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Judul Artikel : ***ANTIBACTERIAL ACTIVITY OF 3,6-BIS[(4-HYDROXY-3-METHOXYPHENYL) METHYLIDENE]PIPERAZINE-2,5-DIONE AND 3,6-BIS[(4-HYDROXY-3,5-DIMETHYLPHENYL)METHYLIDENE]PIPERAZINE-2,5-DIONE TO* Staphylococcus aureus, Escherichia coli, *AND* Bacillus subtilis.**
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INSTITUT TEKNOLOGI BANDUNG * SEKOLAH FARMASI (SCHOOL OF PHARMACY) *

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ANTIBACTERIAL ACTIVITY OF 3,6-BIS[(4-HYDROXY-3-METHOXYPHENYL) METHYLIDENE]PIPERAZINE-2,5-DIONE AND 3,6-BIS[(4-HYDROXY-3,5-DIMETHYLPHENYL)METHYLIDENE]PIPERAZINE-2,5-DIONE AGAINST *Staphylococcus aureus*, *Escherichia coli*, AND *Bacillus subtilis*

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ABSTRACT

Curcumin has antibacterial activity. Modification of curcumin to obtain a more potent compound, stable, safe, effective, and has a better activity has been widely applied. Some of them are derived compounds of pentagamavunon (PGV) and derivatives of piperazine-2,5-dione. Molecule of 3,6-bis[(4-hydroxy-3-methoxy)metiliden]piperazine-2,5-dione (DKP1) and 3,6-bis[(4-hydroxy-3,5-dimetilphenyl)metiliden]piperazine-2,5- dione (DKP2) are derivates of piperazine-2,5-dione. This study aims to determine the antibacterial activity of DKP1 and DKP2 against *Staphylococcus aureus*, *Escherichia coli,* and *Bacillus subtilis*. The antibacterial assay was done using Kirby Bauer diffusion method. Dics were impregnated with curcumin, PGV-0, PGV-1, DKP1 and DKP2. The discs were place on Mueller Hinton media that has been inoculated with the test bacteria. Antibacterial activity was determined by measuring inhibition zone around the discs. The results showed that DKP2 has weak antibacterial activity against *Escherichia coli, Staphylococcus aureus,* and *Bacillus subtilis*. Pentagamavunon-0 (PGV-0) has activity against *E. coli* and along with PGV-1 against *Staphylococcus aureus*. Curcumin and DKP1 have no antibacterial activity against *Staphylococcus aureus*, and along with PGV-0 and PGV-1 against *Bacillus subtilis*.

Keywords: curcumin, diketopiperazine, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis*

Introduction

Infectious diseases are one of the health problems from a long time ago that continue to evolve and increasing resistance incident on distressing level. Infection can be transmitted by bacteria or other microorganism. Medication errors of infection causing bacterial resistance was more frequently reported (Santosaningsih et al, 2008; Mardiastuti et al, 2007; Cha et al, 2005). Furthermore it also followed by the development of discovery of new compounds that have antibiotic efficacious from natural product has increased disproportionately. These compounds have been widely generated either by the process of isolation natural compound or inventions in the synthesis of novel compounds with the support a combination of computational technology and laboratory test.

The research dan development of curcumin, isolated compound from *Curcuma* sp. rhizome have been done. Modification of its functional group have produced many compounds of curcumin derivates and its analogues, and also corrrelated to its biological activity. Curcumin has very weak antibacterial activity and its activity is influenced by functional group of phenolic in curcumin molecule (Naz et al, 2010; Sunilson et al, 2009; Rai et al, 2008; Cikrikci et al, 2008). The modification of curcumin has been widely applied to obtain compounds which are more potent, stable, safe, effective, and has a better activity.

Two compounds of diketopiperazine derivates, analogues of curcumin as first lead compound and similar structure to pyrazoline have been synthesized and one of

them has antioxidant activity (Santoso and Supardjan, 2010; Santoso and Supardjan, 2011). They are 3,6-bis[(4-hydroxy-3-methoxy)metiliden]piperazine-2,5-dione (DKP1) and 3,6-bis[(4-hydroxy-3,5-dimetilphenyl)metiliden]piperazine-2,5- dione (DKP2). Previous research stated that there is a strong correlation between 3D molecular docking result with antibacterial activity against *S. aureus* of the pyrazoline analogues and DKP2 with predicted activity (Santoso, 2012; Santoso, 2011; Bhatia et al, 2010). The aim of the study is to determine the antibacterial activity of DKP1 and DKP2 against *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis*.

Method

Materials: DKP1, DKP2 (obtained from synthesis), curcumin, PGV-0, PGV-1 (obtained from Da'i), *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* (obtained from Microbiology Laboratory, Universitas Muhammadiyah Surakarta), chloramphenicol disc (Oxoid).

The antibacterial activity of the compounds was determined by Kirby-Bauer agar diffusion method. Fifteen mililiter of Mueller Hinton Agar (MHA) medium was dispensed into pre-sterilized Petri dishes and allowed to solidify at room temperature. *Staphylococcus aureus*, *Escherichia coli*, and *Bacillus subtilis* were inoculated on MHA. Sterile discs were impregnated with the compounds and then placed on inoculated MHA. Control antibiotics were placed on inoculated media as well. Petri dishes were incubated for 18-24 hours at 37°C. Inhibition zones around discs that are indicated by clear area were measured.

Result and Discussion

There are two groups of series of concentrations used in this study are intended to fulfill the capacity of the two types of blank discs. Selection of concentration series of compounds based on the solubility of the compounds. The greater the concentration of the compounds will be more insoluble in DMSO 5%, preferred solvent used in this study. In addition, more greater the concentration of the compounds, the compound's impregnation ability into the blank disc increasingly hard to be obtained then used a low concentration series as its solution. The results of the antibacterial activity of the compounds against *Staphylococcus aureus, Escherichia coli*, and *Bacillus subtilis* are presented in Table 1-3.

Table 1. Inhibition zone (mm) the compounds against *Staphylococcus aureus* using blank disc type 1

Compound	DMSO 5%	0.01%	0.05%	0.1%	0.2%	0.3%	0.5%	K+
DKP1	-	-	-	-	-	-	-	
DKP2	-	9 (ir)	10 (ir)	10 (ir)	15 (ir)	14 (r)	16 (r)	
PGV-0	-	-	20 (ir)	17 (ir)	18 (ir)	20 (ir)	23 (ir)	
PGV-1	-	-	-	-	-	13.5 (ir)	14.4 (ir)	
curcumin	-	-	-	-	-	-	-	
control (+)	-							18 (r)

(r)= radical zone; (ir)= irradical zone; (K+) = chloramphenicol

Table 1 and 2 informed that DKP2 gave zone of inhibition against all the bacteria even though give inconsistent results and is not proportional to the concentrations used. The PGV-0 also gave zone of inhibition on *Staphylococcus aureus* and *Escherichia coli*. Blank discs type 1 are used in the antibacterial assay of *Staphylococcus aureus* and *Escherichia coli* have a larger capacity than the blank disc type 2. The constraints in giving similar volume in discs type 1 lead to

disproportionate results. The solubility and the absorptive capacity of compounds also affect the results.

Table 2. Inhibition zone (mm) the compounds on *Escherichia coli* using blank disc type 1.

Compound	DMSO 5%	0.01%	0.05%	0.1%	0.2%	0.3%	0.5%	K+
DKP1	-	12 (ir)	-	-	-	-	-	
DKP2	-	9 (ir)	10 (ir)	10 (ir)	10 (ir)	8 (r)	9 (r)	
PGV-0	-	8.3 (ir)	9.5 (ir)	-	-	12.5 (ir)	9.6 (ir)	
PGV-1	-	-	-	-	-	-	-	
curcumin	-	10 (ir)	11 (ir)	-	-	-	9.8 (ir)	
control (+)	-							8 (r)

(r)= radical zone; (ir)= irradical zone; (K+) = chloramphenicol

Cherkasov (2005) proved that there is a relationship between the chemical structure of antibiotic and its antibacterial activity. The activity of a hydroxy group on the side chain of curcumin was also influenced by the other functional groups in curcumin. It causes weak antibacterial activity. Therefore, modification of the center and side cluster of curcumin has been done. In this study, they are PGV-0, PGV-1, DKP1, and DKP2.

Table 3. Inhibition zone (mm) the compounds on *Bacillus subtilis* using blank disc type 2.

Compound	DMSO 5%	0.025%	0.05%	0.1%	0.2%	0.4%	K+
DKP1	-	-	-	-	-	-	
DKP2	-	-	7.5 (ir)	8 (ir)	9 (r)	10 (r)	
PGV-0	-	-	-	-	-	-	
PGV-1	-	-	-	-	-	-	
curcumin	-	-	-	-	-	-	
control (+)	-						14 (r)

(r)= radical zone; (ir)= irradical zone; (K+) = chloramphenicol

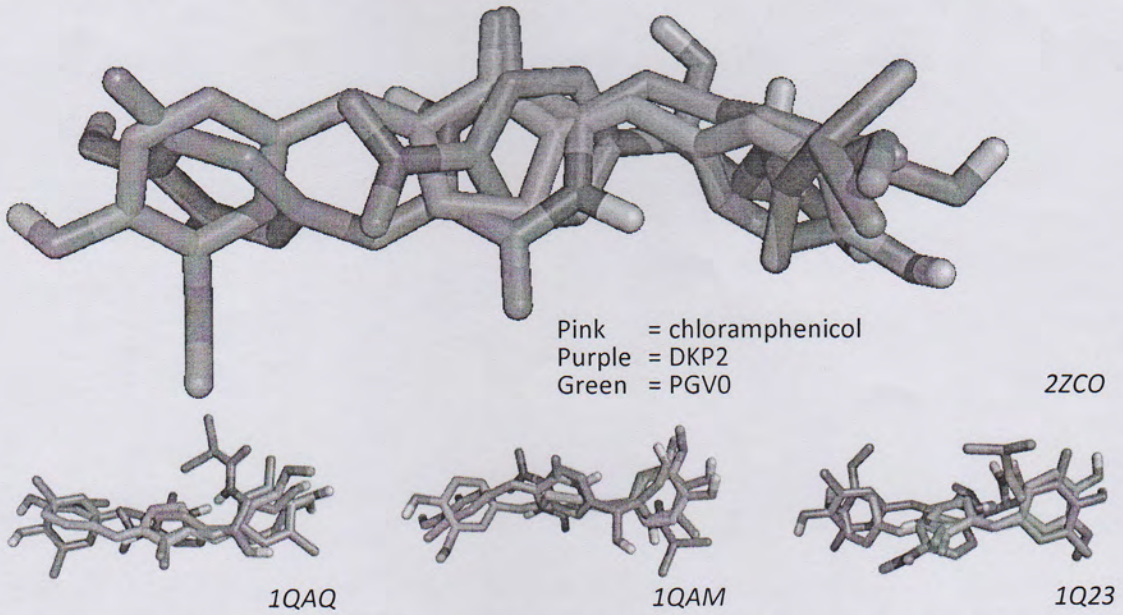

Figure 1. 3D Conformation of Chloramphenicol, DKP2, and PGV0 (reproduced by PyMol)

Modification of the middle ring and the side of curcumin influenced the activity of its new compounds. Middle ring of curcumin was replaced by pentanone or

diketopiperazine and its side chain was substituted with different groups. These modifications lead to different antibacterial activity. Molecules of PGV-0, PGV-1, DKP1 and DKP2 have different chemical structures and antibacterial activity. Based on the chemical structure of PGV-0, there is one hydroxyl group on each side of molecule which support its antibacterial activity. Molecule of PGV-0 has a larger molecular space volume than chloramphenicol and the number of carbon atoms (nonpolar), then PGV-0 may has low antibacterial activity. On the other side, PGV-1 contains hydroxyl group that flanked by two methyl group caused steric hindrance as a reason why its antibacterial activity is very weak.

The synthesized compounds of diketopiperazine derivatives (DKP1 and DKP2) also has its own chemical characteristics, the middle ring molecule has two ketone groups containing oxygen which has reactive lone pair electron. Molecule of DKP1 have 2 hydroxy and methoxy side chain whereas DKP2 only have groups of hydroxy which flanked by a methyl group (hydrophobic) allows the molecule to be reactive as compared to DKP1 as antibacterial.

Previous research stated that the antibacterial activity of a molecule against bacteria *Bacillus subtilis* is affected by the interaction of hydrophobic factor, whereas electrostatic interactions have no effect for the activity. Similarly postulated by Bhatia et al. (2010) for dehydrosqualene synthase protein of *Staphylococcus aureus*. Based on chemical structure, chloramphenicol is more potent as antibacterials because it contains halogen atom (2 chlorine) and hydroxy groups, whereas in DKP2 hydroxy group is hindered by steric hindrance of 2 methyl located side by side. Methyl group gives the non-polar effect to DKP2. This effect caused it has antibacterial activity. The absence of non-polar effect also causes DKP1 have no antibacterial activity. Molecule side of DKP1 does have hydroxy groups, but the presence of a methoxy group did not influence the hydrophobicity.

Conclusion

The results showed that DKP2 has weak antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*. Pentagamavunon-0 (PGV-0) has activity against *E. coli* and along with PGV-1 against *Staphylococcus aureus*. Curcumin and DKP1 have no antibacterial activity against *Staphylococcus aureus*, and along with PGV-0 and PGV-1 against *Bacillus subtilis*.

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