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Article

Pharmacophore-Based Virtual Screening from Indonesian Herbal Database to Find Putative Selective Estrogen Receptor Degraders

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Abstract: Most breast cancer cases are luminal subtypes which are estrogen receptor-sensitive or progesterone receptor-sensitive. Common treatments include surgery and adjuvant endocrine therapy by prescribing selective estrogen receptor degraders (SERD). SERD is a type of medication that inhibits estrogen receptor (ER) activity by degrading it, and as a result, downregulating it. The current FDA-approved SERD can only be administered through intramuscular injection. The aim of this study is to find orally non-toxic and bioavailable herbal alternatives of SERDs in Indonesian Herbal Database by doing virtual screening using LigandScout. The hit compounds were further analyzed using a molecular docking tool, AutoDock. Three compounds that gave the best results in molecular docking, namely kuwanon T, mulberrin, and curcumin, were analyzed in terms of their toxicity and drug-likeness. Based on toxicity and drug-likeness study, curcumin is considered to be the best candidates for SERD alternatives. This result is further supported by molecular dynamic simulation outcome in which curcumin is the most stable while binding with estrogen receptors.

Keywords: Estrogen receptor degraders, breast cancer, molecular simulation

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1. Introduction

According to the Global Cancer Observatory in 2020, around 11.7% of worldwide new cases of cancer are breast cancer. Approximately 6.9% of deaths by cancer were caused by breast cancer in 2020 all around the world.[1] In Indonesia, 19.2% of cancer cases are breast cancer, making it the most prevalent cancer.[2] Breast cancer has been recorded as the type of cancer that causes the highest mortality in women due to its high incidence. Most breast cancer cases are luminal subtypes which are estrogen receptor-sensitive or progesterone receptor-sensitive. About 70% of breast cancers express estrogen receptor alpha (ERα), encoded by estrogen receptor-1 (ESR1)[3]. Common treatments include surgery and adjuvant endocrine therapy[4]. Adjuvant endocrine therapy is done by prescribing selective estrogen receptor modulators (SERMs), selective estrogen receptor degraders (SERDs) and aromatase inhibitors[5]. As SERMs diffuse into cells, they bind to receptor subunits, dimerizing them and causing structural changes in the receptor. Consequently, SERMs will have an easier time interacting with estrogen response elements, resulting in activation of estrogen-inducible genes and mediating estrogen-related effects[6]. It is thought to be a better treatment for premenopausal patients. On the other hand, SERDs work by binding to the estrogen receptor (ER) and, in doing so, degrading and downregulating the ER.[5]. According to some studies, SERD can be an effective and safe treatment option for recurrent hormone-sensitive breast cancer in postmenopausal women[4]. However, the search for SERD that can be orally ingested and has high bioavailability continues as Fulvestrants, the current SERD accepted by FDA, has poor solubility and has to be administered through intramuscular injection.[7][8] The aim of this

study is to find bioavailable herbal alternatives of SERDs in Indonesian Herbal Database that are orally non-toxic by doing in silico molecular simulation.

2. Results

2.1. Structure-Based Pharmacophore Modeling

The pharmacophore model was created using structure-based pharmacophore modeling tools in LigandScout. The pharmacophore model (Fig. 1) contained 9 features; 4 hydrophobic interactions, 1 positive ionizable, 2 H bond acceptor, and 2 H bond donor.

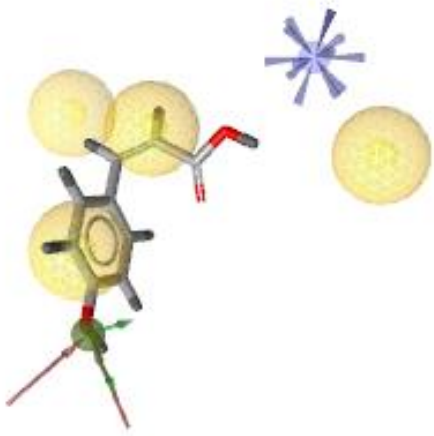


Figure 1. Pharmacophore model with native ligand molecule

2.2. Virtual Screening of Indonesian Herbal Database

The virtual screening was carried out by omitting 5 features of the pharmacophore model. The screening of the Indonesian herbal database yielded 79 hit compounds. The top 20 hit compounds in regards to their pharmacophore fit score were then chosen to be subjects in molecular docking.

Table 1. Top 20 hit compounds and their respective pharmacophore fit score.

Compound name	Pharmacophore-fit score
Nerolidol	76.74
Morindone	76.10
5-HETE	76.06
Geraniol	75.20
Formononetin	68.68
7,3′,4′-Trihydroxyflavone	68.53
Eugenol	68.11
Isoeugenol	67.76
Beta santalol	67.70
(-)-Matairesinol	67.53
Mulberrin	67.45
(Z,Z,Z)-3,6,9-Dodecatrien-1-ol	67.43
Trans-4-Coumarate	67.43
P-Coumaric Acid	67.43
Kuwanon T	67.39
Curcumin	67.35
[6]-Paradol	67.15
(R)-beta-Citronellol	67.11
15-HETE	67.05
Carvacrol	66.99

2.3. Molecular Docking

The molecular docking parameters were optimized by redocking the native ligand using three different sizes of grid box, 40x40x40, 50x50x50, and 60x60x60. Binding energy, RMSD, and inhibition constant of the native ligand redocked to the crystal macromolecule are shown in table below (Table 2).

Table 2. Docking parameters optimization

	40x40x40	50x50x50	60x60x60
Binding Energy	-14.34 kcal/mol	-14.32 kcal/mol	-12.82 kcal/mol
RMSD	0.932 Å	0.9 Å	1.88 Å
Inhibition constant	30.98 pM	31.87 pM	399.22 pM

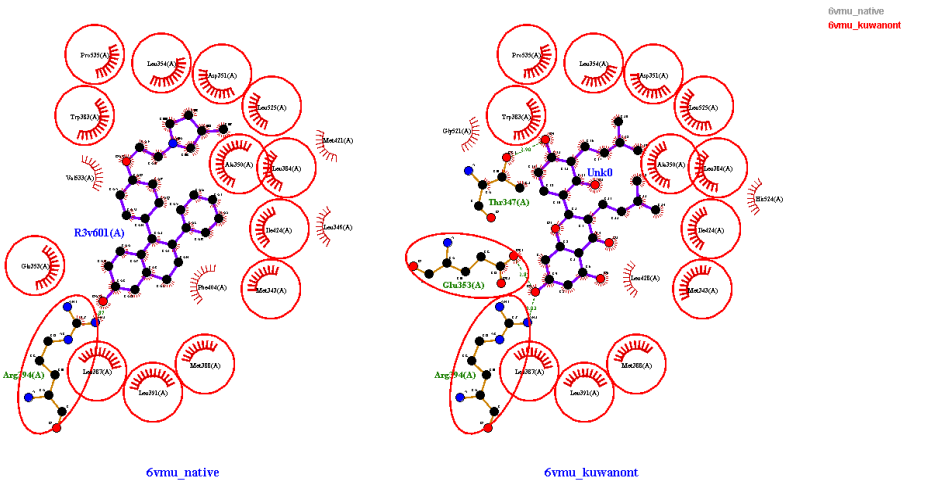
The basic criteria of good docking results are low binding energy, RMSD < 2 Å, and small inhibition constant. All of the redocking results passed all of those criteria. However, binding energy as low as -14 kcal/mol might indicate that the ligand is concerningly toxic. So, the chosen size for the grid box was 60x60x60.

To determine interactions between estrogen receptor and each ligand, molecular docking with a 60x60x60 grid box was carried out using AutoDock version 1.5.6. The 3D structure of 20 ligands were retrieved from the PubChem database and cleaned in MarvinSketch version 20.6. The top 3 ligands based on their docking results (binding energy and inhibition constant) are shown in table below (Table 3).

Table 3. Top 3 ligands based on their docking results =

Compound name	Binding energy (kcal/mol)	Inhibition constant (uM)
Kuwanon T	-11.5	0.00374
Mulberrin	-9.91	0.05475
Curcumin	-8.85	0.32677

For better visualization of interactions between the top 3 ligands and the estrogen receptor protein, LigPlot+ version v.2.2.4 and PyMOL were used. Comparison of the top 3 herbal ligands' molecular interactions to the native ligand's molecular interactions with the same estrogen receptor protein (Fig. 2A, B, and C) may explain the docking results.



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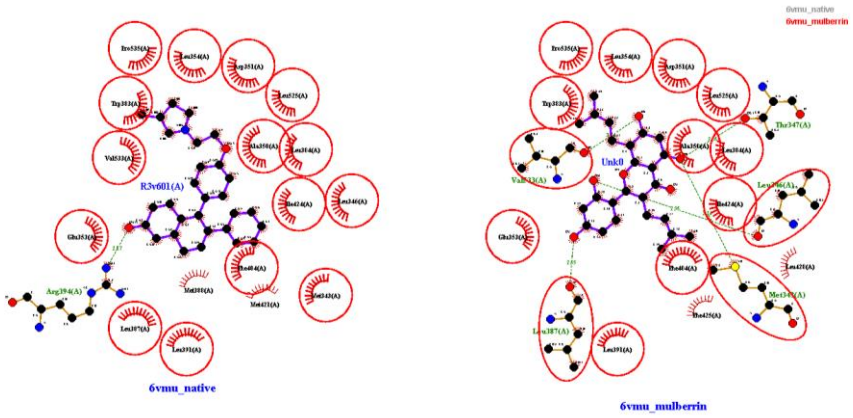

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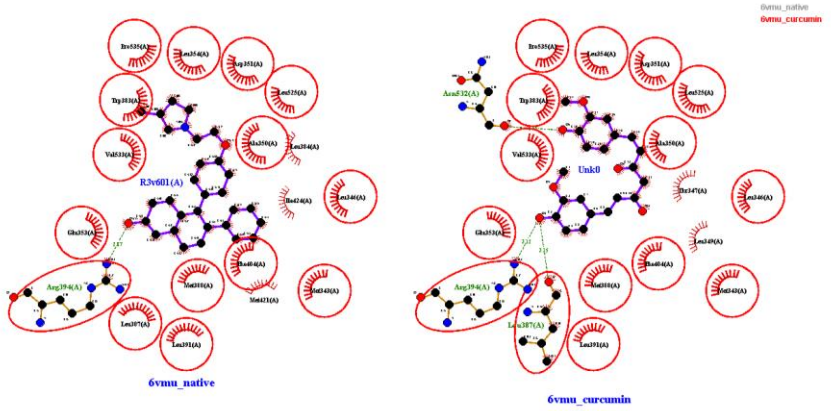

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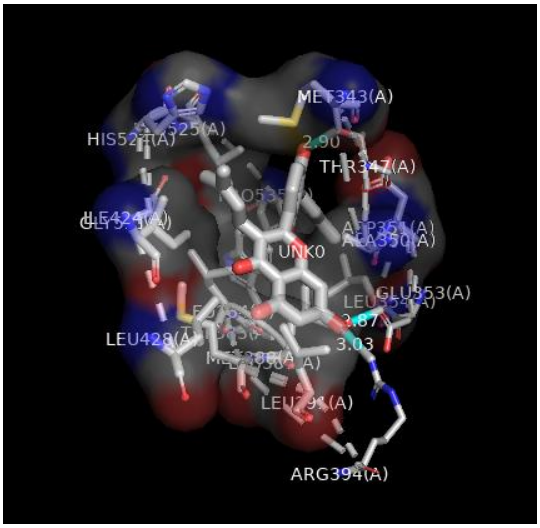
Figure 2. 2D visualization of ligands' molecular interaction with estrogen receptor protein. (a) Molecular interactions comparison of kuwanon t's to native ligand's, (b) Molecular interactions comparison of mulberrin's to native ligand's, (c) Molecular interactions comparison of curcumin's to native ligand's.

Table 2. Docking parameters optimization

Residue	Native	Kuwanon t	Mulberrin	Curcumin
Pro535	✓	✓	✓	✓
Leu354	✓	✓	✓	✓
Asp351	✓	✓	✓	✓
Leu525	✓	✓	✓	✓
Trp383	✓	✓	✓	✓
Val533	✓	-	3.14 Å	✓
Ala350	✓	✓	✓	✓
Leu384	✓	✓	✓	-
Glu353	✓	2.87 Å	✓	✓
Ile424	✓	✓	✓	-
Met421	✓	-	-	-
Met343	✓	✓	3.24 Å	✓
Leu346	✓	-	2.5 Å	✓
Phe404	✓	-	✓	✓
Leu387	✓	✓	3.65 Å	3.15 Å
Leu391	✓	✓	✓	✓
Met388	✓	✓	-	✓
Arg394	2.87 Å	3.03 Å	-	3.12 Å



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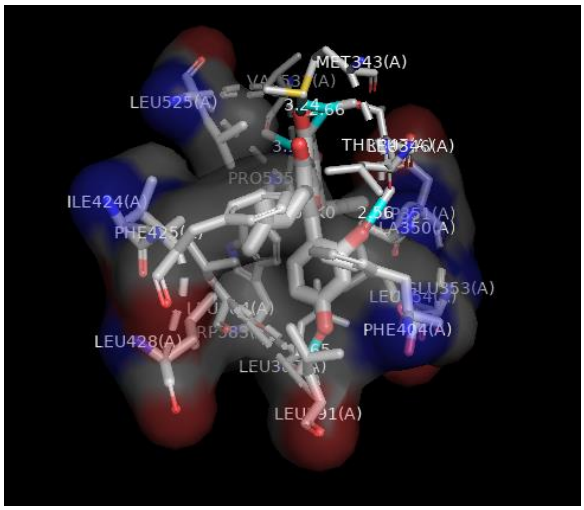

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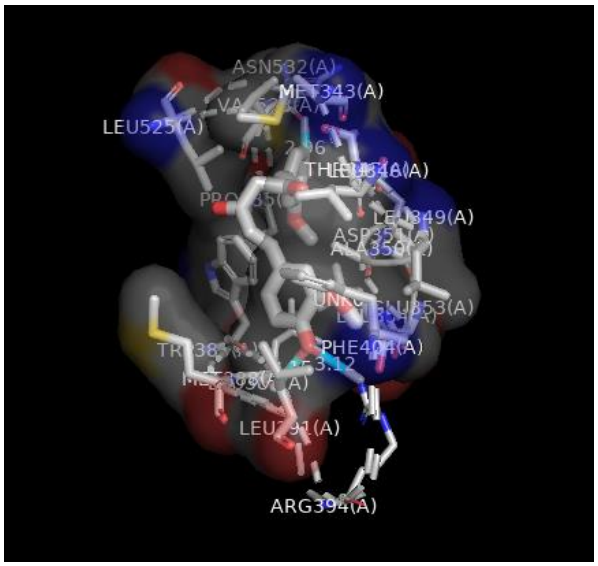

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Figure 3. 3D visualization of ligand docking. (a) Kuwanon t, (b) Mulberrin, (c) Curcumin

2.4. Toxicity & Drug-likeness Study

The toxicity of kuwanon t, mulberrin, and curcumin was predicted using AdmetSAR.

Table 5. Toxicity study

Toxicity parameter	Kuwanon t	Mulberrin	Curcumin
AMES	-	-	-
Carcinogenesis	-	-	-
AOT	III	III	-

Neither kuwanon t, mulberrin, nor curcumin was predicted to be toxic or carcinogens. However, kuwanon t and mulberrin belong to AOT category III, which is slightly toxic if orally ingested.

The drug-likeliness of the compounds was analyzed using SwissADME. The results are shown below (Table 6).

Table 6. Drug-likeness study

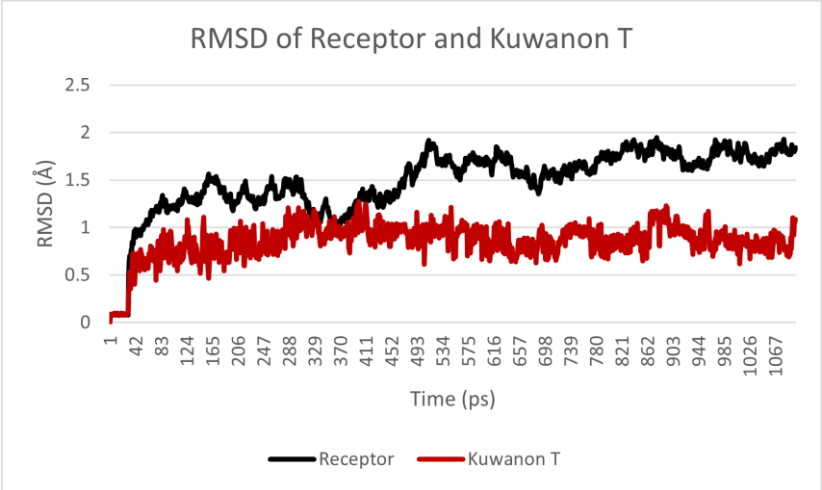
Toxicity parameter	Kuwanon t	Mulberrin	Curcumin
MW (g/mol)	422.47	422.47	368.38
HBA	6	6	6
HBD	4	4	2
LogP	4.39	4.54	3.03
GI absorption	Low	Low	High
Bioavailability score	0.55	0.55	0.55
Water solubility	Poorly soluble	Poorly soluble	Moderately soluble
Lipinski	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	No
CYP2C19 inhibitor	No	No	No
CYP2C9 inhibitor	No	No	Yes
CYP2D6 inhibitor	No	No	No
CYP3A4 inhibitor	No	No	Yes

The optimal range of MW for drugs is between 150 and 500 g/mol[17], in which the three compounds' MWs fall into. Based on the number of hydrogen bond acceptors and hydrogen bond donors, the compounds are capable of forming hydrogen bonds which is important in drug-target interaction. LogP indicates lipophilicity of the compounds. A drug-like molecule should have logP between -0.4 to 5.6[17]. Therefore, all three compounds potentially act like drugs. GI absorption can be an indicator whether administering the compound orally would be effective or not. Only curcumin was predicted to be highly absorbed in the GI tract. The interaction of the compounds with cytochromes P450 (CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4) was identified. These isoenzymes play a major role in drug elimination through metabolic biotransformation. If these enzymes are inhibited, the drug or its metabolites may accumulate in the body, leading to toxic effects [17]. Kuwanon t and mulberrin were predicted as non-inhibitor to any of the isoenzymes. Unfortunately, curcumin turned out to be a CYP2C9 and CYP3A4 inhibitor.

2.5. Molecular Dynamics Simulation

One ns period of MDs were used to investigate the stability of complex interactions as well as the dynamic behavior observed between ligands and receptors. The system was probed for stability and structural adjustability of the ligands in the binding domains of the selected receptor using statistical metrics such as the root mean square deviation (RMSD), root mean square fluctuation (RMSF), and radius of gyration (Rg). RMSD was used to evaluate the stability of the receptor-ligand complexes (Fig. 4). Because of the initial binding to the receptor within 0.01 ns, the RMSD of C and side-chain atoms for each ligand rapidly increases, as seen in the plot. However, differentiating RMSD between the three protein–ligand complexes' interactions is difficult. As a result, we used logarithmic

trendlines to fit those RMSD trajectories (Fig. 4C). Thus, the slope of the curve can be estimated from the fitting. The steeper the curve, the longer it takes for the ligand-protein complex to reach its stability[18].



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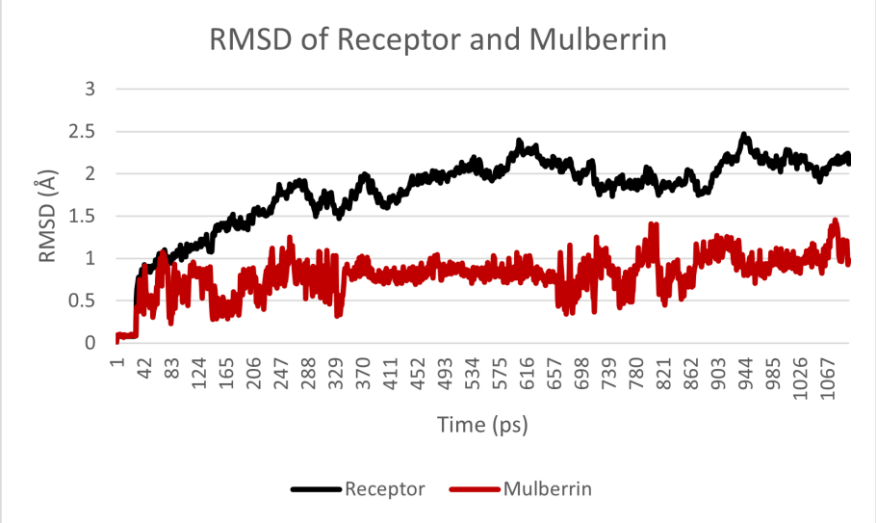

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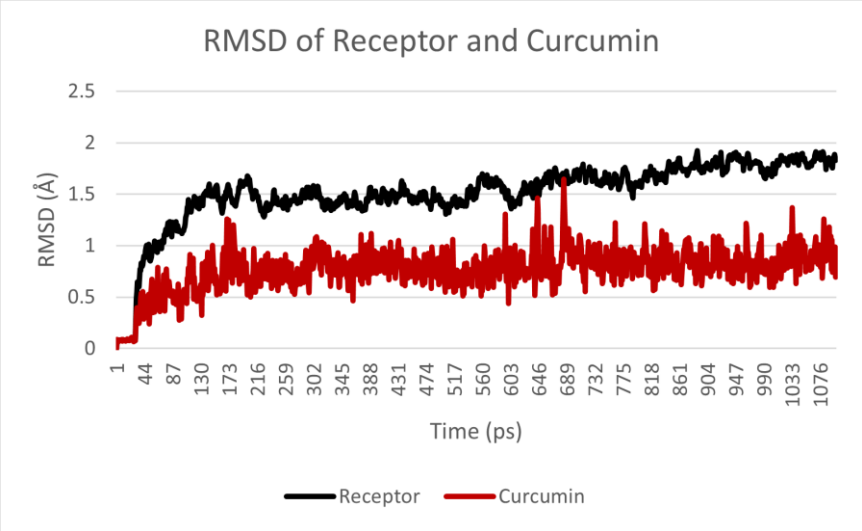

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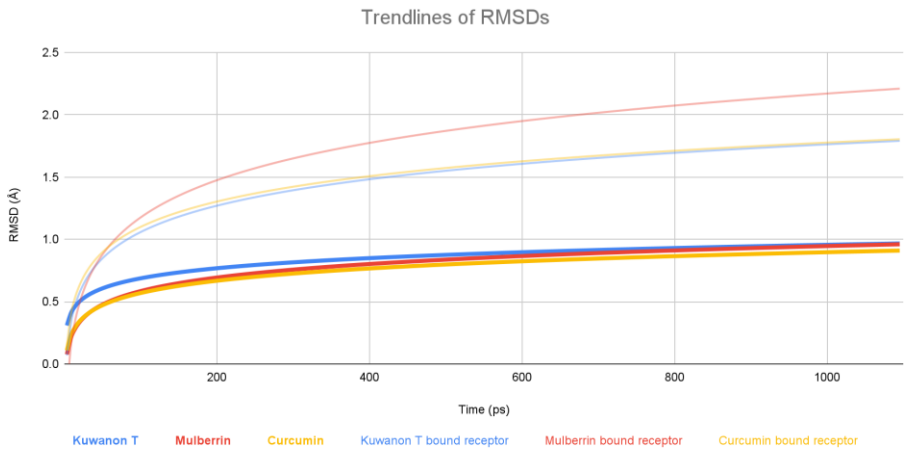
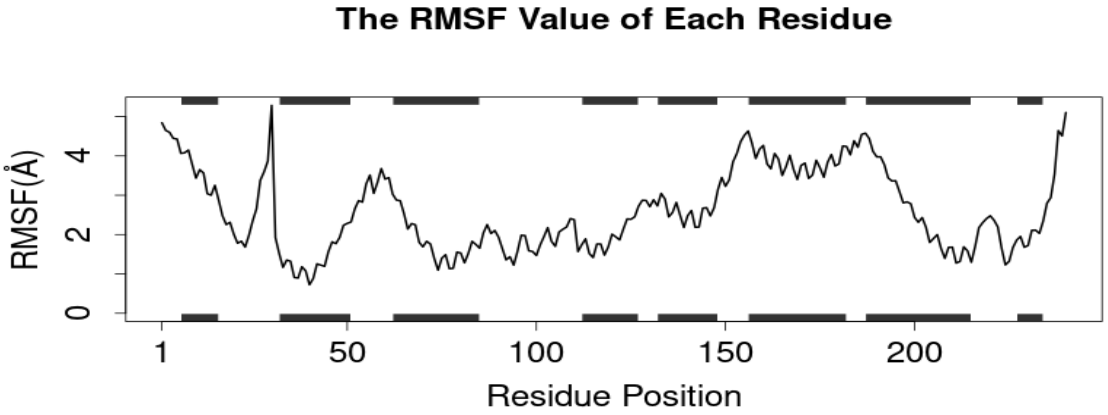


(d)

Figure 4. RMSD of receptor and ligands. (a) Kuwanon T, (b) Mulberrin, (c) Curcumin, (d) Logarithmic trendlines of RMSD

Residual flexibility of each receptor-ligand complex was studied by RMSF (Fig. 5) to assess how contact deviation impacts estrogen receptor binding to kuwanon T, mulberrin, and curcumin. The CA atom of each residue is used to calculate the fluctuation of each residue. The secondary structure schematic is placed on the figure's top and bottom margins (helices are black, strands are gray and loops are white). The bigger fluctuation for loop regions is worth noting. Because the terminal sites change more than other sites, the RMSF value in these sites is expected to be higher than that in the in-between part. However, some sites in the in-between region are observed to have high fluctuation, which means these sites are unstable. The RMSF plot is consistent with the RMSD plot, meaning when the RMSF plot fluctuates a lot, then the RMSD plot shows instability. It is well understood that RMSF represents local residual fluctuation, whereas RMSD represents global fluctuation[14]. Based on the receptor's RMSD, the receptor shows less stability while binding to mulberrin, even though the mulberrin's RMSD shows good results. The RMSF plot (Fig. 5) confirms that mulberrin indeed has higher fluctuation than the rest of compounds of interest.



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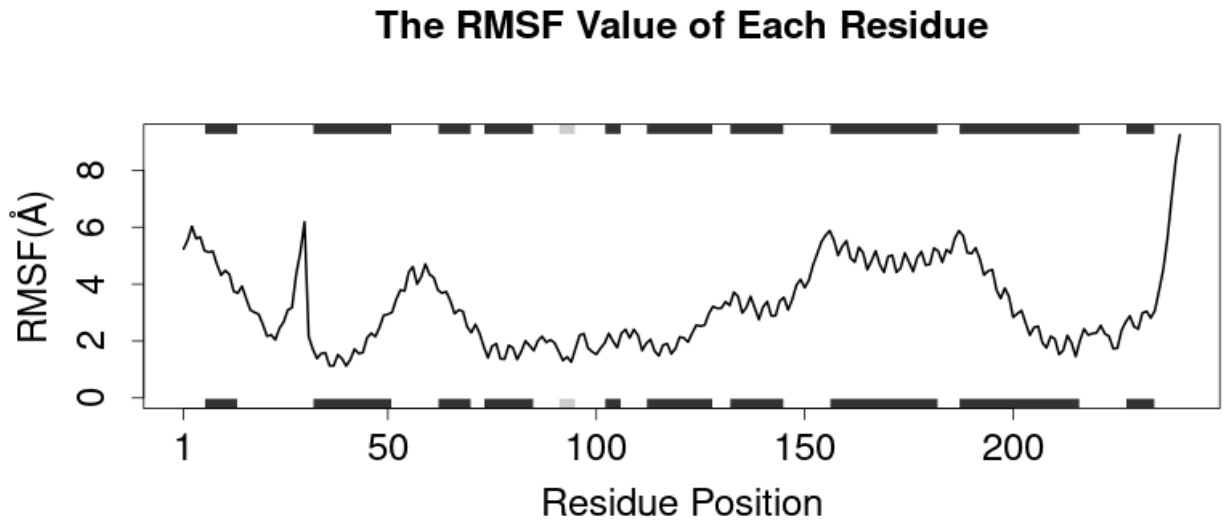

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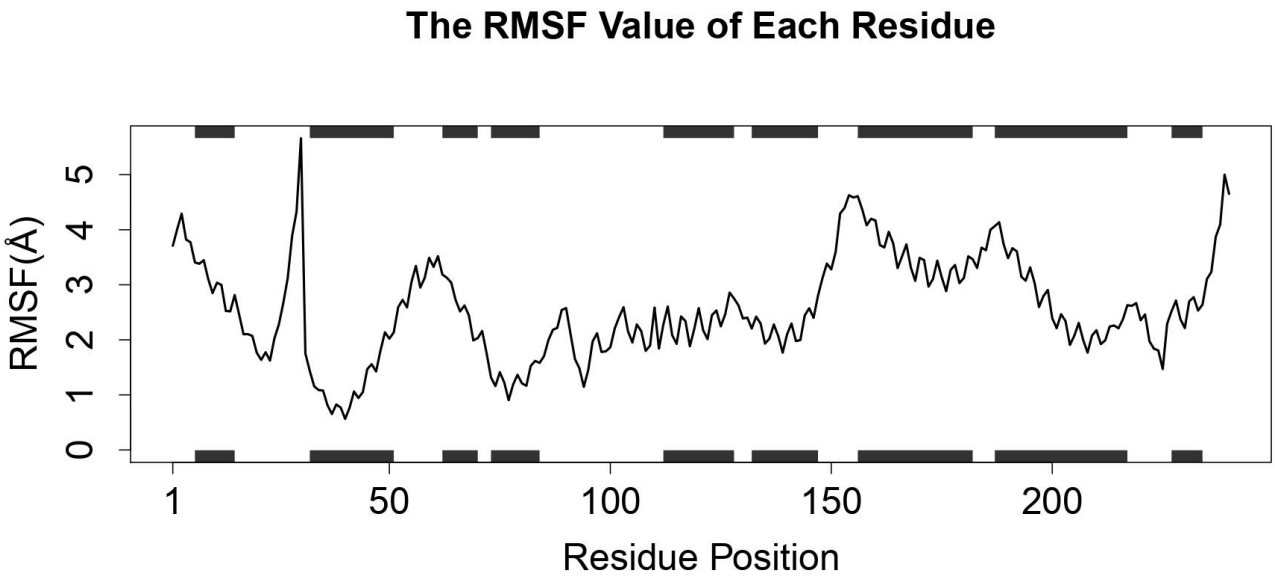

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Figure 5. Protein RMSF for (a) Kuwanon T, (b) Mulberrin, (c) Curcumin

Furthermore, radius of gyration (Rg) was used to predict the structural activity of the protein-ligand complex and to assess compactness of the complex. The Rg provides useful information about the likelihood of protein to expand its structure during dynamic modeling (Fig. 6). The higher the Rg, the less compact the protein and the less stable it is when folded[18]. The system's Rg profile does match the RMSD and RMSF profiles for complex fluctuation, showing that curcumin has the most compact structure, while mulberrin is less compact.

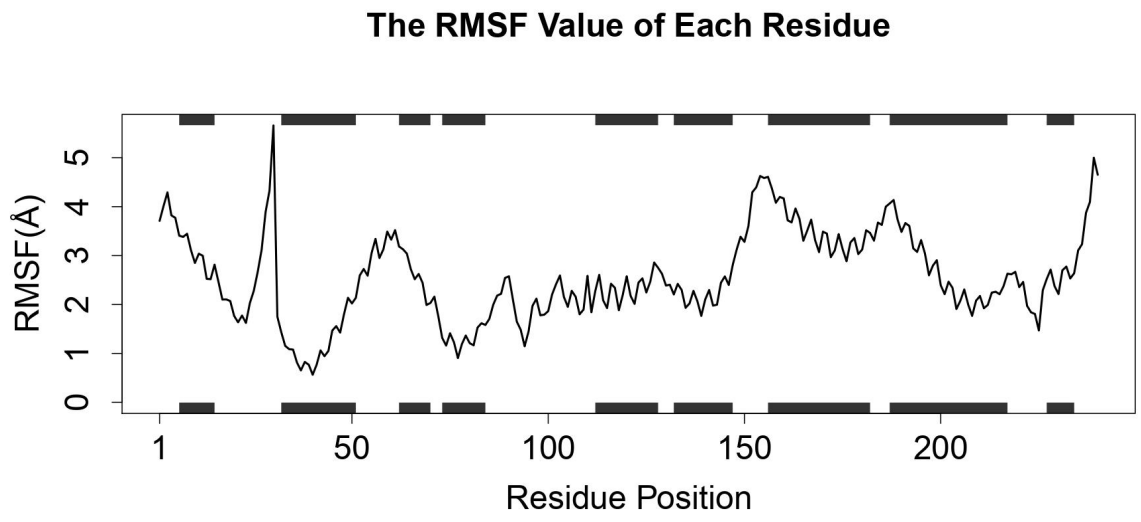

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Figure 6. Radius of gyration for kuwanon T and mulberrin.

5. Conclusions

Virtual screening of the Indonesian herbal database on estrogen receptor protein (PDB ID: 6VMU) using LigandScout yielded 79 hit compounds, the top 20 of them based on pharmacophore-fit score were further analyzed to determine binding affinity and molecular interaction using a molecular docking tool, AutoDock. Docking parameters were optimized by redocking the native ligand. Three compounds that gave the best results in molecular docking, namely kuwanon T, mulberrin, and curcumin, were analyzed in terms of their toxicity and drug-likeness. Based on toxicity and drug-likeness study, curcumin is considered to be the better candidate for SERD alternative as it is orally non-toxic and has higher GI absorption compared to kuwanon t and mulberrin. For the final evaluation, we did molecular dynamics simulation to inspect the stability, fluctuation, and complex compactness of kuwanon T-receptor complex, mulberrin-receptor complex, and curcumin-receptor complex. Overall, curcumin has better stability, lower fluctuation, and is highly compact when forming a complex with an estrogen receptor. However, due to the nature of in silico prediction, it is not conclusive yet to say that curcumin can be a SERD alternative. In vitro and in vivo tests are highly recommended to confirm these results.

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