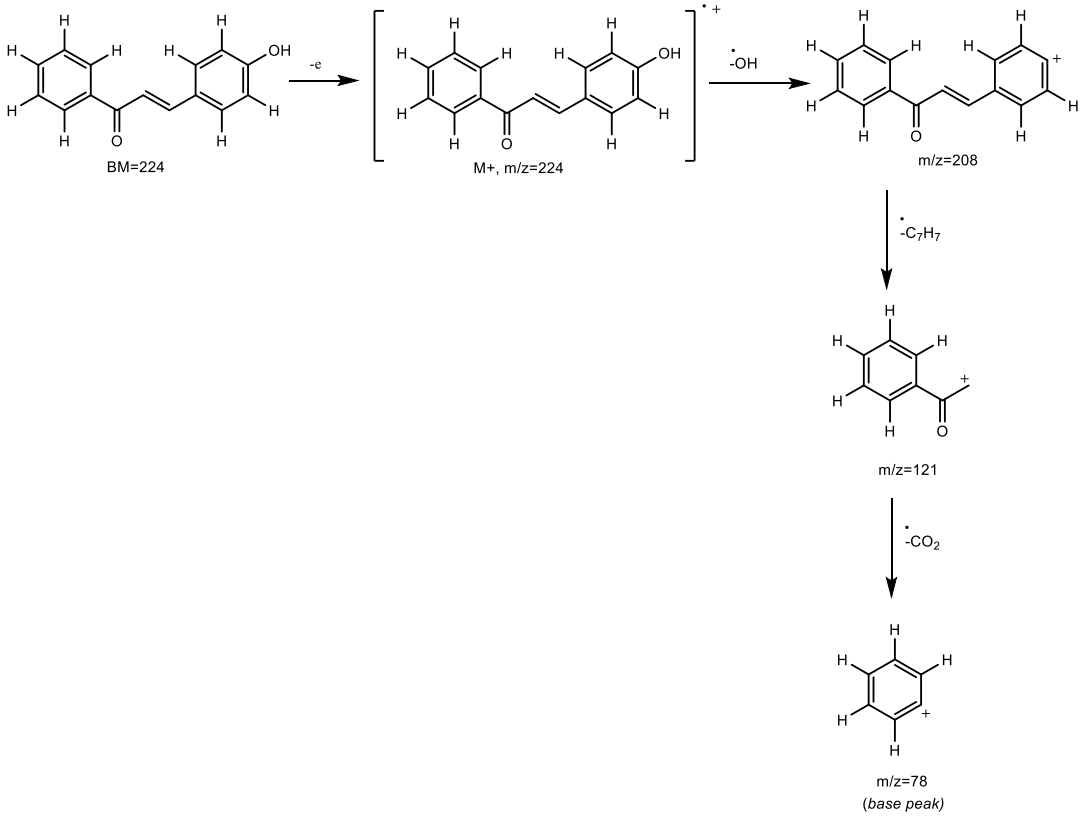

Figure 5. Fragmentation pattern of 4-hydroxychalcone

After synthesizing the chalcone compound, then the chalcone were tested its antibacterial activity against bacterial isolate from PRC, *Staphylococcus epidermidis* and *Staphylococcus aureus*. Exposure the chalcone towards each bacterial isolate had been done in the incubator for 24h. After the incubation time was over, the antibacterial activity was obtained by measuring clear zone formed by growth inhibition of bacteria toward chalcone. The clear zone and the inhibition power data can be seen at Table 1.

Table 1. Inhibition Power of Derivate Chalcone Compound towards Bacterial Isolate from PRC

Derivate Chalcone Concentration	*Staphylococcus aureus*	
	Clear Zone (mm)	Inhibiiton Power (%)
5,0%	15,7	71,36
2,5%	11,1	50,45
1,25%	9,8	44,55
Positive Control	22,0	100,0
Negative Control	0	0

Some previous studies proved that the chalcone compound has various pharmacology characteristics, including antibacterial activity (Avila et al., 2008). Chalcone compound has α, β unsaturated ketone group, namely ethylene ketone group (-CO-CH=CH-) and has the inhibition activity against the bacteria. In this study, three variations of derivate chalcone concentration are tested toward Staphylococcus aureus from PRC. At the concentration 5,0%, the derivate chalcone produces clear zone 15,7mm with inhibition power 71,36%; at the concentration 2,5% it produces clear zone 11,1mm with inhibition power 50,45%; and at concentration 1,25% it produces clear zone 9,8 mm with inhibition power 44,55% toward *Staphylococcus aureus*. Compared to (Sepvianti, Wiwit; Kusumaningrum, 2022) at the concentration of 5,0% of pure chalcone had 11,2 mm of clear zone and inhibition power 64,0%, thus the clear zone and inhibition power produced by pure chalcone lower than in this study. Another concentration of pure chalcone in the previous study also produces clear zone and inhibition power lower than this study, even in the same concentration. It is proved that the derivate chalcone with hydroxyl group in this study showed higher antibacterial activity than pure chalcone. It is suspected that adding the OH group at para position number 4 adds the aromatic ring stability and improves the antibacterial activity against positive gram bacteria. The derivate chalcone with OH group tends to be able to disrupt the cell wall of positive gram bacteria since the peptidoglycan was not surrounded by outer membrane.

Conclusions and Recommendations

In conclusion, PRC is one of the blood product that has susceptibility to bacterial contamination. The bacteria isolate was obtained from the PRC was gram positive bacteria, *Staphylococcus aureus* based on its phenotypic character. The 4-hydroxychalcone had successfully synthesized and has antibacterial activity against *S. aureus* isolated from PRC, with the biggest inhibition power was 71,36% at 5,0% of chalcone concentration.

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References

Astuti, D., & Maharani, E. A. (2014). Identifikasi bakteri yang mengontaminasi konsentrat trombosit. *Dewi Astuti, Eva Ayu Maharani*, *2*(IDENTIFIKASI BAKTERI YANG MENGONTAMINASI KONSENTRAT TROMBOSIT), 61–67.

Attarde, M., Vora, A., Varghese, A., & Kachwala, Y. (2014). Synthesis and evaluation of chalcone derivatives for its alpha amylase inhibitory activity. *Organic Chemistry: An Indian Journal*, *10*(5), 192–204.

Avila, H. P., Smânia, E. de F. A., Monache, F. D., & Smânia, A. J. (2008). Structure-activity relationship of antibacterial chalcones. *Bioorganic & Medicinal Chemistry*, *16*(22), 9790–9794.

https://doi.org/10.1016/j.bmc.2008.09.064

Delaney, M., Wendel, S., Bercovitz, R. S., Cid, J., Cohn, C., Dunbar, N. M., Apelseth, T. O., Popovsky, M., Stanworth, S. J., Tinmouth, A., Van De Watering, L., Waters, J. H., Yazer, M., & Ziman, A. (2016). Transfusion reactions: prevention, diagnosis, and treatment. *Lancet (London, England)*, *388*(10061), 2825–2836. https://doi.org/10.1016/S0140-6736(15)01313-6

Depcik-Smith, N. D., Hay, S. N., & Brecher, M. E. (2001). Bacterial contamination of blood products: factors, options, and insights. *Journal of Clinical Apheresis*, *16*(4), 192–201. https://doi.org/10.1002/jca.10004

Farzad, B. B., Farshad, B., Zahra, B., Nahid, A., & Mahsa, K. B. (2016). Bacterial contamination of platelet products in the Blood Transfusion Center of Isfahan, Iran. *GMS Hygiene and Infection Control*, *11*, Doc23. https://doi.org/10.3205/dgkh000283

García-Roa, M., Del Carmen Vicente-Ayuso, M., Bobes, A. M., Pedraza, A. C., González-Fernández, A., Martín, M. P., Sáez, I., Seghatchian, J., & Gutiérrez, L. (2017). Red blood cell storage time and transfusion: current practice, concerns and future perspectives. *Blood Transfusion = Trasfusione Del Sangue*, *15*(3), 222–231. https://doi.org/10.2450/2017.0345-16

Guinet, F., Carniel, E., & Leclercq, A. (2011). Transfusion-Transmitted Yersinia enterocolitica Sepsis. *Clinical Infectious Diseases*, *53*(6), 583–591. https://doi.org/10.1093/cid/cir452

Hassall, O., Maitland, K., Pole, L., Mwarumba, S., Denje, D., Wambua, K., Lowe, B., Parry, C., Mandaliya, K., & Bates, I. (2009). Bacterial contamination of pediatric whole blood transfusions in a Kenyan hospital. *Transfusion*, *49*(12), 2594–2598. https://doi.org/10.1111/j.1537-2995.2009.02344.x

Ikhtiarudin, I., Agistia, N., Frimayanti, N., Harlianti, T., & Jasril, J. (2020). Microwave-assisted synthesis of 1-(4-hydroxyphenyl)-3-(4-methoxyphenyl)prop-2-en-1-one and its activities as an antioxidant, sunscreen, and antibacterial. *Jurnal Kimia Sains Dan Aplikasi; Vol 23, No 2 (2020): Volume 23 Issue 2 Year 2020DO - 10.14710/Jksa.23.2.51-60* . https://ejournal.undip.ac.id/index.php/ksa/article/view/27167

Kumar, K. S., Siddaiah, V., Lilakar, J. D., & Ganesh, A. (2020). An efficient continuous-flow synthesis and evaluation of antimicrobial activity of novel 1,2,3-Triazole-Furan hybrid chalcone derivatives. *Chemical Data Collections*, *28*, 100457. https://doi.org/https://doi.org/10.1016/j.cdc.2020.100457

Kusumaningrum, S. B. C., & Sepvianti, W. (2020). Karakterisasi Bakteri Kontaminan Pada Produk Darah Thrombocyte Concentrate. *Syifa' MEDIKA: Jurnal Kedokteran Dan Kesehatan*, *10*(2). https://doi.org/10.32502/sm.v10i2.2185

McDonald, C. P. (2006). Bacterial risk reduction by improved donor arm disinfection, diversion and bacterial screening. *Transfusion Medicine (Oxford, England)*, *16*(6), 381–396. https://doi.org/10.1111/j.1365-3148.2006.00697.x

Rosa, G. P., Seca, A. M. L., Barreto, M. D., Silva, A. M. S., & Pinto, D. C. G. A. (2019). Chalcones and Flavanones Bearing Hydroxyl and/or Methoxyl Groups: Synthesis and Biological Assessments. In *Applied Sciences* (Vol. 9, Issue 14). https://doi.org/10.3390/app9142846

Sepvianti, Wiwit; Kusumaningrum, S. B. . (2022). SINTESIS SENYAWA 4'-HIDROKSI-4-METOKSIKALKON DAN UJI AKTIVITAS ANTIBAKTERI TERHADAP BAKTERI KONTAMINAN PRODUK DARAH. *Pharma Xplore: Jurnal Sains Dan Ilmu Farmasi*, *7*(1), 11–23. https://doi.org/https://doi.org/10.36805/farmasi.v7i1.2331

Sepvianti, W., Kusumaningrum, B. S., & Christiyani. (2021). Analog Kalkon (E)-1,3-bis(4-hydroxyphenyl)prop-2-en-1-one: Sintesis dan Uji Aktivitas Antimicrobia terhadap Bakteri Kontaminan Produk Darah Analog. *Jurnal Kesehatan Saelmakers PERDANA*, *4*(2), 321–328. https://doi.org/10.32524/jksp.v4i2.287

Sepvianti, Wiwit;, Btari, Serafica;, Kusumaningrum, & Christiyani. (2022). Sintesis Dan Uji Aktivitas Antibakteri Senyawa Kalkon Terhadap Bakteri Kontaminan Produk Darah Gram Negatif Dan Positif Synthesis and Antibacterial Activity Test of Chalcone Against Blood Product Contaminant Bacteria Negative and Positif Gram. *Jurnal Kefarmasian Akfarindo*, *7*(1), 22–28.

Sepvianti, Wiwit & Serafica Btari Christiyani Kusumaningrum. (2021). Sintesis Dan Uji Aktivitas Antibakteri Senyawa Kalkon (E)-3-(4-(Dimethylamino)Phenyl)-1-Phenylprop-2-En-1-One Terhadap Bakteri Kontaminan Produk Darah. *Journal of Health (JoH)*, *8*(2), 75–84. https://doi.org/10.30590/joh.v8n2.p75-84.2021

Tjiptoprajitno, N. A., Aryati, A., & Sudiana, I. K. (2012). Analisis Produk Darah Thrombocyte Concentrate di Palang Merah Indonesia Surabaya. *Jbp*, *14*(3).

Zaaijer, H. L. (2017). Prevention of Transfusion-Transmitted Infections: Dilemmas. *Frontiers in Medicine*, *4*, 221. https://doi.org/10.3389/fmed.2017.00221