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Original Article

Quercetin, rutin, proanthocyanidin, catechin and epitacethin as fatty acid synthase inhibitor using virtual screening

Candra Rini Hasanah Putri[1,2*], Sutiman Bambang Sumitro[3], Setyawati Karyono[4]

[1]Doctoral Program of Medical Science, Faculty of Medicine, Brawijaya University, Malang, Indonesia

[2]Department of Anatomy, Faculty of Medicine, Wijaya Kusuma University, Surabaya, Indonesia

[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, Indonesia

[4]Department of Pharmacology, Faculty of Medicine, Brawijaya University, Malang, Indonesia

Abstract

Fatty acid synthase is known to participate in the occurrence of malignancies, so fatty acid synthase inhibition is expected can restrain malignancy. In this research, virtual screening is done by molecular docking between the active ingredients in *Tamarindus indica* with three domains of FAS. In thioesterase domain, it turns out all of the active ingredients in *Tamarindus indica* can bind thioesterase domain right in the place where orlistat (as a reference inhibitor) bonded, with a higher strength than the orlistat. In enoyl [acyl-carrier-protein] reductase domain, it turns out the binding affinity of quercetin, rutin, catechin and epicatechin against fatty acid synthase are greater than the reference inhibitor, triclosan. In malonyl-CoA / acetyl-CoA-ACP-transacylase domain, it turns out the binding affinity of quercetin, rutine, proanthocyanidin, and catechin against fatty acid synthase is greater than the natural substrate, Malonyl-CoA. The high binding affinity of the active ingredients in *Tamarindus indica* against the two domains of fatty acid synthase that may also can be occupied by reference inhibitors, showed the ability of *Tamarindus indica* as an inhibitor of fatty acid synthase. While the high ability of active ingredients in *Tamarindus indica* to bind to a domain that should be occupied by the natural substrate of fatty acid synthase (malonyl-CoA) demonstrated the ability of *Tamarindus indica* to inhibit fatty acid synthase's work in a way to compete with the natural substrate. This study shows that *Tamarindus indica* may serve as anti-malignancy through its ability to inhibit fatty acid synthase.

Keywords: catechin, epicatechin, fatty acid synthase, proanthocyanidin, quercetin, rutin

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Introduction

Fatty acid synthase is a big multi-enzym that catalyzes various stages of fatty acid synthesis (Maier *et al.*, 2008). This polypeptide contains seven catalytic domains, namely β-ketoacyl synthase, malonyl /acyltransferase (MAT), dehydrogenase, enoyl reductase, β-ketoacyl reductase, acyl carrier protein and thioesterase. The process of fatty acids synthesis begins with condensation of malonyl-coA which is catalyzed by the MAT domain of fatty acid synthase (Liu *et al.*, 2010). Various studies indicate that fatty acid synthase (FAS) has positive roles in the different paths that lead to cell proliferation, for example in lung cancer cells, breast cancer cells, meningioma cells, and ovarian cancer cells (Orita *et al*., 2008; Puig *et al*., 2009; Haase *et al*., 2010; Tomek, K. *et al*., 2011). Fatty Acid Synthase's activity was known to be inhibited on thioesterase domain (Pemble *et al*., 2007) and enoyl [acyl-carrier-protein] reductase domain (Sippel *et al*., 2014) in FAS.

This study is a virtual screening to show the details mechanism of interaction between active substances antioxidants that contained in *Tamarindus indica*, namely quercetin, rutin, proanthocyanidin, catechin and epicatechin (Amir *et al*., 2013; Sudjaroen *et al.*, 2005) with FAS, to determine the mechanism of inhibition of *Tamarindus indica* against FAS. Because of FAS itself has roles in the proliferation and malignancy, so the binding of FAS by the active substance of antioxidants in *Tamarindus indica* shows that *Tamarindus indica* can serve as anti-malignancy.

* Corresponding Author:
Candra Rini Hasanah Putri
Doctoral Program of Medical Science, Faculty of Medicine, Brawijaya University, Jl. Veteran, Malang, Indonesia, 65145
Phone: 0818535071 Fax: (031)5686531
e-mail: candrarinihp75@gmail.com

Method

Retrieval of Sample

All of materials that was used in this research was samples that be taken from the database of web servers on the internet. FAS protein structure was obtained from a database of protein structures in the PDB BANK (www.rcsb.org) (2PX6 and 4W9N) and was modeled by using SWISS Model (for 4W9N). Malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT) domain of FAS also was obtained from PDB BANK (www.rcsb.org) (2JFD). That protein structures then were prepared by using PyMOL program to remove the water and eliminate ligand molecules contained within the complex.

The compounds of quercetin (5280343), rutin (5280805), proanthocyanidin (108065), catechin (9064) and epicatechin (72276) were obtained from the compounds database PubChem (https://pubchem.ncbi.nlm.nih.gov) and were minimized to obtain the most suitable conformation before interacting with the target protein by using OpenBabel in PyRx. A natural substrate of MAT, namely malonyl-CoA, also was obtained from PUBCHEM (https://pubchem.ncbi.nlm.nih.gov), then was prepared by Vega ZZ, and then was minimized to obtain the most suitable conformation before interacting with the target protein by using OpenBabel in PyRx.

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Molecular Docking Process

Molecular docking was done specifically on the active side of the FAS (adjusted by references), by analyzing the binding of inhibitors to the FAS. A natural inhibitor of FAS was separated and then was used as a control in the process of docking. Special on MAT domain, FAS natural substrate (malonyl-CoA) which is used as a control in the process of docking. If the compound has a binding site and its affinity was as high as the reference inhibitor, then the compound can be considered as a potential inhibitor. Docking process was done using Autodock Vina in PyRx 0.8.

Molecular Interaction and Visualization

The molecular interaction and visualization of the docking results were analyzed by using LigandScout 2.0 (for thioesterase domain and enoyl [acyl-carrier-protein] reductase domain) and LigPlot^{+} Ver. v.1.4.5 (for malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT) domain), so all of the amino acids that interact directly can be known

Results

By using virtual screening, turns all of the active ingredients in *Tamarindus indica* that was studied, namely quercetin, rutin, proanthocyanidin, catechin, and epicatechin can bind to the thioesterase domain FAS, where visualization can be seen in Figure 1. In Figure 1, it can be seen that all binding sites of the ligand's bonds that were studied were right where the reference inhibitor (orlistat) was bind with domain thioesterase FAS. Furthermore, from Table 1 it can be seen that the results of binding affinity of all these ligands are more negative than the binding affinity of orlistat.

Table 1. The comparison of binding affinity between ligands with thioesterase domain in Fatty Acid Synthase

Ligand	Receptor	Binding Affinity
Orlistat (reference inhibitor)	Fatty Acid Synthase (Thioesterase Domain)	-6.3
Quercetin (5280343)	Fatty Acid Synthase (Thioesterase Domain)	-7.6
Rutin (5280805)	Fatty Acid Synthase (Thioesterase Domain)	-7.6
Proanthocyanidin (108065)	Fatty Acid Synthase (Thioesterase Domain)	-9.5
Catechin (9064)	Fatty Acid Synthase (Thioesterase Domain)	-6.5
Epicatechin (72276)	Fatty Acid Synthase (Thioesterase Domain)	-7.3

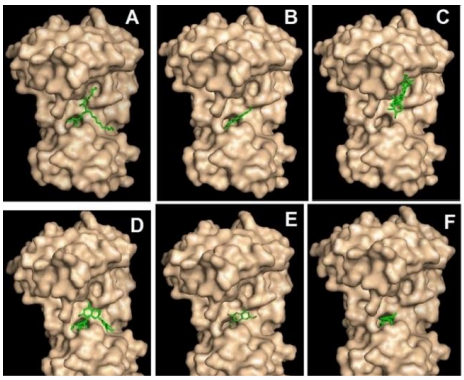

Figure 1. The position of each ligands in association with thioesterase domain in Fatty Acid Synthase. Ligands in green. (A) Orlistat. (B) quercetin. (C) rutin. (D) proanthocyanidin. (E) catechin. (F) epicatechin

Table 2. Comparison of amino acids that involved in the bond between the ligands with Fatty Acid Synthase in Thioesterase Domain

	Orlistat	Quercetin	Rutin	Proanthocyanidin	Catechin	Epicatechin
hydrogen bond	ILE2250	GLN2374	SER2340	TYR2343 -3.12	GLU2251	ILE2250
		ILE2250	SER2308	GLU2251 -2.59		LYS2426 -3.27
		TYR2343	THR2342	LEU2222		ALA2419
		LEU2222	ASP2338	ALA2419 -3.30		
			TYR2462			
			ALA2448			
			THR2450			
hydrophobic bond	TYR2343	PHE2371	TYR2343	PHE2423	PHE2423	LEU2222
	THR2342	PHE2423		PHE2371	LEU2222	PHE2423
	PHE2423			ILE2250	ILE2250	PHE2371
	PHE2371			PHE2370		
	LEU2222					
	ALA2367					
	LEU2427					
	ALA2363					
amino acid similarity		25%	12.50%	50%	37.50%	50%

To ascertain whether the ligands indeed occupy thioesterase domain of FAS, examination of molecular interactions was done to determine the amino acids that interact directly by using LigandScout 2.0 software. In Figure 2, it appears that the interaction between the ligands with FAS turns out to involve 2 to 5 amino acids that similar to amino acids that involved in the interaction between orlistat (as a FAS reference inhibitor) with FAS

When compared with orlistat, it turns out that proanthocyanidin and epicatechin are compounds in *Tamarin-*

dus indica that have the most similarity (in term of its amino acid's bonds) which is about 50%, followed by catechin (37.5%), quercetin (25%) and rutin (12.5%) (Table 2). Although the equation of amino acids is not up 100%, but in proanthocyanidin, quercetin, and rutin, it produces greater bond strength, due to the different types of the bonds of amino acids. On bonding with orlistat, amino acids are bound by hydrophobic bonds, while the proanthocyanidin, quercetin, and rutin, the type of bond are hydrogen bonding that is stronger. Epicatechin also has amino acids similarities up to 50% with orlistat, but it bond strength was almost the same with orlistat.

Meanwhile, in the enoyl (acyl-carrier-protein) reductase domain of FAS, it turns out that all of the active ingredients in *Tamarindus indica* that be studied, namely quercetin, rutin, proanthocyanidin, catechin, and epicatechin also be able to bind in the place where the reference inhibitor (triclosan) binds, as shown in the visualization in Figure 3. In Figure 3, it can be seen that all of the ligands that being studied can bind with FAS right where triclosan can bind FAS. The examination of binding affinity by using software PyRx shows that besides proanthocyanidin, the other active ingredients in *Tamarindus indica* showed bonding capabilities with FAS at enoyl (acyl-carrier-protein) reductase domain which is stronger than the triclosan (more negative) (Table 3). It is also in line with the results of examination of molecular interactions with software LigandScout 2.0, where in addition to proanthocyanidin, the bond between the other ligands on enoyl (acyl-carrier-protein) reductase domain FAS turns out to involve 4 to 5 amino acids that similar to amino acids that were involved in the interaction between triclosan with enoyl (acyl-carrier-protein) reductase domain of FAS.

When compared with triclosan, it turns out that quercetin, catechin, and epicatechin in *Tamarindus indica* are the compounds that have most amino acids bond similarity, which is about 71.4%, followed by rutin (57.1%) (Table 4).

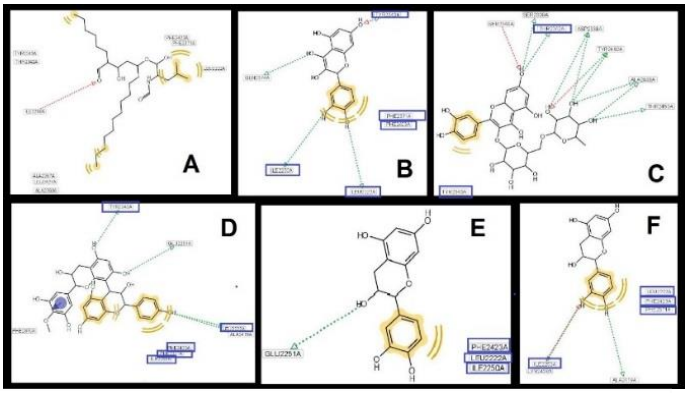

Figure 2. The Amino Acids that are Involved in the Bond between the Ligands and Thioesterase Domain in Fatty Acid Synthase**.** Amino acids that are in the blue box is the same amino acids in the orlistat (as reference inhibitor) that also hold the bond with thioesterase domain in Fatty Acid Synthase (A) Orlistat. (B) quercetin. (C) rutin. (D) proanthocyanidin. (E) catechin. (F) epicatechin.

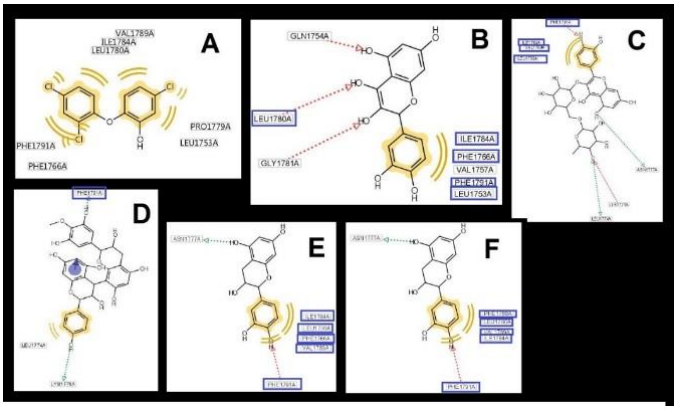

Figure 4. The Amino Acids that are Involved in the Bond between the Ligands and Enoyl [acyl-carrier-protein] Reductase Domain in Fatty Acid Synthase. Amino acids that are in the blue box is the same amino acids in the reference inhibitor (triclosan). (A) Triclosan (B) quercetin. (C) rutin. (D) proanthocyanidin. (E) catechin. (F) epicatechin.

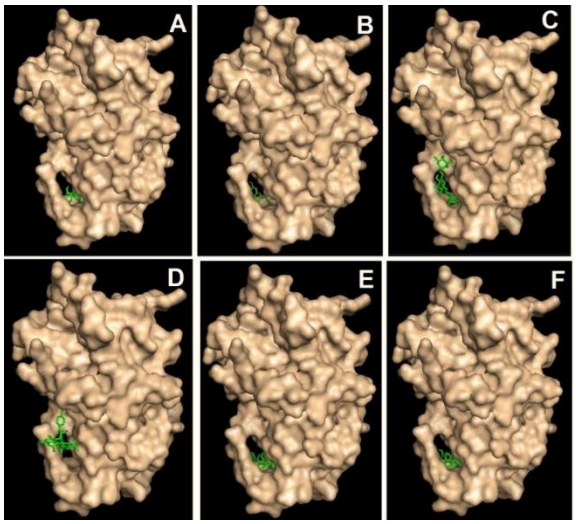

Figure 3. The position of each ligands in association with Fatty Acid Synthase in enoyl [acyl-carrier-protein] reductase domain. Ligands in green. (A) Triclosan. (B) quercetin. (C) rutin. (D) proanthocyanidin. (E) catechin. (F) epicatechin.

Table 3. The comparison of binding affinity between ligands with Enoyl (Acyl-Carrier-Protein) Reductase domain in Fatty Acid Synthase

Ligand	Receptor	Binding Affinity
Triclosan (reference inhibitor)	Fatty Acid Synthase (Enoyl (Acyl-Carrier-Protein) Reductase Domain)	-7.7
Quercetin (5280343)	Fatty Acid Synthase (Enoyl (Acyl-Carrier-Protein) Reductase Domain)	-8.3
Rutin (5280805)	Fatty Acid Synthase (Enoyl (Acyl-Carrier-Protein) Reductase Domain)	-9.2
Proanthocyanidin (108065)	Fatty Acid Synthase (Enoyl (Acyl-Carrier-Protein) Reductase Domain)	-6.7
Catechin (9064)	Fatty Acid Synthase (Enoyl (Acyl-Carrier-Protein) Reductase Domain)	-8.4
Epicatechin (72276)	Fatty Acid Synthase (Enoyl (Acyl-Carrier-Protein) Reductase Domain)	-8.1

Table 4. Comparison of amino acids that involved in the bond between the ligands with Fatty Acid Synthase in Enoyl [acyl-carrier-protein] Reductase Domain

	Triclosan	Quercetin	Rutin	Proanthocyanidin	Catechin	Epicatechin
hydrogen bond		GLN1754	PHE1791	PHE1791	PHE1791	PHE1791
		LEU1780	ASN1777	LYS1771	ASN1777	ASN1777
		GLY1781	LYS1771			
			LEU1774			
hydrophobic bond	VAL1789	ILE1784	ILE1784	LEU1774	ILE1784	PHE1766
	ILE1784	PHE1766	VAL1789		LEU1780	LEU1780
	LEU1780	VAL1757	LEU1780		PHE1766	VAL1789
	PRO1779	PHE1791			VAL1789	ILE1784
	LEU1753	LEU1753				
	PHE1791					
	PHE1766					
amino acid similarity		71.40%	57.10%	14.28%	71.40%	71.40%

Proanthocyanidin also binds to FAS on the enoyl (acyl-carrier-protein) reductase domain. This is indicated by the presence of similarity of one amino acid that was involved in the bond between proanthocyanidin and FAS with an amino acid that was involved in the bond between triclosan and FAS in enoyl (acyl-carrier-protein) reductase domain, namely PHE1791. Especially for proanthocyanidin, turns out that the binding affinity of this ligand with FAS on enoyl (acyl-carrier-protein) reductase domain was weaker than the bond between triclosan with FAS. Although this type of bond of PHE1791 of proanthocyanidin is a stronger hydrogen bonding, but the percentage of the amino acid bond equation was only 14.28%.

In malonyl-CoA / acetyl-CoA-ACP-transacylase (MAT) domain of FAS, it turns out that all of the active ingredients in *Tamarindus indica* that was studied, namely quercetin, rutin, proanthocyanidin, catechin, and epicatechin can also bind in places where the natural substrate (malonyl -CoA) binds. It is also in line with the results of the examination of molecular interactions with software LigPlot + Ver. v.1.4.5, where the bond between the ligand with malonyl-CoA/acetyl-CoA-ACP-transacylase (MAT) domain of FAS turns out to involve 7 to 9 amino acids that similar to amino acids that involved in the interaction between malonyl-CoA with malonyl-CoA- / acetyl-CoA-ACP-transacylase (MAT) domain of FAS (Table 6).

The examination of binding affinity by using software PyRx showed that aside from epicatechin, other active ingredients in *Tamarindus indica* showed bonding capabilities with FAS in the domain malonyl-CoA- / acetyl-CoA-ACP-transacylase (MAT), which is stronger than the malonyl-CoA (more negative) (Table 5).

When compared with malonyl-CoA, turn out that rutin is a compound in *Tamarindus indica* that has the highest percentage of amino acids bond similarity, which is as much as 69%, while the other compounds having an equal percentage, namely 54% (Table 6).

Epicatechin, evidently, its binding affinity with malonyl-CoA- / acetyl-CoA-ACP-transacylase (MAT) domain was weaker than malonyl-CoA, although the percentage of the amino acid bond equations is equal with other compounds, such as catechin, quercetin, and proanthocyanidin.

Table 5. The comparison of binding affinity between ligands with malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT) domain in Fatty Acid Synthase

Ligand	Receptor	Binding Affinity
Malonyl CoA (substrate)	Malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT)	-6.3
Quercetin (5280343)	Malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT)	-6.7
Rutin (5280805)	Malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT)	-7.2
Proanthocyanidin (108065)	Malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT)	-7.1
Catechin (9064)	Malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT)	-6.4
Epicatechin (72276)	Malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT)	-6.1

Table 6. Comparison of amino acids that involved in the bond between the ligands with malonyl-CoA-/acetyl-CoA-ACP-transacylase (MAT) Domain in Fatty Acid Synthase

	Malonyl-CoA	Quercetin	Rutin	Proanthocyanidin	Catechin	Epicatechin
hydrogen bond	LEU805-3.15	GLN483-2.90	GLN483-3.08	LEU805-3.31		
	THR780-3.17	THR780-2.70	GLN483-3.05			
	THR780-3.29		THR780-3.24			
	THR780-3.20		THR780-3.23			
	ARG802-2.88	ARG490-3.01	ARG802-3.12	ARG802-3.04		
	ARG802-3.25	ARG802-3.16		ARG802-3.06	ARG806-2.80	ARG802-2.94
	ARG802-2.83					
	ILE781-2.91		ILE781-2.91			ILE781-2.88
	ILE781-2.97		ILE781-2.91			
	LEU775-3.01					
	LYS772-3.24		LYS772-3.09			
	PRO777-2.74		PRO777-3.21			
				CYS779-3.25		
				TYR470-2.83		
hydrophobic bond	PRO486	PRO486				
	ARG490		ARG490			
	SER778			THR780		THR780
	GLN483	GLN484		GLN483		
	SER806	SER806	SER806	SER806		
	LYS776	LEU805	LEU805		LYS776	LYS776
				PRO777	PRO777	PRO777
				ILE78I	ILE78I	
				GLU481	LEU775	LEU775
					THR780	
					LYS772	LYS772
					CYS779	CYS779
amino acid similarity		54.00%	69.00%	54.00%	54.00%	54.00%

Discussion

There were various studies which stated that *Tamarindus indica* can inhibit malignancy (Aravind *et al*., 2011; Aravind *et al*., 2012; Ng *et al*., 2012; Nie and Deters, 2013; Razali *et al*., 2015; Ruby-Celsia *et al*., 2016; Shirisha and Varalakshmi, 2016). From previous studies, it can be found that the active ingredients in *Tamarindus indica* are quercetin, rutin, proanthocyanidin, catechin and epicatechin (Amir *et al*., 2013; Chong *et al*, 2012). All of those compounds (hencefort we call them as ligands) are part of flavonoids, which are already known to have antioxidant activities so that they also have anti-malignancy activities (Amir *et al*., 2013; Bhadoriya *et al*., 2011). On the other hand, there was a study which claimed that *Tamarindus indica* can inhibit FAS (Azman *et al*., 2011), so in this study, the focus of discussion is on the activity of flavonoids in *Tamarindus indica* that mentioned before as FAS inhibitor. FAS can enhance the ability of metastasis of malignant cells through Epithelial-Mesenchymal Transition (EMT) (Jiang *et al*., 2014, Li *et al*., 2014) so that the drag on the FAS activity is expected to also inhibit EMT.

To determine the inhibitory ability of each of these ligands on FAS, comparisons with the reference inhibitors of FAS, that are orlistat and triclosan, were done on two different domains, namely thioesterase domain and enoyl (acyl-carrier-protein) reductase domain (Pemble *et al*., 2007; Calvarho *et al*., 2008; Sippel *et al*., 2014). In this study, it can be stated that the binding of ligands that was studied to the thioesterase domain FAS was stronger than the binding of reference inhibitor (orlistat). Figure 2 shows that all of the ligands truly occupy the thioesterase domain of FAS in their interactions, as well as orlistat. On thioesterase domain, proanthocyanidin's bond strength is the strongest one, exceeds the bond strength of orlistat to FAS, probably due to differences in the type of bonding. Pemble *et al* (2007) stated that in his research, orlistat can bind Ser2308 of the catalytic triad within subdomain A with covalent attachment, that produces a stabilized acyl-enzyme intermediate, which then inhibits the function of FAS. In this study, one of the ligands in *Tamarindus indica* namely rutine, turns out also can bind to Ser2308, so that might also produce an acyl-enzyme intermediate, although the hydrogen bonds are weaker than covalent

bonds. The peptidyl moiety of orlistat also interacted with a cavity at the interface that is also evident in the apoenzyme structure of FAS, including with Leu2222, Ile2250, Gln2374, Phe2370, Phe2371, Phe2375 and Phe2423 (Pemble *et al*., 2007). In this study, there are also bonds between the ligands contained in *Tamarindus indica* with most of the amino acids that are mention before (besides Phe2375) either by hydrogen or hydrophobic bonds, so that the combined ligands in *Tamarindus indica* can work as an inhibitor of FAS with the same mechanism as orlistat.

Different circumstance occurs on enoyl (acyl-carrier-protein) reductase domain, where the bond strength of proanthocyanidin against FAS was the weakest, even weaker than the triclosan's bond (the reference inhibitor). Differences in percentage of amino acids that are involved in this bond seem to cause bonding between the proanthocyanidin with enoyl (acyl-carrier-protein) reductase domain FAS less intense. When compared with triclosan, although the amino acid similarity is not up 100%, but it turns out that the other four ligands (quercetin, rutin, catechin, and epicatechin) have a greater bond strength. This may due to the different types of the bond of amino acids, which is when FAS bind with triclosan, the amino acids are bound by hydrophobic bonds, while in the other ligands, a type of bond is stronger hydrogen bonding. On the previous study, it had been known that triclosan was only bound by van der Waals interactions with hydrophobic side chain residues from both chains, while in A chain the bonding occur at Leu1753, Leu1780, Ile1784 and Phe1791, whereas the B chain the bonding occur at Leu1753, Leu1780, and Phe1791 (Sippel *et al*., 2014). The location of the bond between triclosan and the domain enoyl (acyl-carrier-protein) reductase turned out to be far away from the active site. It is an indication that the inhibition happened is an allosteric inhibition. In this study, it appears that the ligands in *Tamarindus indica* also bind to Leu1753, Leu1780, Ile1784 and Phe1791. Quercetin turns out can bind to four of the amino acids with the higher binding affinity which is more than the triclosan's binding affinity, although its binding affinity is not the highest compared to other ligands. The ligand that binds to domain enoyl (acyl-carrier-protein) reductase with highest binding affinity is rutin, but it only binds to the three of the four amino acids mentioned above (Leu1780, Ile1784 and Phe1791). While proanthocyanidin, in accordance with its weakest binding affinity, it only binds to Phe1971 only. The same binding site with triclosan indicates the possibility that *Tamarindus indica* can also inhibit FAS with the allosteric mode, the same mechanism as triclosan did.

The next stage is also carried out a comparison between the bonding ability of the compound to FAS compared with the bond between malonyl-CoA (as the original substrate) to FAS. On malonyl-CoA / acetyl-CoA-ACP-transacylase (MAT) domain, rutin is the strongest ligand that can bind to malonyl-CoA / acetyl-CoA-ACP-transacylase (MAT) domain. It also might be due to differences in the type of amino acids bond.

All of the five active ingredients in *Tamarindus indica* (quercetin, rutin, proanthocyanidin, catechin, and epicatechin) may act as an inhibitor of FAS, starting from the beginning of the catalytic domain until to the last catalytic domain at FAS. In malonyl-CoA-/ acetyl-CoA-ACP-transacylase domain (MAT) of FAS, quercetin, rutin, proanthocyanidin, and catechin were expected to work competitively with malonyl-CoA as a natural substrate. In the thioesterase domain of FAS, all of the five active ingredients in *Tamarindus indica* (quercetin, rutin, proanthocyanidin, catechin, and epicatechin) were expected to work by the same mechanism with orlistat in inhibiting FAS. While in the enoyl [acyl-carrier-protein] reductase domain of FAS, only four active ingredients of *Tamarindus indica* (quercetin, rutin, catechin, and epicatechin) that can act as an inhibitor of FAS with estimates mechanisms such as triclosan. FAS itself has known to have roles in the proliferation and malignancy, so the binding of FAS by all of the five substances of antioxidants in *Tamarindus indica* shows that *Tamarindus indica* can inhibit malignancy.

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