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International Conference On Natural And Social Science (ICNSSE) 2020

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LETTER OF ACCEPTANCE

Dear,

NUMLIL KHAIRA RUSDI

Assalamu'alaikum wr wb.

Greetings from ICNSSE 2020: International Conference On Natural And Social Science Education

We are pleased to inform you that your abstract entitled **SOYBEAN EXTRACT WITH TARGETED LUNASIN (ET-LUN) AS AN ANTI-BREAST CANCER: STUDIES IN SILICO AND IN VITRO** has been **accepted** for:

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We are looking forward for welcoming you on ICNSSE 2020 by virtually.

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STUDY *IN SILICO* AND *IN VITRO* ANTI-BREAST CANCER NATIVE INDONESIAN SOYBEAN EXTRACT WITH TARGETED LUNASIN

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ABSTRACT

Background / Objective: Breast cancer is the most common type of cancer in Indonesia, as well as in the world. Nowadays, the study of medicinal plants as anticancer becoming popular. To prepare anticancer medicines from natural resources, cytotoxic compounds assay, and screening active compounds which have a pharmacological effect is necessary. The aim of this study was to investigate the anti-breast cancer of soybean extract with targeted lunasin (ET-Lun) in vitro and to dock different ligands of its constituents to target protein of ESR1, ESR2, HER2 and EGFFR as molecular biomarkers in breast cancer. **Method:** In vitro assay, the extract cytotoxic activity was observed using the MTT 3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide test against MCF-7 breast cancer cells. In silico testing was using the Autodock Vina program. The active compounds in the soybeans are genistein, daidzein, coumestrol, carotene, phytoalexin, and lunasin as ligands, were being binding to the ERS1, ESR2, HER2 and EGFR as a cancer protein target. **Result:** In vitro studies showed that ET-Lun was cytotoxic against breast cancer cells with a CC50 value of 103 µg / mL. In silico studies showed that all the active compound from the soybean had activity to ESR1, ESR2, and EGFR. Lunasin and Genistein had better activity to ESR1, ESR2, and EGFR. Daidzein and Genistein had the best activity to HER2 while lunasin has no activity to HER2. **Conclusion:** These results indicate that the ET-Lun has anti-breast cancer activity in vitro assay. In silico assay showed that all the active compounds from the soybean seed had activity as anti-breast cancer by agonis ESR2 and inhibiting ESR1, HER2, and EGFR except Lunasin has not activity to HER2.

Keywords : *in-silico, in-vitro*, soybean, breast cancer

INTRODUCTION

In 2020, the incidence and mortality of breast cancer (BC) in the word highly.[1] The initial steps for designing and developing novel anti-cancer drugs are *in silico* study.[3] This has become methods to improve efficiency for compound activity optimization which are widely used in novel drug discovery.

Estrogen Receptor (ER), Progesterone receptor (PR) and Human Epidermal Growth Factor Receptor-2 (HER2), and Epidermal Growth Factor Receptor-1/ EGFR status examinations required to achieve precise therapy.[5] Moreover, ER, PR and HER2 are specific predictions and prognostic molecular biomarkers in breast cancer patients.

ET-Lun is a crude extract containing lunasin and the isoflavones genistein, daidzein, and glycitein (data not shown). Soybean (Glycine max (L.) Merr.) is one of the plants whose activity is being developed as anticancer. The active compounds in soybeans that have pharmacological activity include the isoflavones genistein, daidzein, and glycitein, coumestrol, carotene, phytoalexin, and protein of lunasin. Isoflavones and proteins are the main bioactive phytochemicals found in soybean plants.

The aim of this study was to investigate the anti-breast cancer of soybean extract with targeted lunasin (ET-Lun) in vitro and to dock different ligands of its constituents to target protein of ESR1, ESR2, HER2 and EGFFR as molecular biomarkers in breast cancer. This study explores the use of native Indonesian soybean varieties “Grobogan” which has a high protein and amino acid content. In silico assay, the active compound of soybean will be analyzed to ER (ESR1 and ESR2), HER2, and EGFR using AutoDock vina Tools, while *in vitro* assay were evaluated cytotoxic ET-Lun to the MCF-7 cancer cell lines using MTT assay to determine the CC50 value.

MATERIAL AND METHODS

Plant material identification and extraction

The procedure identification and extraction in accordance with previous studies.[14,16] The extract was kept at 2-8^{0}C.

In silico assay

Docking was performed using Autodock Vina software by performing ligand and protein preparation. The ligands that will be evaluated were lunasin, carotene, coumestrol, daidzein, genistein, and phytoalexin; which were active compounds found in soybean plants. The ligands were made in 2D and 3D structure. The 2D ligand structure was obtained from the PubChem / chem spider site/ Chemsketch,[18] while for the lunasin the 2D structure was obtained from the Swiss Model. To get a 3D format was by Marvin's sketch. ER protein preparation; ESR1 (5T92), ESR2 (1X7B), HER2 (5MY6) and EGFR (1XKK), as macromolecules were obtained from https://www.rcsb.org/ and https://www.ncbi.nlm.nih.gov. After obtaining the 3D structure of the ligands and receptors, then docking was performed. The output of docking was in the form of a ligand pose on the active side and its affinity score. The docking results were ligand poses in active sites and affinity scores. Analysis of ligand docking was performed to residues that interact with the ligand, Gibbs binding free energy parameter (ΔG), structure conformation, affinity and hydrogen bonds between ligand and receptor (ER, HER2, and EGFR).

In vitro/ Cytotoxicity Assay

Cytotoxicity assay was performed using a 96-well microplate. The step was started by seed 100 µL of MCF7 cell suspension to each well of tissue culture vessel and incubated 24 hours under standard culture conditions (5 % CO2, 37 ºC). The medium was removed afterward through well plates invention. The assay was then continued by adding 100 µL of assay solution to each well with various concentrations in triplicate, followed by 24 hours incubation

under standard culture conditions (5 % CO2, 37 ºC). The medium was then removed and replaced by of 100 µL of MTT reagent to each well and continued by 4 hours incubation under standard culture conditions (5 % CO2, 37 ºC). Lastly, the solution were removed and replaced by 100 µL of DMSO to each well to dilute the formazan crystal. The assay was conduct by measured the absorbance in 630 nm using ELISA plate reader.[4,17]

RESULTS AND DISCUSSION

In silico Assay

In this study, observed parameters were Gibss free energy (ΔG binding), hydrogen bonds, amino acid residues, and ligand structure conformation (lunasin, genistein, daidzein, coumestrol, carotene, and phytoalexin) in correspond receptors (ER, HER2, and EGFR). The stability and non-covalent interaction in ligand-receptor complexes were shown by free energy released during the interaction of ligand complexes formation.

All the ligands had a bond affinity with ERα (ΔG <0). Lunasin and Genistein had better activity towards ESR1. Lunasin had the highest affinity value, which was 12.426 and had more hydrogen bonds and amino acid residues. Genistein also had good activity to ESR1/ ERα because it had the lowest Gibbs free energy (-9.5476 kcal/mol). Genistein had activity with ESR2/ ERβ because it had the lowest ΔG (-10.4002 kcal/mol) and Lunasin have the biggest affinity value (pKI = 13.376). Daidzein had the best activity to HER2 because it had the lowest ΔG (-7.3067 kcal/mol), while lunasin had no affinity because Gibb's free energy was greater than 0**.** Lunasin had the greatest affinity for EGFR (pKi = 25.183), while Genistein also had a good affinity for EGFR because it possessed the smallest Gibbs free energy (Table 1).

High throughput Screening (HTS) or Virtual High-throughput Screening (vHTS) has been applied to accelerate efficiency in drug development and discovery.[3,18] One approach of vHTS was using in silico study to determine ligand affinity toward corresponding proteins.[24] In silico study has become applied methods to initiate novel drug discovery and increase efficiency in compound activity optimization. The advantages of in silico study are determinate, hypothesize, and deliver novel drugs or advances towards therapy and treatment. One approach of in silico is by docking the candidate molecule to targetted receptors. Docking is an effort to tune ligand which is a small molecule to proteins receptor which is greater protein.[3,24]

Molecular docking may predict molecule orientation from molecules to other molecules while bind to form a stable complex and methods for exploring optimal ligand position to the active site of target proteins (receptor). The scoring function was predicted interaction affinity between macromolecule (receptor) to ligands. Lower Gibbs free energy (ΔG) will increase binding stability.

In this studies the results showed that Lunasin and Genistein had the best activity to ESR1, ESR2, and EGFR because they had the lowest Gibbs free energy and the largest inhibition constant. On the other hand, the best activity to HER2 was shown by Daidzein and Genistein. The ligand affinity for the receptor was determined by the ΔG and pKi values. The more negative of Gibbs free energy and the greater of the pKi indicates the higher ligand affinity.[25] Analysis of bond Gibbs free energy (ΔG) and affinity (pKi) relates to binding affinity. Binding affinity is a measure of the drug's ability to bind to the receptor. The value of ΔG < 0 was indicates that the ligand has not an affinity for the active site of the receptor.

Beside ΔG and inhibition constant to predict the affinity of complexes binding between receptor and ligand formations, other parameters were hydrogen bonds and hydrophobic contact. Hydrogen bonds were intermolecular or intramolecular forces that occurred in between atom with high electronegative with hydrogen atom that covalently binds to an electronegative atom. The results showed that lunasin had the most hydrogen bonds with amino acid residues to

ESR1, ESR2, and EGFR (Table 1,2,4). The more hydrogen bonds indicated the best ligand-receptor interactions.

Hydrogen bonding is an electrostatic interaction between a weak acid donor group and the receptor atom with the formation of a lone pair. This hydrogen bond is formed between fellow molecules in a polypeptide (intermolecular) or between polypeptide molecules and water. The internal hydrogen bonds are arranged in a way that allows all hydrogen bonds to be formed. Hydrogen bonds are the main bonds that maintain protein stability.[26]

The ligands with amino acid residues and hydrogen bonds that are sama to natural ligands show similar types of interactions and illustrating similar activities.[27] The results of literature study explain that the active site ESR1 according to RSCB data (https://www.rcsb.org/) is at positions 347, 350, 353, 384, 387, 388, 394, 404, 421, 424, 524 and 525, whereas NCBI data (https://www.ncbi.nlm.nih.gov/) shows the ESR1 ligand-binding site at positions 346, 351, 353, 387, 394, 521, 524 and active site at positions 309-546. All ligands had a bond on the active site of the receptor, which was at position 309-546, except for Daidzein which was bound at the Lys 362 position (Table 1).

Active site ESR2 according to RSCB data (https://www.rcsb.org/) is at positions 295, 298, 305, 339, 340, 343, 346, 378, 475, 476, while the NCBI data (https: // www .ncbi.nlm.nih.gov /) shows the ligand-binding site ESR2 at positions 298, 303, 305, 339, 346, 473, 476. Lunasin has active site to receptor ESR2 at position Asp 303. Other ligans showed there were no ligan bind to active site of ESR2. Furthermore, the HER2 active site according to RSCB data (https://www.rcsb.org/) is at positions 187, 188, 192, 193, 194, 195, 252, 259, 404, 405 , 429, 459, and the NCBI data (https://www.ncbi.nlm.nih.gov/) shows the HER2 ligand binding site at positions 726, 727, 729, 734, 751, 753, 774, 798, 799, 801 , 849, 850, 852, 863, 720-976. There are no ligands that bind to the active site of the HER2 receptor (Table 3). It was mean, they did not show similar activity to natural ligands.

In addition, the active site of EGFR based on RSCB data (https://www.rcsb.org/) is at positions 743, 745, 766, 775, 776, 777, 788, 790, 791, 793, 797, 799, 800, 803, 832, 833, 834, 836, 844, 854, 855, 856, 860, 913, and the NCBI data (https://www.ncbi.nlm.nih.gov/) shows the ligand-binding site EGFR on positions 718-723, 745, 791-841, 842, 855, 876-880, 885, 889-968. All the ligands have an active side to bind with EGFR. Lunasin has 3 active binding sites at positions Arg 803, Arg 841, and Lys 913. Carotene, coumestrol, daidzein, genistein, and phytoalexin have bonds on the active side of Gln 791.

Cytotoxicity Assay

This study was in vitro experiment to the observed the impact of ET-Lun treatment compared to 5-Fluorouracil (5-FU) as control positive, and negative control to MCF7 cancer cell lines. Cytotoxicity assay was performed to determined optimal concentration to inhibits 50% of MCF7 cell population or cytotoxicity concentrations (CC50) between ET-Lun and 5-FU. From this experiment, ranges of toxic effect concentrations from compound to the cancer cell were determined. The concentrations of soybean extract and 5-FU were between 250-0.9 µg/mL in 24 hours. The curve showed that ET-Lun treatment to MCF7 breast cancer cell culture may induce cell deaths. The effect was observed with parallel decrease to amount of concentrations between both compounds to viable cells of MCF7. However, 5-FU were not respond to 50% of cell deaths. (Figure 1)

The results of in vitro assay shown of the percentage of MCF7 viable cells using MTT with a gradual concentration of ET-Lun were shown in figure 1. In MCF7, the curves showed that ET-Lun and 5-FU towards MCF-7 induced cell deaths. This was demonstrated with MCF-7 cell percentage were not parallel with increase concentration of either extract or 5-FU. The correlation between soybean extract concentration treatment to the percentage of viable MCF-7 cells was induced by cell deaths. This was R2=0,9284 while 5-FU was R2=0,8599. The R2

value that approach 1 shown good value and relations between two variables[19], therefore relations between soybean extract and 5-FU (assay compound) percentage shown a decrease in viable MCF-7. The greater compound test concentration, then the MCF7 viable cells percentage were smaller.[20]

Cytotoxicity of compounds may be observed from CC50 using linear regression equation of curves, that is y=-0,1852x + 69,06 for ET-Lun and y=-0,0547 + 65,311 for FU (Figure 1). The CC50 was obtained from x variables by put 50 (standard value were collected from 50% inhibition concentration) to the variable y in regression equation of both compound thus CC50 were obtained from soybean extract were 103 µg/mL and 280 µg/mL for 5-FU. Based on the cytotoxic assay, a soybean extract compound was found to has three times cytotoxic activity compared to the 5-FU. Research conducted by Dia et al.,[21] showing a concentration range of 1-100 µM of Lunasin from soybean extract were cytotoxic towards HT-29 cells by induced apoptosis and induced cell cycle arrest on G2/M phase by increasing p21 expression. Lunasin was also cytotoxic in MCF7 by induced ROS for DNA fragmentation, increase p53/p21 regulations, induced cell cycle arrest to G1/S phase and activated caspase 9/3.[22] 5-FU that was applied as a positive control on MCF-7 were not cytotoxic to the cell as a range of concentration were unable to reduced half of MCF-7 cell population thus need to used other positive control as tamoxifen.[23]

CONCLUSIONS

In silico assay showed all the active compounds from the soybean seed had activity as anti-breast cancer by inhibiting ESR1 and EGFR, and agonist of ESR2. The results of in vitro study demonstrated that the ET-Lun has anti-breast cancer.

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COMPETING OF INTEREST

The authors declare that they have no competing interest.

REFERENCES

1. Kementerian Kesehatan RI. Hasil utama RISKESDAS 2018. In: Riskesdas 2018. Jakarta.

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca Cancer J Clin. 2018;68:394-424.

3. Jansen F. Introduction to Computational Chemistry. 2nd ed. Odense: Denmark; 2007.

4. Cancer Chemoprevention Reaserch Center (CCRC). A fix procedure of cytotoxicity testing: MTT methods. Yogyakarta. UGM Faculty of Pharmacy. 2013;p 1-8

5. Schettini F, Buono G, Cardalesi C, Desideri I, De Placido S, Del Mastro L. Hormone Receptor/Human Epidermal Growth Factor Receptor 2-positive breast cancer: Where we are now and where we are going. Cancer Treat Rev 2016;46:20–6.

6. Eberhard DA, Johnson BE, Amler LC, Goddard AD, Heldens SL, Herbst RS, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and

prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. J Clin Oncol 2005;23(25):5900–9.

7. Wolff AC, Hammond MEH, Hicks DG, Dowsett M, McShane LM, Allison KH, et al. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of American pathologists clinical practice guideline update. Arch Pathol Lab Med 2014;138(2):241–56.

8. Changavi AA, Shashikala A, Ramji AS. Epidermal Growth Factor Receptor Expression in Triple Negative and Nontriple Negative Breast Carcinomas. 2015;79–83.

9. Hsu JL, Hung M. The role of HER2, EGFR, and other receptor tyrosine kinases in breast cancer. 2017;35(4):575–88.

10. Lule VK, Garg S, Pophaly SD, Hitesh, Tomar SK. Potential health benefits of lunasin: A multifaceted soy-derived bioactive peptide. J Food Sci. 2015;80(3):485–94.

11. Liu J, Jia SH, Kirberger M, Chen N. Lunasin as a promising health-beneficial peptide. Eur Rev Med Pharmacol Sci. 2014;18(14):2070–5.

12. Vuyyuri SB, Shidal C, Davis KR. Development of the plant-derived peptide lunasin as an anticancer agent. Curr Opin Pharmacol 2018;41:27–33.

11. Serra A, Gallart-Palau X, See-Toh RSE, Hemu X, Tam JP, Sze SK. Commercial processed soy-based food product contains glycated and glycoxidated lunasin proteoforms. Sci Rep 2016;6(November 2015):1–12.

12. Wijiasih, Kusmardi, Elya B. The Effect of Soybean and Soybean Meal Extract on COX-2 and iNOS Expression in Colon Preneoplasia of Mice Induced by Azoxymethane and Dextran Sodium Sulfate. Int J ChemTech Res 2017;10(1):39–46.

15. Putri AS, Elya B, Kusmardi K. Inhibitory Activity of Indonesian Soybean in comparison with Soybean Meal on Goblet Cell Count and Micro Vessel Density in Colitis-Associated Mouse Colon Carcinogenesis. Int J PharmTech Res. 2017;10(1):9–18.

16. Amalia AW, Kusmardi K, Elya B, Arsianti A. Inhibition of carcinogenesis by seed and soybean meal extract in colon of mice: Apoptosis and dysplasia. Asian J Pharm Clin Res 2017;10(4):22–7.

17. Basedia DP, Shrivastava B, Dubey BK, Sharma P. Synthesis and Anticancer Activity of Novel Substituted 1,3,4-oxadiazolo-[3,2-a]-1,3,5-triazine and 1,3,4-thiadiazolo-[3,2-a]-1,3,5-triazine Derivatives. Asian J. Research Chem. 2014. 7(3):310–5.

18. Patel RN, Patel UY, Patel KM, Patel JS, Patel AD, Sen DJ. Latest Strategy of Medicinal Chemistry Implements In-silico After In-Vivo and In-Vitro. Asian J Res Chem. 2011;4(2):167–80.

19. Schneider A, Hommel G, Blettner M. Linear regression analysis—part 14 of a series on evaluation of scientific publications. Dtsch Arztebl Int. 2010; 107(44): 776–82

20. Wijayanti PA, Puspaningtyas AR, Pangaribowo DA. Proliferation and cytotoxicity assay of 1-(4- rifluorometilbenzoiloksimetil)-5-fluorourasil compound to MCF-7 breast cancer cells. e-Journal of Medical Libary. September 2015;3(3):1-5.

21. Dia VP, Mejia EG. Lunasin promotes apoptosis in human colon cancer cells by mitochondrial pathway activation and induction of nuclear clusterin expression. Cancer Lett. 2010 Sep 1;295(1):44-53.

22. Zafar A, Singh S, Naseem I. Cytotoxic activity of soy phytoestrogen coumestrol against human breast cancer MCF-7 cells: Insights into the molecular mechanism. Food Chem Toxicol. 2017 Jan;99:149-161.

23. Jordan VC. Tamoxifen treatment for breast cancer: concept to gold standard. Oncology (Williston Park). 1997 Feb;11(2 Suppl 1):7-13.

24. Khurshid Ahmad MH. Drug Discovery and In Silico Techniques: A Mini-Review. Enzym Eng. 2014;04(01):1–3.

25. Kim R, Skolnick J. Assessment of Programs for Ligand Binding Affinity Prediction. J Comput Chem 2012;32:174–82.

26. Zhao H, Huang D. Hydrogen bonding penalty upon ligand binding. PLoS One 2011;6(6).

27. Cosconati S, Forli S, Perryman AL, Harris R, Goodsell DS, Olson AJ. Virtual screening with AutoDock: Theory and practice. Expert Opin Drug Discov 2010;5(6):597–607.

28. Ferreira R, Lopes AC, Oliveira PA, Faustino-Rocha AI, Colaço B, Alvarado A, et al. Prognostic factors in MNU and DMBA-induced mammary tumors in female rats. Pathol - Res Pract 2017;213(5):441–6.

29. Kerdelhué B, Forest C, Coumoul X. Dimethyl-Benz(a)anthracene: A mammary carcinogen and a neuroendocrine disruptor. Biochim Open 2016;3:49–55.

30. Kumar A, Sunita P, Jha S, Pattanayak SP. 7,8-Dihydroxycoumarin exerts antitumor potential on DMBA-induced mammary carcinogenesis by inhibiting ERα, PR, EGFR, and IGF1R: involvement of MAPK1/2-JNK1/2-Akt pathway. J Physiol Biochem 2018;74(2):223–34.

31. Moi LL, Flågeng MH, Gjerde J, Madsen A, Røst TH, Gudbrandsen OA, et al. Steroid receptor coactivators, HER-2 and HER-3 expression is stimulated by tamoxifen treatment in DMBA-induced breast cancer. BMC Cancer. 2012;12:1-12.

32. Shou J, Massarweh S, Osborne CK, Wakeling AE, Ali S, Weiss H, et al. Mechanisms of tamoxifen resistance: Increased estrogen receptor-HER2/neu cross-talk in ER/HER2-positive breast cancer. J Natl Cancer Inst 2004;96(12):926–35.

33. Pietras RJ. Interactions between estrogen and growth factor receptors in human breast cancers and the tumor-associated vasculature. Breast J. 2003;9(5):361–73.

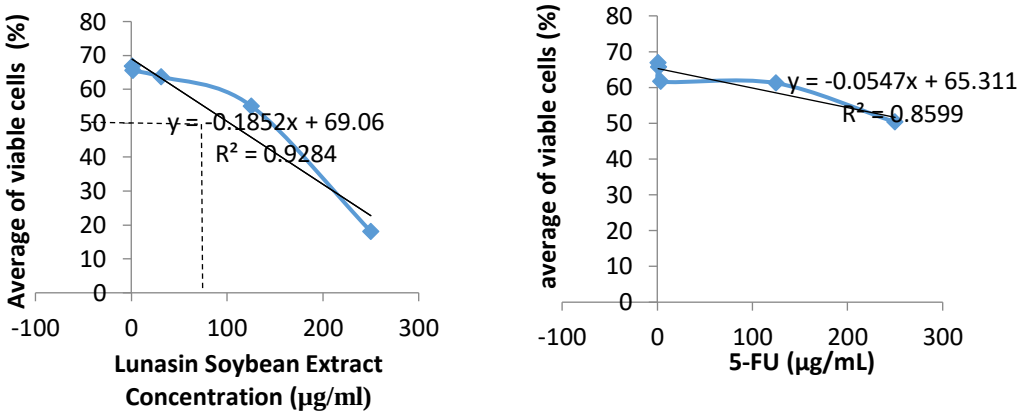
34. Rusdi NK, Purwaningsih EH, Hestiantoro A, Elya B, Kusmardi K. In Vivo Antimammary Tumor Effects of Soybean Extract with Targeted Lunasin (ET-Lun). Pharmacogn J. 2021;13(5).

TABLE AND FIGURE

Tabel 1. Gibbs Free Energy (ΔG), Inhibition constant, and Hydrogen bonds and amino acid residues of ligans to ESR1/ ERα, ESR2/ ERβ, HER2, dan EGFR receptor.

Ligan	Energi bebas Gibbs (kcal/mol)	Afinitas (pKi)	Jumlah atom H	Ikatan Hidrogen
Reseptor ERα (5T92)				
Lunasin*	-5,0005	**12,426**	14	Asp 321, Phe 367, Asp 369, Asp 369, Asp 369, Leu 370, Thr 371, Gln 375, Glu 397, Glu 443, Ala 307, Leu 308, Ile 326, Lys 362
Carotene	-6,8835	3,908	-	-
Coumesterol	-8,0939	7,093	1	Glu 353
Dadzein	-8,8523	5,753	2	Leu 370, Lys 362
Genistein*	**-9,4439**	4,293	1	Asp 321
Phytoalexin	-6,7596	5,954	1	Thr 347
Reseptor ERβ (1X7B)				
Lunasin*	-2,8762	**13,376**	14	Ser 283, Ser 286, Ser 286, Asp 303, Asp 303, Asp 489, Asp 489, Thr 613, Ala 287, Lys 300, Lys 300, Lys 304, Lys 605, Lys 605
Carotene	-6,0757	3,040	-	-
Coumesterol	-8,4775	4,377	2	Asp 489, Lys 300
Dadzein	-8,5833	4,695	1	Asn 496
Genistein	**-10,4002**	9,246	2	Glu 389, Ser 423
Phytoalexin	-6,7754	3,606	-	-
Reseptor HER2 (5MY6)				
Lunasin	2,7079	5,838	7	Gln 362, Gln 362, Asn 388, Gln 398, Gln 398, Glu 401, Glu 401
Carotene	-6,2611	7,703	-	-
Coumesterol	-6,1025	8,466	-	-
Dadzein	-7,3067	7,823	2	Thr 68, Thr 68
Genistein*	-6,3292	**8,979**	1	Lys 368
Phytoalexin	-	-	-	-
Reseptor EGFR (1XKK)				
Lunasin*	-3,1974	**25,183**	14	Glu 709, Glu 709, Glu 749, Leu 782, Ser 784, Ser 784, Glu 709, Arg 803, Arg 841, Val 876, Val 876, Lys 913, Lys 913, Lys 913
Carotene	-8,3510	12,139	5	Gln 791, Gln 791, Lys 846, Lys 846, Lys 852
Coumesterol	-9,7183	14,323	5	Gln 791, Gln 791, Lys 846, Lys 846, Lys 852
Dadzein	-9,2115	13,109	5	Gln 791, Gln 791, Lys 846, Lys 846, Lys 852

Genistein*	**-10,4368**	12,695	5	Gln 791, Gln 791, Lys 846, Lys 846, Lys 852
Phytoalexin	-8,0671	11,828	5	Gln 791, Gln 791, Lys 846, Lys 846, Lys 852



(a) **(b)**

Figure 1 (a). Standard curves of citotoxicity assay of soybean extract and 5-FU (**b**). Gradual concentration of 5-FU to MCF7 cell shown CC50 concentration were 280 µg/mL.