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Research paper

Antibacterial Activities Test of the Xanthorrhizol Bioactive Compound in the Endophytic Bacteria of *Curcuma xanthorrhiza*

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Abstract

The objective of this study is to understand the potential of xanthorrhizol bioactive compound of the endophytic bacteria of Curcuma xanthorrhiza rhizome as an antibacteria in vitro and the molecular docking simulation. The potency of two endophytic bacteria isolates (ET 1 and ET 2) as the antibacterial producer was tested, then the xanthorrhizol compound was separated using the Thin Layer Chromatography (TLC) and the contact-autobiography TLC. Furthermore, a docking simulation analysis of the xanthrorrhizol compound as an antibacteria was made. The endophytic bacteria isolate ET1 shows its antibacterial activities to Escherichia coli (6.40 mm) and Staphylococcus aureus (21.48 mm with the inhibiting power under category of very strong), while the endophitic bacteria isolate ET2 shows its antibacterial activities to E. coli (4.52 mm) and S. ureus (8.56 mm). The results of the bioautography -TLC test of the bioactive compound of the endophitic bacteriais isolate ET1 (Rf 0.57) and isolate ET2 (Rf 0.57) the visualization of 254 UV lamp is assumed to be the xanthorrizol compound, does not show antibacterial activities in E. coli and S. aureus. Based on docking simulation results can be seen that xanthorrhizol compounds have the potential to interact with the target protein in E. coli and S. aureus, but it is lower than the control antibiotic.

Keywords: *xanthorrhizol, docking, endophytic bacteria, antibacterial*

1. Introduction

Indonesia is generally known as a tropical country with rich medicinal plants, one of them is temulawak (Curcuma xanthorrhiza) known among the people to be able to cure various diseases, because most of its chemical components possess some secondary metabolites specific in plants serving as medicines and medicinal materials, including antibacterial substances. One of the newest way of producing the secondary metabolite compounds specially existing in plants is by making use of endophytic microbes living in plants tissues (Strobel, 2002). The extract of the C. xanthorrhiza rhizome may inhibit the growth of Candida albicans (13,07 mm), S. aureus (15,75 mm) and E. coli (31,56 mm). The diameter of the inhibiting zone of the C. xanthorrhiza rhizome to the E. coli is wider than the diameter of the positive control chloramphenicol; 29 mm (Adilla et al., 2013). The C. xanthorrhiza rhizome contains starch, fiber, curcuminoid and volatile oils (phelandren, kamfer, tumerol, cyneol, and xanthorrhizol) (Siagian, 2006).

Xanthorrhizol is a compound specific in the C. xanthorrhiza rhizome under the molecular formula of C15H22O with the molecular weight of 218.335 g/mol. This compound with the name of IAPAC 5-5- (1,5-dimetylheks-4-enil)-2-metilphenol has the following characteristics: no color, being stable to heat, having bitter taste and being soluble in DMO and ethanol 96% (Hwang 2000). According to Oon et al (2015), the xanthorrhizol campound has potential as antimicrobial as an antibacterial (Actinomyces viscosus, Porphyromona gingialis, Streptococcus mutans, S. aureus, methicillin-resistant S. aureus, E. coli, Propionibacterium acnes), anticandidal (Candida albicans, C. glabrata, C. guilliermondii and C. parapsilosis), antifungal (Malassezia species, Aspergillus flavus, A. fumigatus, A. niger, Fusarium oxysporum, Rhizopus oryzae and Trichophyton mentagrophtes).

Endopythic microbe is referred to any microbes (fungi and bacteria) living in plant fibers by making some colonies for a certain period from its life cycle without giving any harm to the main plant. This microbe may be isolated from the plant fibers of which its surfaces have been sterilized or the fibers from the inner part of the plant (Hallmann et al, 1997). In the medium of fermentation, endophytic microbe will produce some compounds with the type of those of the mother plant (Shibuya et al, 2005). Xanthorrhizol is known to to be able to possess some anti-bacterial activities. Therefore, it is necessary to study about the potency of xanthorrhizol that may produce endophytic bacteria from the C. xanthorrhiza rhizome that can be used as an antibacteria.

2. Materials and Methods

2.1. Fermentation of the Endophyte Bacteria

A piece of the colony of endophytic bacteria that had been incubated in an NA (Nutrient agar) medium for 24-48 hours under the temperature of 370C was taken and moved into 5 ml MHB (Muller-Hinton Broth) medium. One ml of the suspension of the endophytic

bacteria colony was moved into 9 ml MHB medium in an 12 ml ependof tube, incubated under temperature of 300C using an inkubator shaker with 130 rpm for 16 hours (isolate ET1) nd 18 hours (isolate ET2). Then it was centrifubalized with the speed of 3800 rpm, under the temperature of 4˚C, for 20 minutes. The obtained supernatan was used to test the antibacterial acivities (Utami, 2011).

2.2. Antibacterial Activity Testing

The test of the antibacterial activities was made by Kirby-Bauer method, using sterile paper disc was plunged into the testing solution (the supenatan produced from the fermentation of endophyt fungi) for 60 minutes. Acceptically, the paper disc was putn on the MHA (Muller Hinton agar) medium surface that has contained the testing bacteria (E. coli and S. aureus). After being incubated in the tempature of 37˚C for 24 hours, the inhibiting zone was measure (Utami, 2011).

2.3. The Extraction of Xanthorrhizol Compound Using TLC

The supernatan extraction from the results of the endophyt bacteria fermentation was extracted twice with the solvent of ethyt accetat (1:1 v/v) placed in a tube for 20 minutes. The obtained extract was then evaporated by adding N2 for the next tests (Inamandar and Pallit, 2003).

2.4. The Separation of Xanthorrhizol Compound Using TLC

The chromatography of the antibacterial compound made use of the TLC in gel 60F254 silica plate as silent phase. The extract of the ethyl acetate from the endophytic bacteria bioactive compound was given to each point for 15 spots. The phase of movement was made by the comparison between N-hexan: ethyl acetat eluent = 10:1 (Asriani, 2010). Each plate was then intered into the TLC containing eluent for some minutes until it reaches the uppter level of (1 cm from the lower and upper ends), then it was dried and observed under the UV 254 nm and 365 nm. The patterns of the spots were observed and drawn, and then the Rf value was calculated (Sherma and Faried, 2003).

2.5. The TLC-Bioautoghrapy Testing

The testing was made using a contact method by placing the plate from the TLC results on the surface of the MHA medium that had been mixed with the suspension of the testing bacteria of 5 µl and was kept for 60 minutes. Then the plate was taken and moved, and incubated in temperature of 37˚Cfor 24 hours. The antibacterial activities were indicated by the formation of the inhibiting zone around spotsfrom the TLC results (Yulianty et al, 2011).

2.6 The Simulation of the xanthorrhizol docking compound as anti-bacteria

The target/receptor protein in the 3D structure is obtained from the Protein Data Bank through http://www.rcsb.org/pdb/explore. The used target protein is the Penicilin Binding Protein (PBP) enzyme from (PBP) enzyme from S. aureus (PDB ID 3hun), Dihydrofolate Reductase (DHFR) enzyme from S. aureus (PDB ID 2w9s), and DNA gyrase enzyme from E. coli (PDB ID 5l3j). The ligand/chemical compound used are obtained from http://pubchem.ncbi.nlm.nih.gov/, namely xanthorrhizol, ampicilin, trimetoprim, and ciprofloxacin compounds. The parDOCK program was employed for the docking process (Gupta et.al., 2007). Pymol was used to protein and ligand preparation. The Poseview program (http://poseview.zbh.uni-hamburg.de/) was used for the visualization of the docking results, and the Lipinski Role of Five http://www.scfbio iitd.res.in/software/ drugdesign/ lipinski.jsp for the bioavaibility of the xantorrhizole compound (Yang, 2013).

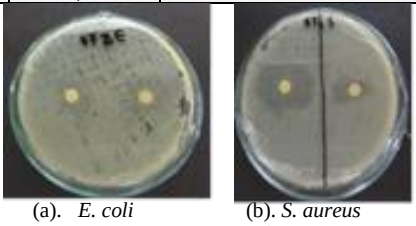
3. Discussion and Conclusion

3.1. The Antibacterial Activities of the Bioactive Compound of Endophytic Bacteria

The diameter of the inhibiting zone of the bioactive compound of endophytic bacteria of the isolate ET1 was 6.40 mm for E. Coli and 21.48 mm for S. Aureus, while for that of the isolate ET2 was 4.52 mm for E. Coli and 8.56 mm for S. Aureus. The diameter of the inhibiting power of the endophytic bacteria in the rhizome C. xanthorrhizol may be grouped on the based on the inhibiting power category by Davis and Stout (1971), namely when the formed inhibiting zone in the gel diffusion testing < 5 mm (weak), 5-10 mm (medium), 10-19 (strong) and ≥20 20 mm (very strong). The bioactive compound of the endophytic bacteria of the isolate ET1 may categorized into very strong in inhibiting the growth of S. aureus bacteria (21,48 mm) since exceeds the very strong inhibiting power category namely ≥20 mm (Table 1, Figure 1 and 2).

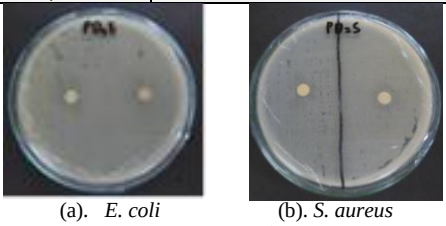
Table 1: Diameter of the inhibiting zones

Bacteria isolates	Diameter of the inhibiting zone (mm) in the testing bacteria			
	E. coli	Inhibiting power Category	*S. aureus*	Inhibiting power category
ET1	6,40	Medium	21,48	Very strong
ET2	4,52	Weak	8,56	Medium



(a). *E. coli* (b). *S. aureus*

Fig 1: Inhibition zone of isolate ET1



(a). *E. coli* (b). *S. aureus*

Fig2: Inhibition zone of isolat ET2

3.2. The Separation of Xanthorrizol using TLC

To obtain the results of the separation of the xanthrorrizol compound with TLC, N-hexan : ethyl acetate (10:1) eluen was applied to the endophytic bacteria isolate ET1with the visualization of 254 UV, resulting 2 spots with the Rf values of 0,57 and 0,77, while that of 365 UV, resulting 1 spot with the Rf value of 0,57. To the isolate ET2, the visualization of 254 results in 3 spots, with the Rf values of 0,19; 0,57; and of 0,77. Visualization with the lamp of 365 UV showed two spots with the Rf values of 0,54 and 0,60 (Table 2).

The identification of the separation of the zanthorrhizol compound in the spots and the Rf values resulted from this research was compared with the standard Rf of the xanthorrhizol using the same eluen. The Rf values in the spots of the bioactive compound of the endophytic bacteria isolate ET1 (Rf 0,57) and isolate ET2 (Rf 0,57) and the visualization of the 254 UV lamp is assumed to be the xanthorrhizol compound. It is in line with previous researches that in the separation of the xanthrorrizol compound with TLC from C. xanthorrhiza using the same eluen as an N-hexan: ethyl acetate (10: 1), spots with the Rf value of 0,54 with the purity content of 99,9% (Hwang et al., 2000), those with the Rf value of 0,56 with the purity content of 99.5% (Asriani, 2010) and those with theRf value of 0,54 with the purity content of 87.4% (Herdiyanto, 2014) are produced.

Table 2: The TLC analysis of the ethyl ecetate extract of endophytic bacteria

Bacteria isolates	Rf (UV_{254})	Rf (UV_{365})	color (UV_{365})	Rf standard xanthorizol
ET1	0,57	0,57	Blue	0, 54 (Hwang *et al.*, 2000)
	0,77			
ET2	0,19	0,54	Blue	0, 56 (Asriani, 2010)
	0,57	0,60	Violet	
	0,77			0, 54 (Herdiyanto, 2014)

3.3. Result of Bioautografi TLC Test

Bioautographic testing of the contact with the TLC plate to the results of separating the endophytic bioactive bacteria Isolate ET1 (Rf 0,57) and ET2 (Rf 0,57) did not produce any inhibiting zones in the E.coli and S. aureus (Figure 3). Radical or irradical inhibiting zones around the spots in the Rf values are not formed due to the transfer of bioactive compounds of the endophytic bacteria in the contact bioautography which is not maximum. It might be caused by the plate of chromatogram which did not stick well on the surface of the gel medium, and might also be resulted from the little amount of the bioactive compound left on the plate so that much of it was in the chromatogram and this resulted in too thin zone to observe. Some compounds on tie to matrices with the matrix of chromatogram plates, especially the silica-based matrices so that it cannot diffuse with the gelatin (Verbitsky et al. 2006, in Kusumaningtyas et al. 2008).

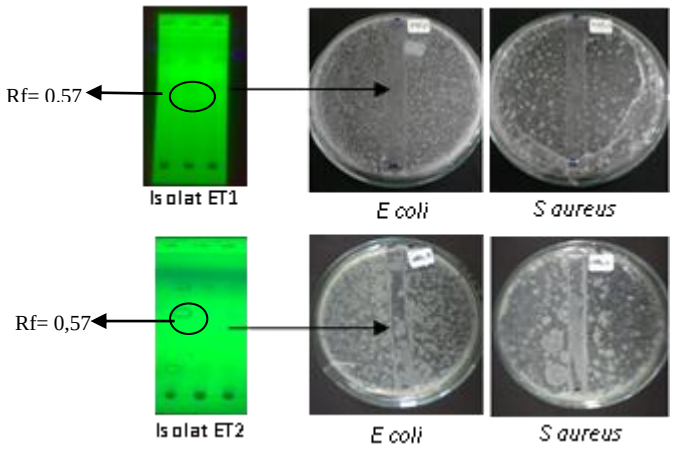


Fig 3: Result of bioautografi-TLC of endophytic bacteria on *E. coli and S. aureus*

3.4. Docking Simulation of the Xathorrhizol Compound

On the basis of the calculation result of the binding affinity (ΔG) value (Table 3), it is known that the xanthrorrhizol compound possess some potency to interact with the target protein in the testing of bacteria (PBP, DHFR and DNA gyrase enzymes). The enzymes are very important to the biosynthetic process of the bacterial cells, so that if their work are inhibited by certain compounds, the cell biosynthesis will be disturbed and this may result in the death of the cell. The PBP enzyme catalyzed the end process in the cell wall synthesis and is the main target of the antibiotic β-lactam. The DHFR (dihydrofolate reductase) enzyme is a key enzyme related to the metabolism of pholate acid. The DNA gyrase enzym is a multi-subunit one playing roles in the process of the DNA replication (Yang et al. 2013). The ampicilin, trimethoprim, and cyprofloxacin antibiotics are used as the control control since they are commercial antibiotics known as antibacterial medicines with good ability in inhibiting the target protein.

Table 3: The binding affinity (ΔG) of the test control ligands and the target protein in the bacteria

No	Ligand compound	Molekul target/ reseptor		
		PBP (kcal/mol)	DHFR (kcal/mol)	DNA gyrase (kcal/mol)
1	Ampicilin	-4.19	-	-
2	Trimethoprim	-	-6.40	-
3	Ciprofloxacin	-	-	-6.85
4	Xanthorrhizol	-3.20	-4.50	-4.81

The lowest ΔG value is the interaction between xanthorrizole and DNA gyrase (-4,81 kcal/mol) compared with that of PBB (-3.20 kcal/mol) and DHFR (-4.50 kcal/mol). According to Arwansyah et al (2014), the lower binding affinity value, the more stable between

the ligand and the receptor will be and this results in a harmonious condition to interact one another. The DNA gyrase is the target of anti-bacteria good for the antibiotic under the fluroquinolon (ciprofloxacin) group since this enzyme merely exists in the procariotic cell and is important for the growth of bacteria (Fabrega et all, 2009). The DNA gyrase plays an important role in the DNA replication serving to organize the DNA topology in cell, so that if the DNA gyraze is bound by the antibiotic, the DNA synthesis will be inhibited and at last resulted in the death of the cells (Mohamed et. al., 2013). But if the ΔG value of xanthorrhizol and the DNA gyrase is -4.84 kcal/mol is still above the ΔG value of the control antibiotic, -6.85 kcl/mol. The ΔG value between xanthrorrizole and the PBP and the DHFR is also higher than the positive control antibiotic. This shows that the potential of xanthorrhizol to interact with the target protein is lower that the that of the control antibiotic.

The interaction between xanthrorrhizol and the target protein is visualized using the Poseview program. On the basis of the visualization result, each has an interaction of the same amino acid between xanthorrizol and the control antibiotic in the hydrogen tie. In the PBP the same amino acid is Phe217, in the DHFR, Leu20, and in the DNA gyraze, Ile64. Therefore, it may be assumed that xanthorrhizol also has potency as an antibacterial substance like trimethoprim in inhibiting DHFR from S. aureus, ciprofloxacin in inhibiting DNA gyrase from E. coli, ampicilin in inhibiting PBP from S. aureus. (Table 4).

Table 4: Results of test and control ligand docking at the target protein

Protein target/ receptor	Ligand	Hydrogen tie	Hydrophobic tie
PBP	Xanthorrhizol	Phe217	Phe217
	Ampicilin	Phe217	Ser51,Ser115,Ser92,
DHFR	Xanthorrhizol	Leu20, Ile50, Phe92	-
	Trimethoprim	Leu20	Trp22
DNA gyrase	Xanthorrhizol	Asp35, Ile64	Asn32
	Ciprofloxacin	Ile64	Thr133

The potency of bioavability of xanthorrhizol in the body may be identified by analyzing the Role of Five (Ro5). In general, a medicine may actively work orally if it fulfills the following criteria: the donor of the hydrogen tie ≤5, the acceptor of the hydrogen tie ≤10, the molecular weight ≤500 Da, and lipophilicity (logarithm from n-octanol/water partition coefficient)5 (clogP≤ 5) (Lipinski, 2004). Based on the Lipinski criteria, it is predicted that xanthorhizol has a high potency of bioavability for the body.(Table 5). According to Veber et. al. (2002), bioavability is an ability of a medicine to be absorbed and distributed in the body.

Table 5: The results of *Role of Five* (Ro5) of the xantorrhizol compound

No	Ro5 Xantorrhizole compound	
1	Molecular weight	290 Da
2	Donor of the hydrogen tie	4
3	Receptor of the donor tie	7
4	LogP	1,26

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