Molecular docking compounds of essential oil isolated from *Curcuma mangga Val.* toward EGFR

R S Sudibyo[1] and H Purnomo[1,2]

1. Lab. Medicinal Chemistry, Faculty of Pharmacy, Gadjah MadaUniversity, Yogyakarta
2. Dept. Pharmaceutical Chemistry, Faculty of Pharmacy, Gadjah MadaUniversity, Yogyakarta

E-mail: retno.sudibyo@ugm.ac.id

Abstract. EGFR is a rational target in a strategic study of antitumor. Activated EGFR will stimulate the growth and progress of tumor through some mechanisms, such as cell proliferation, angiogenesis, invasion, metastatic induction, apoptotic inhibition, cell adhesiveness and differentiation. In colorectal cancer, EGFR expression has been related to the disease aggressiveness and bad prognosis. *Curcuma mangga* Val. compounds have been reported to have cytotoxic effect against some cancer cell lines. In this study molecular dockings had been conducted between Lapatinib/Native ligand and *C. mangga* Val. compounds toward EGFR (1XKK.PDB) with RMSD = 1,9592 Å. The results showed that the Docking Score (DS) of Lapatinib was -144.18(6); while the *C. mangga* Val. compounds were: cis Nerolidol -83.59(8), β Mirsene -66.24(4), trans β Ocimene -65.95(2), cis β Ocimene -65.66(5), Caryophyllene oxide-64.85(2), limonene -63.17(10), β Caryophyllene -62.66(7), Eucalyptol -58.25(2), camphene -56.73(5) dan β Pinene -55.45(1). Although the affinity of the *C. mangga* Val. compounds to EGFR was weaker comparing that of Lapatinib, but the similarity of the binding sites of the EGFR was predicted to have some roles in the cytotoxic activity toward cancer cells.

1. Introduction

Based on the in vitro tests, it was reported that the *Curcuma mangga* Val. compounds were having cytotoxicity effect toward breast cancer cell lines [1-4]. To find out the activity mechanism of the compounds of the *C. mangga* Val. toward the breast cancers, a molecular docking analysis can be conducted using several receptors related to the breast cancer developments. There are three different pathways of breast cancer [5,6], those are: i) The estrogen dependent pathway, ii). The HER2 pathway, and iii) the triple negative cell pathway. Since *C. mangga* Val. was reported to have cytotoxic effect toward breast cancer [7,8,9], so that this study was to analyze the potency of *C. mangga* Val. compounds interaction with the HER2 receptor of 1XKK (an EGFR receptor kinase domain complexed with a quinazoline inhibitor- GW572016), by docking analyses of the *C. mangga* Val. compounds in the EGFR [10-13].

Validation of the 1XKK.PDB was performed using PLANTS (Protein Ligand ANT System). The interactions between the amino-acids of the EGFR and the *C. mangga* Val. compounds were analyzed using MOE software. The best poses of the compounds to the receptor were compared to that of the native ligand, Lapatinib, using YASARA.

2. Experiment section

2.1. Materials

Molecule structures of *C. mangga* Val.: Caryophyllene oxide, *Trans* Ocimene, Limonene, Eucalyptol, *Cis* Ocimene, Caryophyllene, β Pinene, β Myrcene, cis-Nerolidol and Camphene, obtained from *C. mangga* Val Research Group of Gadjah Mada University. The reference ligand of Lapatinib and the 1XKK were obtained from Protein Data Bank (PDB). Softwares of YASARA, Marvin Sketch, PLANTS, mingwm 10.dl, npp.5.6.8.Installer, and cmd.

2.2. EGFR(1XKK.PDB) Validation

2.2.1. Preparations ofProtein.mol2 and ref_ligand.mol2

The EGFR protein (1XKK) was obtained from the PDB through www.rcsb.org/structure/1xkk. The protein (1XKK.PDB) was then uploaded to the YASARA, removed the water molecules appeared and added hydrogen molecules to the receptor's structures,then stored it as the Protein.mol2 of 1XKK.YOB. The ref_ligand.mol2 (lapatinib) was prepared by removing the FMM (Lapatinib) residue from the1XKK.YOB, then take the negate name so that the FMM ligand was still appeared and stored as the ref_ligand.mol2.

2.2.2. Preparation of the ligand.mol2 of lapatinib

On the Marvin Sketch the ref_ligand.mol2 was loaded, and was conditioned into body pH of 7.4 and then stored as the ligand2D.mrv. Set the software for 10 of most stable energies of the ligand2D.mrv and then switched the file into mol2-form, and stored it as the ligand.mol2.

2.2.3 Docking process

In the cmd software wrote: *plants–mode bind ref_ligand.mol2 5 protein.mol2* to put the reference ligand for binding to the receptor. Then wrote:*–mode screen pc_1xkk.txt* for showing up the 1XKK. PDB on the screen. For docking process and getting the best pose of docking (with lowest docking score), it was written: *cd results* and *more bestranking.csv*, respectively. Select the lowest docking score of the reference ligand, and determined its RMSD. Stored the results as the 1XKK.PDB validation folder.

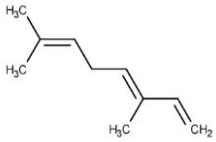
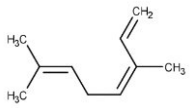
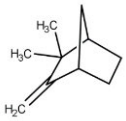
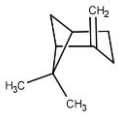
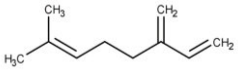
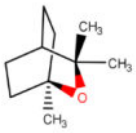
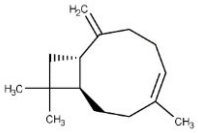
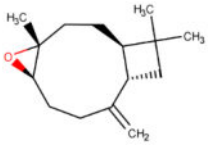
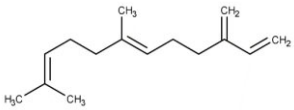
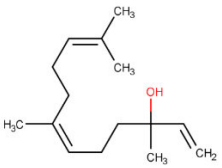
2.3. Docking process of the C. mangga Val compounds to the 1XKK.PDB

Chemical structures of the 10 *C. mangga* Val compounds were performed using MarvinSketch, and they were then conditioned into the body pH of 7.4 and set theminto their 10 conformations with the lowest energies, and stored them as the ligand.mol2 of compounds. Use theprotein.mol2 dan ref_ligand.mol2 from the validation process. The docking process of each compounds toward 1XKK.PDB was conducted as that of the reference ligand (lapatinib). Select the lowest docking score of each compound, and determined as the compound docking score.

3. Discussions

Chemical structures of *C. mangga* Val. 10 compounds are in the Table 1.

Table 1. Chemical structures of 10 selected *C. mangga* Val. compounds.

No.	Compound	Chemical structure
1	Trans β- Ocimene	
2	Cis β -Ocimene	
3	Camphene	
4	β -Pinene	
5	β -Myrcene	
6	Eucalyptol	
7	Trans β-Caryophyllene	
8	Caryophyllene oxide	
9	β -Farnesene	
10	Cis Nerolidol	

From the validation process of 1XKK.PDB using reference ligand of Lapatinib (anti-cancer drug), it was resulted RMSD 1.9592 Å which was valid because < 2 Å (Figure 1).

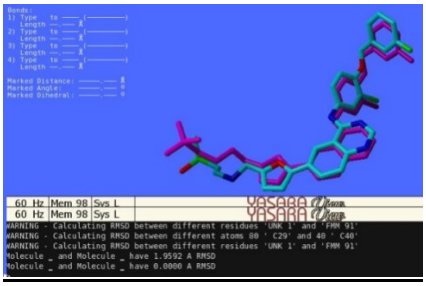
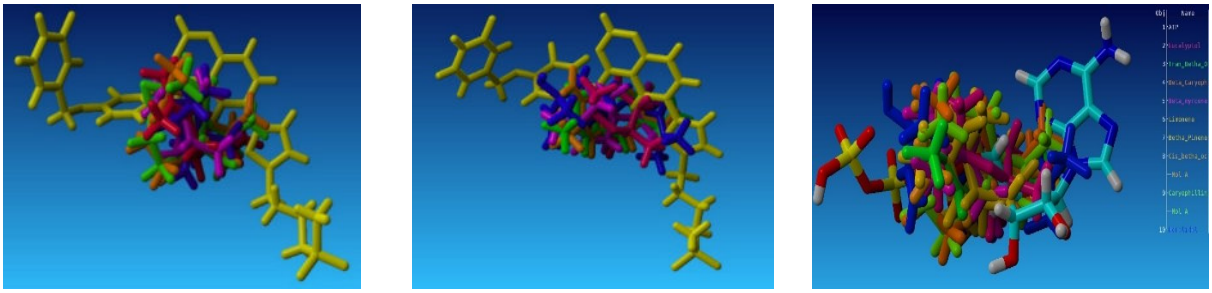


Figure 1. Validation of 1XKK.PDB with ligand of lapatinib. RMSD 1.9592 Å.

The results of the docking processes are in Table 2, and the docking visualizations are in Figures 2.



(a) (b) (c)

Figure 2. Docking visualizations of interactions between EGFR and the reference ligand (Lapatinib), native ligand (ATP) and the 10 compounds of *C. mangga* Val.
(a) Lapatinib (yellow), β-Caryophyllene (brown), β -Myrcene (red), β -Pinene (violet), Camphene (dark blue), Caryophyllene-oxide (green).
(b) Lapatinib (yellow), Cis β-Ocimene (brown), Eucalyptol (red), Limonene (violet), *cis* Nerolidol (dark blue), trans β-Ocimene (green).
(c) ATP (light blue), *trans* β -Ocimene (green), Limonene (violet), Eucalyptol (red), *cis* β-Ocimene (brown), β-Caryophyllene (brown), and *cis* Nerolidol (dark blue).

It is shown in Table 2 that Lapatinib as the reference ligand had interactions with 14 amino-acids within EGFR with docking score (DS) of -144.18.Itmeans that Lapatinib has much stronger affinityto EGFR than ATP as the native ligand with DS of -100.85. So that Lapatinib can strongly replace the ATP from its complex with EGFR. All compounds of the *C. mangga* Val.having interactions with amino-acid residues within EGFR, however only 8 out of 10 compounds were having interactions at the same sites as those of ATP and Lapatinib; those were:Caryophyllene,*trans* β Ocimene,Limonene, Eucalyptol, *cis* β Ocimene, β Caryophyllene, β Pinene, and β Myrcenewhich having interactions with respectively 26,26,14,13, 11, 11, 11,9 amino-acids within EGFR. While *Cis*-Nerolidol and Camphene each was having interaction with 8 amino-acids of EGFR at different sites from those of ATP and Lapatinib (Table 2).

Table 2. The results of docking processes of EGFR (1XKK.PDB) with native ligand (ATP), reference ligand (Lapatinib) and the 10 selected compounds of *C. mangga* Val

EGFR Amino acid Residues	Native ligand	Ref. ligand	10 Selected ligands from *Curcuma mangga* Val.									
	ATP	Lapatinib	Caryophyl-lene oxide	*trans* β - Ocimene	Limonene	Eucalyptol	*cis* β - Ocimene	β-Caryo-phyllene	β -Pinene	β - Myrcene	*cis* Nerolidol	Camphene
ARG 705											ARG 705	ARG 705
PRO 733											PRO 733	PRO 733
VAL 738											VAL 738	VAL 738
LYS 739											LYS 739	LYS 739
ILE 740											ILE 740	ILE 740
PRO 741											PRO 741	PRO 741
HIS 773			HIS 773	HIS 773								
VAL 774			VAL 774	VAL 774								
GLN 791											GLN 791 H-acc, 16.3%	GLN 791
CYS 797	CYS 797		CYS 797	CYS 797				CYS 797				
LEU 798	LEU 798		LEU 798	LEU 798	LEU 798							
LEU 799			LEU 799	LEU 799								
ILE 821		ILE 821			ILE 821	ILE 821						
GLY 824			GLY 824	GLY 824								
MET 825	MET 825	MET 825			MET 825	MET 825		MET 825				
LEU 828	LEU 828		LEU 828	LEU 828								
LEU 833			LEU 833	LEU 833								

EGFR Amino acid Residues	Native ligand	Ref. ligand	10 Selected ligands from *Curcuma mangga* Val.								
	ATP	Lapatinib	Caryophyl-lene oxide	*trans* β - Ocimene	Limonene	Eucalyptol	*cis* β - Ocimene	β-Caryo-phyllene	β -Pinene	β - Myrcene	*cis* Nerolidol	Camphene
HIS 835	HIS 835	HIS 835	HIS 835	HIS 835	HIS 835	HIS 835	HIS 835		HIS 835	HIS 835		
ASP 837	ASP 837	ASP 837			ASP 837				ASP 837			
LEU 838	LEU 838	LEU 838			LEU 838	LEU 838	LEU 838	LEU 838	LEU 838	LEU 838		
ALA 839	ALA 839 H-acc 43.9%	ALA 839	ALA 839	ALA 839	ALA 839	ALA 839	ALA 839	ALA 839	ALA 839	ALA 839		
ALA 840	ALA 840	ALA 840	ALA 840	ALA 840	ALA 840	ALA 840	ALA 840	ALA 840	ALA 840	ALA 840		
ARG 841	ARG 841	ARG 841	ARG 841	ARG 841	ARG 841	ARG 841	ARG 841	ARG 841	ARG 841	ARG 841		
ASN 842	ASN 842 H-acc 13.5%	ASN 842	ASN 842	ASN 842	ASN 842	ASN 842	ASN 842	ASN 842	ASN 842	ASN 842		
VAL 843	VAL 843	VAL 843	VAL 843	VAL 843	VAL 843	VAL 843	VAL 843	VAL 843	VAL 843	VAL 843		
LEU 844	LEU 844	LEU 844	LEU 844	LEU 844		LEU 844	LEU 844	LEU 844				
VAL 845	VAL 845											
LYS 846											LYS 846 H-acc 22.7% H-acc 69.9%	LYS 846
VAL 851			VAL 851	VAL 851								
LYS 852	LYS 852	LYS 852	LYS 852 H-acc 10.0%	LYS 852 H-acc 10.0%	LYS 852	LYS 852	LYS 852	LYS 852	LYS 852			
ILE 853	ILE 853	ILE 853	ILE 853	ILE 853	ILE 853	ILE 853	ILE 853	ILE 853	ILE 853	ILE 853		
THR 854	THR 854	THR 854	THR 854	THR 854	THR 854	THR 854	THR 854	THR 854	THR 854	THR 854		
ASP	ASP		ASP 855	ASP 855								

EGFR Amino acid Residues	Native ligand	Ref. ligand	10 Selected ligands from *Curcuma mangga* Val.									
	ATP	Lapatinib	Caryophyl-lene oxide	*trans* β - Ocimene	Limonene	Eucalyptol	*cis* β - Ocimene	β-Caryo-phyllene	β -Pinene	β - Myrcene	*cis* Nerolidol	Camphene
855	855											
PHE 856	PHE 856		PHE 856 H-acc 21.1%	PHE 856 H-acc 21.1%								
TRP 880			TRP 880 H-acc 40.8%	TRP 880 H-acc 40.8%								
VAL 902			VAL 902	VAL 902								
THR 903	THR 903		THR 903	THR 903								
GLU 906			GLU 906	GLU 906								
SER 912			SER 912	SER 912								
Binding Amino acids	20	14	26	26	14	13	11	11	11	9	8	8
Docking Score	-100.85	-144.18	-64.85	-65.95	-63.17	-58.25	-65.66	-62.66	-55.45	-66.24	-83.59	-56.73

Caryophyllene and*t rans* β Ocimene showed a bit strong affinity to EGFR because both were hydrogen acceptor with moderate strength. While *cis*-Nerolidol with DS of -83.59 showed strongest affinity to EGFR due to its ability as strong hydrogen acceptor (Table 2).The docking visualizations showed that all the *C. mangga* Val. compounds were binding in the same pocket of EGFR as Lapatinib (Figures 2A and 2B) and ATP (Figure 2C). Although all compounds of *C. mangga* Val. having interactions with binding sites of EGFR, however the docking score of all the compounds was much weaker than that of the native ligand (ATP) and even the reference ligand (Lapatinib), except *cis*-Nerolidol (DS= -83.59) (Table 2). Among the *C. mangga* Val. compounds *cis*-Nerolidol was having the strongest affinity to the EGFR, but it did not bind at the same amino acid sites of EGFR as those of ATP, the native ligand (Table 2). So that *cis*-Nerolidol could not be strong EGFR inhibitor. It seems that based on the number of binding amino-acids and DS is not enough to conclude the exact roles of the ligands toward the EGFR. Study on the pharmacophoric binding sites of EGFR (using MOE software) are necessary determine the findings. Because all the *C. mangga* Val. compounds could bind to the receptor, so it may be suggested that they could be kind of "steric-hindrance" for the native ligand,although their EGFR affinity were weak.

4. Conclusion

Based on the study findings, it was concluded that cytotoxic activity of the *C. mangga* Val. compounds may follow the HER2 pathway.It was suggested to study the other breast cancer pathways (the ERα or triple negative pathway) for these *C. mangga* Val. compounds.

References

[1] Nurrokhman 2004 Efek antiproliferasi dan induksi apoptosis minyak atsiri *C. mangga* Val. pada Epithelial *cervical cancer cell lines* (HeLa dan Siha) *Thesis* (Yogyakarta: Universitas Gadjah Mada)
[2] Verlianara I 2004 Efek in vitro minyak atsiri *C. mangga* Val. pada sitotoksisitas, antiproliferatif dan apoptosis sel raji dan mieloma *Thesis* (Yogyakarta: Universitas Gadjah Mada)
[3] Rumiyati, Sudibyo R, S Sismindari, Jenie U A, Mubarika S, and Kardono L B 2007 Selective cytotoxicity of essential oil of *C. mangga* Val. on cell lines and its effect on expressions of p53 and Bcl-2 *Proceeding of The International Symposium on Recent Progress in Curcumin Research* Faculty of Pharmacy Gadjah Mada University Indonesia 107-114
[4] Astuti 2014 Mekanisme molekuler antikanker senyawa aktif dalam rimpang *Curcuma mangga Val*., *Dissertation* (Yogyakarta: Universitas Gadjah Mada)
[5] Song D, Cui M, Zhao G, Fan Z, Nolan K, Yang Y, Lee P, Ye F and Zhang D Y 2014 Pathway-based analysis of breast cancer *Am. J. Transl. Res.* **6**(3) 302-311
[6] Brown M and Attardi L D 2005 The role of apoptosis in cancer development and treatment response *Nat. Rev. Cancer* **5** 231-236
[7] Budiman H 2001 Uji sitotoksisitas kandungan metabolit sekunder rimpang *c. mangga* val. pada sel hela-s3 dan raji serta identifikasi struktur kimianya *Thesis* (Yogyakarta: Universitas Gadjah Mada)
[8] Chen Y C 2015 Beware of docking trends in pharmacological sciences *Opinian* **34**(2)
[9] Gibbs J B 2000 Anticancer drug targets:growth factor and growth factor signaling *J Clin Invest* **105** 9-13
[10] Korb O, Stützle T, and Exner T E 2006 PLANTS: Application of ant colony optimization to structure-based drug design *Lecture Notes in Computer Science* **4150** 247-258
[11] Korb O, Stützle T, and Exner T E 2007 An ant colony optimization approach to flexible protein-ligand docking *Swarm Intell.* **1** 115-134

[12] Korb O, Stützle T, and Exner T E 2009 Empirical scoring functions for advanced protein-ligand docking with PLANTS *J. Chem. Inf. Model.* **49** 84-96
[13] Hari P 2013 Kimia komputasi untuk farmasi dan ilmu terkait: uji in siliko senyawa antikanker (Yogyakarta: Penerbit Pustaka Pelajar)