ORIGINAL ARTICLE

Computational docking study of multitarget bioactive compounds in Indonesia traditional herbal medicine for tuberculosis therapy

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Abstract

Introduction: Tuberculosis (TB) is one of the leading infectious diseases in the world. It is commonly infected by *Mycobacterium tuberculosis* (TB) and can rapidly spread through droplet transmission. Poverty and malnutrition cause immunodeficiency, and thus, it increases the risk factor for TB. Indonesia traditional herbal medicine, jamu, has been using for long time to treat diseases involving TB. This research makes new jamu formulation from *Curcuma xanthorrhiza* Roxb., *Tamarindus indica* L., *Citrus aurantifolia*, and *Zingiber officinale* var. *rubrum* and analyzes the formulation with docking method. **Materials and Methods:** Protein targets used were from human matrix metallopeptidase 1 and Src and form MTB PknB and catalase-peroxidase. Compound-target proteins and protein-protein docking were conducted by PatchDock and FireDock. **Results and Discussion:** The docking results were analyzed and visualized using LigPlot$^+$ and PyMoL. Lipinski's rule and toxicity were checked by SwissADME and AdmetSAR. The result showed that 6 compounds from 223 compounds (not 222 compounds, but 223 compounds) analysed could play as multitarget compounds inhibiting four target proteins. In addition, two compounds were found which could change the binding location of Src and PknB coproteins. **Conclusion:** According to the results, the new jamu formulation has the potential to utilize as TB therapy.

Keywords: Jamu, molecular docking, multitarget compounds, tuberculosis

INTRODUCTION

Tuberculosis (TB) is one of the infectious respiratory diseases caused by airborne bacteria, *Mycobacterium* TB. TB causes main primary high mortality and morbidity in the world, and numerous new TB cases are arised annually. In 2014, it has been recorded that 9.6 million incident cases were discovered.[1] However, more than 95% of death patients of TB occurs in low- and middle-income countries, and it means that there is high correlation between poverty and TB infection.[2] Poverty causes deployment of TB, the majority through (1) living condition like living in the overcrowded place, slum, and poorly ventilated home, (2) prolong delaying checkup, and (3) malnutrition and/or HIV infection.[3] These facts match with TB dissemination case in Indonesia, which the regions with high TB transmission have high populated area, malnutrition cases, and HIV infection.[4] In addition, according to the WHO annotation, the reason of failure TB treatment is caused by the degree of poverty, difficulty to reach medical facilities, lack of medical staff, the high cost of TB drugs, and complicate procedure.[5] Malnutrition has a close link with infection; it causes immunodeficiency and enhances TB risk factor. Based on animal studies, insufficient nutrition intake reduces helper T cell 1 (Th1) cytokine secretion such as interferon-γ, interleukin-2, and tumor necrosis factor-α which had a role as mycobacteria infection control, reduce NO production, and also gain transforming growth factor beta production suppressing inflammation cytokine to eradicate mycobacteria.[6]

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The use of common TB medication causes TB cases which have been developing lately. For a long time, common medicine for treating TB has been isoniazid, rifampicin, pyrazinamide, and ethambutol (first-line drug). Unfortunately, these drugs cause rapid evolution and result resistant to MTB. Furthermore, this case leads to multidrug-resistant TB (MDR-TB) and makes TB more serious and difficult to treat.[7] Second-line drugs such as aminoglycosides, polypeptides, fluoroquinolones, thioamides, cycloserine, and para-aminosalicylic have been used to treat MDR-TB; however, so far, these drugs cause mutation and emerge extensively drug-resistant TB (XDR-TB) case. XDR-TB is described as MTB not only resistant to first-line drugs but also to second-line drugs.[8] From these reasons, it can be concluded TB therapy focusing only to exterminate bacteria cause mutation and make diseases more severe and hard to cure. Nowadays, for overcoming TB cases, multiple therapies which can eradicate mycobacteria, improve nutrition, and balance immunity and the human system should be developed.

Jamu is Indonesia traditional herbal medicine that has been used for a long time ago in Indonesia community for maintaining health and treating diseases. Jamu is a traditional medication from ancestor and still popular in rural and urban areas.[9] In jamu production, people use various plants which are easy to found in their environment. For resolving TB case, this study tries to make new jamu formulation form Indonesia medical plants that used Indonesia local people to treat TB, *Curcuma xanthorrhiza* Roxb., *Tamarindus indica* L., *Citrus aurantifolia*, and *Zingiber officinale* var. *rubrum*. Some reports have shown that all of these plants had the ability as antimicrobial and immunostimulation.[10-12] For analyzing the effect toward TB, *in silico* docking method was used in this study. The targets protein selected were not only from MTB for eradicating mycobacteria but also from a human for regulating defense mechanism, matrix metallopeptidase 1 (MMP1), tyrosine-protein kinase Src, protein kinase (PknB), and catalase-peroxidase (KatG).

MMPs are a member of zinc-dependent protease that has two conserved domains as a predominant and a catalytic domain. MMPs can degrade components of extracellular matrix-like collagens, laminin, fibronectin, vitronectin, and proteoglycans. MMP activity is controlled by the gene expression and proenzyme activation. Tissue inhibitor of metalloproteinase is an inhibitor of MMPs. High activity of MMP can induce diverse pulmonary disease caused by extracellular matrix destruction.[13] In TB patients, MMP1 has been found had upregulation, and MTB caused high expression of MMP1. The excessive of MMP1 leads to granuloma degradation, thus causing mycobacteria disseminate to another part of the human body.[14,15]

Src protein-tyrosine kinase, a non-receptor protein–tyrosine kinase, is a proto-oncogene that is important for cell morphology, motility, proliferation, and survival. Src structure contains the SH3 domain, a protein-tyrosine kinase domain, and SH2 domain, C-terminal regulatory tail.[16] PknB is a transmembrane signaling kinase which has a signal recognition domain and an intracellular kinase domain. PknB plays as cell growth and division regulator in MTB. PknB encoded by *PknB* which is part of the operon carrying cistron coding involved cell shape control.[18,19]

KatG is a multifunctional catalase-peroxynitrite and NADH oxidase. By the KatG enzyme, INH is changed into INH-NAD which can interfere with activation inhibiting NADH-dependent enoyl-ACP reductase (inhA) in mycolic acid biosynthesis process. Mutation in *katG* and *inhA* is associated with isoniazid resistance. Reduction of catalase or peroxidase activity is the result of *katG* mutation in which most common mutation is in S315T. In addition, mutation in *inhA* causes resistance to isoniazid and ethionamide. *inhA* mutation occurs commonly in its promoter region and it associates with monoresistant strains.[20]

MATERIALS AND METHODS

Ligand Preparation

There were 55 compounds of *C. xanthorrhiza* Roxb., 59 compounds of *Z. officinale* var. *rubrum*, 55 compounds of *T. indica L*., and 54 compounds of *C. aurantifolia.* All three-dimensional (3D) structure of the compounds and other 3D chemicals such as mitoxantrone, cyanidin, dasatinib, morin, and isoniazid were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

Protein Preparation

Target proteins used were PknB and KatG from MTB and MMP-1 and Src from human. Protein 3D structures were obtained from Protein Data Bank (http://www.rcsb.org/); the PDB code is as follows: PknB (2FUM), ForkHead Associated A (FhaA) (3PO8), KatG (1SJ2), MMP1 (3SHI), Src (1FMK), and PIK3 (3L54). The controls used were determined by the following: Mitoxantrone-PknB bond, isoniazid-KatG bond, doxycycline-MMP1 bond, and saracatinib-Src bond. The water molecules in proteins were omitted using Discovery Studio 2016 before docking process.

Docking Simulation and Interaction Analysis

Patchdock was used for docking protein-bioactive compounds and complex protein-protein.[21,22] It presents geometry-based molecular docking algorithm and gives geometry shape complementarity score, area, atomic contact energy (ACE), and 3D transformation outputs; nevertheless, the highest geometry shape complementarity score was chosen. Root mean square deviation (RMSD) value 1.5 was

used for docking protein compounds, and RMSD value 4.0 was used for docking complex protein-protein. For protein-protein docking, Firedock was used to refine protein-protein docking. It shows binding energy or global energy value, attractive and repulsive Van der Waals force value, ACE, and the contribution hydrogen bounds to global binding energy (HB), but only the highest binding energy was used in this study.[23,24] LigPlot+ was used to analyze the ligand-protein structure and binding after docking process.[25] This program showed 2D structure of position and interaction ligand in protein. The results showed H-bond interactions and distance, hydrophobic interaction, and external binding. The result of protein-protein dockings were visualized with PyMoL.

Chemical Information and Toxicity

Swiss ADME was used to calculate Lipinski's rule of five. The toxicity was analyzed with AdmetSAR.[26,27]

RESULTS

Active Compound Docking

According to the docking screening of 223 compounds' result, it has been chosen highest ten top best scores of each protein-ligand docking by PatchDock [Table 1]. 17 compounds were selected based on ten highest score from docking result. Each of the compounds had various pattern scores while docked with target proteins. Phenol compounds such as curcumin and demethoxycurcumin had supreme binding energy score while docked with MTB PknB and KatG and human MMP1, whereas an organic compound, oleic acid, had the first score when docked with Src of human.Moreover, the docking results presented that curcumin, demethoxycurcumin, phytol, oleic acid, and linoleic acid [Figure 1] could bind with four target proteins; it might be concluded that these compounds were multitarget compounds. For more exploring, binding interaction and position were analyzed with LigPlus.

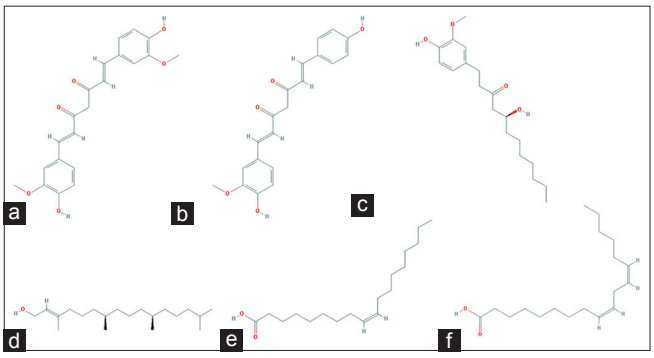

Figure 1: Active compounds of (a) curcumin, (b) demethoxycurcumin, (c) 8-gingerol, (d) phytol, (e) oleic acid, (f) linoleic acid

Active Compounds - human Proteins

According to Figure 2, the compounds bound with a catalytic domain which is in residues of 106–261 MMP1.[28] Curcumin, demethoxycurcumin, and phytol [Figure 2a,b,d] had one external binding, respectively, with Asn 143, Gln 135, and Thr 148. In addition, curcumin had one hydrogen bound interaction with Tyr201 (2.76 Å), while demethoxycurcumin [Figure 2b] had two hydrogen binding interactions with Phe 149 (2.24 Å) and Arg 202 (2.72 Å). These interaction numbers were less than the control that had three hydrogens bound in Asp124 (2.97 Å) and Ser 142 (2.23 Å and 3.33 Å). In contrast, 8- gingerol, oleic acid, and linoleic acid [Figure 2c,e,f] had none of the external binding and hydrogen binding, and it tended to be hydrophobic while contact with MMP1.

When compounds docked with Src, phenol compounds, curcumin, demethoxycurcumin, and 8-gingerol [Figure 3a-c] had the same interaction residues with control [Figure 3f], Thr 247, Phe 150, Leu 161, Ile 153, and Val 399. Moreover, these compounds also interacted with Src in SH3 domain (83–142) and SH3-SH2 interaction domain (142–146).[29,30] Curcumin and demethoxycurcumin had one hydrogen binding, Lys104 (2.07 Å), Val 339 (3.19 Å), and one external binding Lys 104 and Val 339. 8-gingerol and control had one hydrogen binding interaction with Thr 247 (3.02 Å) and Asn397 (2.84 Å). However, phytol and oleic acid had the same position when interacting with Src, Asn 391, Leu 273, Ser 345, Asp 404, Leu 393, and Asp 348. Oleic acid had a hydrogen binding with Asp 386 (2.97 Å), while phytol and linoleic acid tended to be hydrophobic while interacting with Src.

Active Compounds - MTB Proteins

Based on Figure 4, active compounds had the same position with control, eventhough the binding scores were lower than the control. All the compounds are contact with N-terminal lobes of PknB, Leu 17, Gly 18, Val 25, Ala 38, Met 92, Glu 93, and Val 95 and C-terminal of PknB lobes (exclude curcumin), Met145, and Met155. It has been evident that the connection in this position could suppress the activity of PknB in MTB.[31] Curcumin [Figure 4a] had two hydrogen binding with Thr149 (2.81 Å) and Ser147 (3.23 Å); 8-gingerol, phytol, and oleic acid [Figure 4c and d] had one hydrogen binding, respectively; Glu 93 (3.15 Å), Asn143 (2.67 Å), and Asp (102), control only had an external binding with Asp126, and linoleic acid had none of the hydrophobic and external binding.

In KatG docking term, the result described that the compounds had different location binding sites [Figure 5]. Curcumin and control [Figure 5a and g] had interaction with Gly 421, Asp 440, Gln 439, and Gly 490, demethoxycurcumin and 8-gingerol interacted with Glu 709, Arg 705, Arg 145, Arg 128,

Table 1: Ten highest score of screening result

Plants	Compounds	Class	Binding energy score (kcal/mol)			
			MMP1	Src	PknB	KatG
Curcuma xanthorrhiza Roxb.	Curcumin	Phenol	5118	5140	5572	5414
Curcuma xanthorrhiza Roxb.	Demethoxycurcumin		4802	4920	5108	5692
Curcuma xanthorrhiza Roxb.	Bisdemethoxycurcumin		-	5060	-	-
Zingiber officinale var. *rubrum*	8-gingerol		5222	5146	5482	5430
Zingiber officinale var. *rubrum*	8-shogaol		4738	-	5388	5422
Zingiber officinale var. *rubrum*	6-gingerol		-	4892	5002	-
Zingiber officinale var. *rubrum*	6-shogaol		-	-	-	-
Zingiber officinale var. rubrum, *Citrus aurantifolia*	Phytol	Terpenoid	4730	4952	5476	5370
Curcuma xanthorrhiza Roxb.	Citronellyl pentanoate		-	-	-	5320
Tamarindus indica L., Tamarindus indica L., Citrus aurantifolia	Oleic acid	Organic acid	4896	5190	5326	5444
Tamarindus indica L., Citrus aurantifolia	Linoleic acid		4560	5156	5216	5660
Tamarindus indica L.	Heptadecanoic acid		4896	5038	-	5384
Curcuma xanthorrhiza Roxb.	Butyl dodecanoate		4610	-	-	-
Tamarindus indica L.	Myristic acid		-	-	-	-
Citrus aurantifolia	Palmitic acid		-	4976	-	-
Tamarindus indica L.	Palmitoleic acid		4598	-	5230	5394
Tamarindus indica L.; Citrus aurantifolia	Linolenic acid		-	-	5318	-
	Control	-	4856	6770	5906	3088

Table 2: Protein docking by patchdock and firedock

Compounds	Global energy (kcal/mol)	Attractive VdW	Repulsive VdW	ACE	HB
(Src-curcumin)-PI3K	–43.44	–29.21	3.78	3.89	–4.77
(Src-demethoxycurcumin)-PI3K	–45.16	–35.56	13.61	5.67	–6.19
(Src-8-gingerol)- PI3K	–45.16	–35.56	13.61	5.67	–6.19
(Src-phytol)- PI3K	–36.43	–35.92	22.70	6.81	–10.75
(Src-oleic acid)- PI3K	–38.88	–36.67	15.32	2.49	–6.51
(Src-linoleic acid)- PI3K	–43.67	–38.65	23.70	5.77	–7.30
(Src-control)-PI3K	–41.29	–40.88	28.87	6.73	–7.53
Src-PI3K	–43.67	–38.65	23.70	5.77	–7.30
(PknB-curcumin)-FhaA	–46.84	–29.90	14.47	2.99	–8.55
(PknB-demethoxycurcumin)- FhaA	–46.84	–29.90	14.47	2.99	–8.55
(PknB-8-gingerol)- FhaA	–46.84	–29.90	14.47	2.99	–8.55
(PknB-phytol)- FhaA	–47.75	–44.47	24.38	–3.40	–10.64
(PknB-oleic acid)- FhaA	–46.84	–29.90	14.47	2.99	–8.55
(PknB-linoleic acid)- FhaA	–46.84	–29.90	14.47	2.99	–8.55
(PknB-control)-FhaA	–46.84	–29.90	14.47	2.99	–8.55
PknB-FhaA	–46.84	–29.90	14.47	2.99	–8.55

PIK3 (PIK3 not P13K): Phosphoinositide 3-kinase, FhaA: ForkHead Associated A

Gly 297, and Glu 289, whereas oleic and linoleic acid had the same position to connect with Asn 44, Lys 46, Glu 195, Asn 35, Gly 32, Gln 36, Gly 33, and Arg 42. Demethoxycurcumin had three hydrogen bindings with Ser 700 (2.81 Å), Asn 41 (3.04 Å), and Tyr 608 (2.52 Å); 8-gingerol, oleic acid, and linoleic acid had a hydrogen bindings, respectively, with Glu 709 (2.87 Å), Gln 36 (2.44 Å), and Gln 36 (3.01Å); curcumin, linoleic acid, and control had an external binding, but phytol had distinct position binding with others and it only had hydrophobic interaction with KatG.

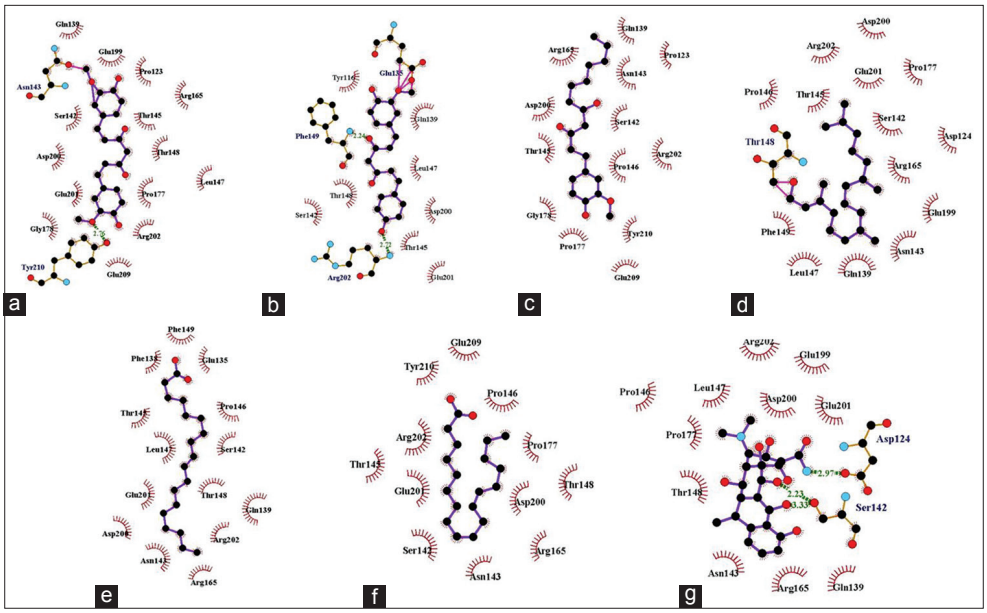

Figure 2: Matrix metallopeptidase 1 - active compounds, (a) curcumin, (b) demethoxycurcumin, (c) 8-gingerol, (d) phytol, (e) oleic acid, (f) linoleic acid, (g) control

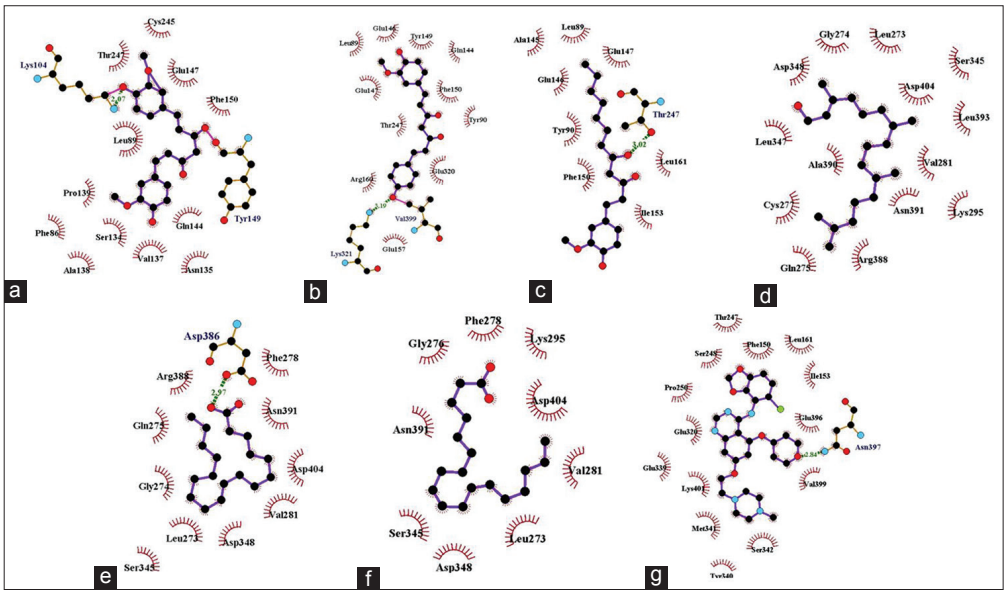

Figure 3: SRC - active compounds, (a) curcumin, (b) demethoxycurcumin, (c) 8-gingerol, (d) phytol, (e) oleic acid, (f) linoleic acid, (g) control

Protein Docking

In Src and PknB term, binding score with control was higher than with active compounds [Table 1], and for more understanding this case, protein docking between protein target-another with downstream protein was conducted. Src was docked with phosphatidylinositide 3-kinases (PI3K), a protein signal transducer phosphorylating the inositol group of phosphoinositides. Src-PI3K complex can active the AKT/mTOR pathway involving negative regulator of autophagy, and it has been shown that inhibitor of Src-PIK3 proven to inhibit the survival of MTB.[17,32] However, based on the docking result [Table 2], the global energy value of control was lower (−41.29 kcal/mol) than phytol (−36.43 kcal/mol) and oleic acid (−38.88 kcal/mol). It was assumed that phytol and oleic acid might be a better inhibitor than saracatinib. PknB was docked with FhaA, the substrate of Ser/Thr protein kinases. It was reported that there was an interaction between PknB and FhaA for MTB growing process.[33] In addition according to the results, it showed that all of the complexes had same value except complex protein with phytol [Table 2], it had the lowest global energy, indicating that it had robust binding rather than the other complexes. Furthermore, in Src term, according to the visualization of protein docking complex protein with phytol and oleic acid, these compounds could change the conformation of the complex protein [Figure 6]. Not only

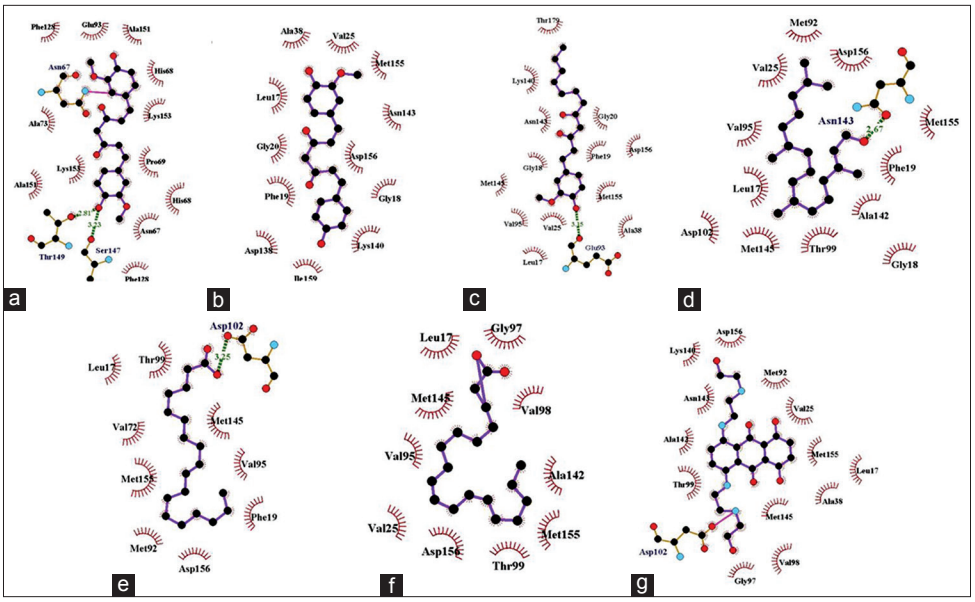

Figure 4: PknB - active compounds, (a) curcumin, (b) demethoxycurcumin, (c) 8-gingerol, (d) phytol, (e) oleic acid, (f) linoleic acid, (g) control

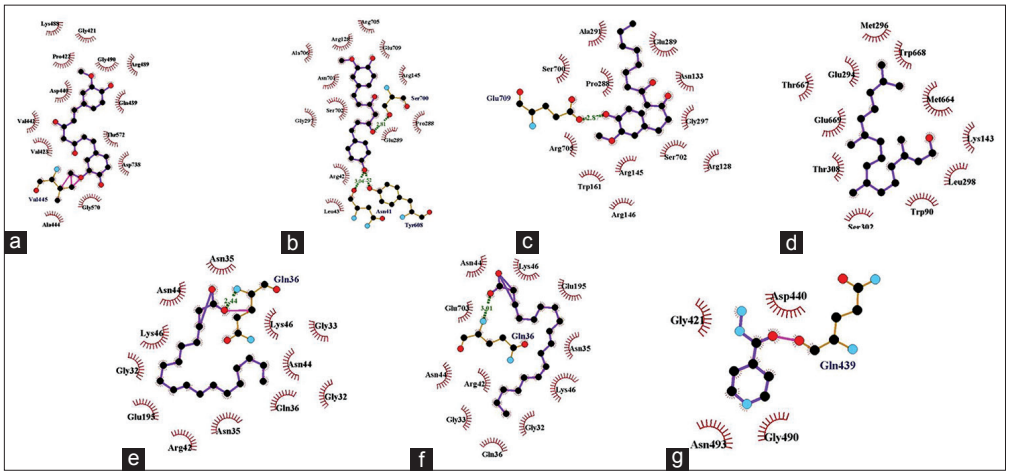

Figure 5: KatG - active compounds (a) curcumin, (b) demethoxycurcumin, (c) 8-gingerol, (d) phytol, (e) control

changing the conformation but also the binding location was different with the control which had the same position with other compounds such as curcumin, demethoxucurcuin, and 8-gingerol. However in PknB case, only phytol could change the conformation of PknB and FhaA. Phytol also had a different position when interacting with PknB, and it seemed that phytol interacts with a distinct chain of PknB.

Lipinski's Rule and Toxicity

The value of Lipinski's score and toxicity could be seen in Table 3, and it represented that phytol, oleic acid, linoleic acid, mitoxantrone, doxycycline, and saracatinib had a violation even it still allowed in Lipinski's rule. Isoniazid and mitoxantrone had a high probability to cause the mutation in bacteria, and it might induce the bacteria resistance with drugs. All of the compounds showed that it did not induce carcinogen.

DISCUSSION

C. xanthorrhiza Roxb., *Zingiber officinale* var. *rubrum*, *T. indica* L., *Ocimum basilicum,* and *C. aurantifolia* are used by Indonesia local people to treat TB. However, not only in Indonesia but also in other counties use these plants for TB. Furthermore, these plants and the constituents have been evaluated to inhibit the cells growth and infection of MTB.[34-36] According to the result, the plants consisted of six compounds: curcumin, demethoxycurcumin, 8-gingerol, phytol, oleic acid, and linoleic acid which could act as multitarget compounds, inhibiting four targets proteins.

Nowadays, multitarget drug is one possible way that can be developed to overcome TB. Multitarget compounds can target many proteins and it is more effective rather than combination compounds with single target, respectively. There are three types of multitarget inhibitors that can design

Table 3: Ligand property and toxicity

Compounds	Lipinski's rule	AMES toxicity	Probability	Carcinogen toxicity	Probability
Curcumin	Yes; 0 violation	Non-AMES toxic	0.9132	Non-carcinogens	0.8689
Demethoxycurcumin	Yes; 0 violation	Non-AMES toxic	0.7747	Non-carcinogens	0.8866
8-Gingerol	Yes; 0 violation	Non-AMES toxic	0.7697	Non-carcinogens	0.9121
Phytol	Yes; 1 violation: MLOGP>4.15	Non-AMES toxic	0.9132	Non-carcinogens	0.5055
Oleic acid	Yes; 1 violation: MLOGP>4.15	Non-AMES toxic	0,9674	Non-carcinogens	0,6568
Linoleic acid	Yes; 1 violation: MLOGP>4.15	Non-AMES toxic	0,9674	Non-carcinogens	0,6568
Isoniazid	Yes; 0 violation	AMES toxic	0,8557	Non-carcinogens	0,7514
Mitoxantrone	Yes; 1 violation: NH or OH>5	AMES Toxic	0.9108	Non-carcinogens	0.8742
Doxycycline	Yes; 1 violation: NH or OH>5	Non-AMES toxic	0.9132	Non-carcinogens	0.8632
Saracatinib	Yes; 1 violation: MW>500	Non-AMES toxic	0,5	Non-carcinogen	0,9215

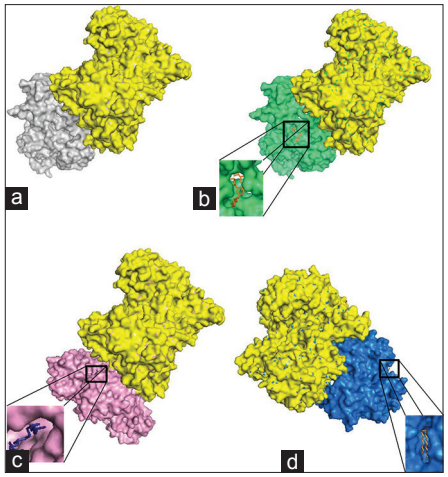

Figure 6: SRC complex (a) SRC-PIK3, (b) (SRC-control)-PIK3, (c) (SCR-phytol)-PIK3, (d) (SRC-oleic acid) - PIK3. Gray structure: SRC wild-type protein; yellow structure: PIK3: green structure: SRC-saracatinib complex; pink structure: SRC-phytol; blue structure: SRC-oleic complex

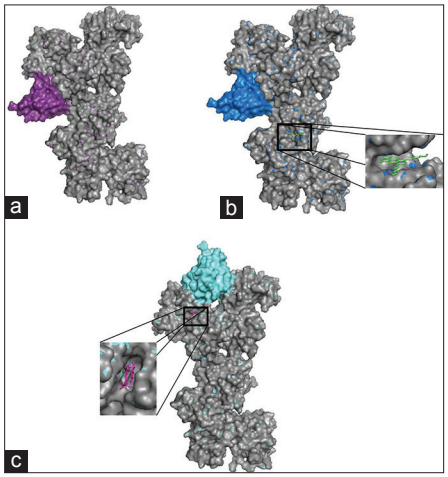

Figure 7: PknB complex, (a) PknB- ForkHead Associated A (FhaA), (b) (PknB - control)-FhaA, (c) (PknB -phytol)-FhaA gray structure: PknB; purple: FhaA in PknB-FhaA complex; dark blue: FhaA in PknB -(PknB -mitoxantrone)-FhaA complex; light blue: FhaA in PknB -(PknB -phytol)-FhaA complex

for treating infectious diseases: (1) Series inhibitor, which can inhibit proteins in the same metabolic pathway; (2) parallel inhibitor, which can inhibit proteins unrelated mechanism; and (3) network inhibitor, which is a combination of series inhibitor and parallel inhibitor.[37] Recently, scientists design new multitarget inhibitor for TB.

Recently, scientists design new multitarget inhibitor for TB called SQ109. It has been reported that could inhibit transporter proteins, MmpL3, manaquinone biosynthesis and ATP synthesis.[38] Nevertheless, it will not effective when only targeting the virulence factor. Otherwise, development of mycobacteria dissemination is also caused by imbalance immune system or other human mechanisms.

From previous studies, oleic acid and linoleic acid could be selective inhibitor of enoyl-acyl carrier protein reductase (FabI) which takes apart in fatty acid synthesis in bacteria.[39] Curcumin has been reported to induce the activation of JNK pathway to activate apoptosis in macrophage.[40] 8-gingerol could inhibit mycobacteria survival through it lipophilicity characteristic.[41] Phytol has been also shown that could suppress the mycobacteria infection.[42] Moreover, all of these compounds were shown to attach in the catalytic domain of MMP1, and these indicated disturbed catalytic activity of MMP1 to break collagen which is constituting granuloma. Curcumin, linoleic acid, oleic acid, and phytol have been evaluated which could also suppress the expression of MMP1 gene.[43-45] In Src inhibition, all of the compounds had a various effect toward the Src-PIK3 complex. Curcumin has been reported which could suppress the activation signaling Src/PIK3 pathway for inducing apoptosis.[46]

CONCLUSION

The novel formulation of jamu for TB therapy contained six compounds, curcumin, demethoxycurcumin, 8-gingerol, phytol, oleic acid, and linoleic acid could bind all of the target proteins. According to docking complex, phytol and oleic acid could change the position of Src while a bond

with PIK3, and in addition, phytol also could change FhaA position while docked with PknB. This novel discovery should be analyzed with advanced simulation to explore the conformation complex. Based on the results, this novel formulation could be TB medication; however, it should be analyzed by *in vitro* and *in vivo* research to ensure the effect.

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