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Original Research Article

In Silico Analysis of Isoflavone Compounds in Soybean (*Glycine max* L) as Anti-Breast Cancer Agents Targeting Estrogen Receptor Alpha

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ABSRTACT

Breast cancer is a main health concern globally and the second leading cause of cancer death in many countries, including developed and developing. Meanwhile, soybean is reported to contain isoflavones, which have properties similar to certain hormonal anti-cancer drugs. This study aimed to investigate the anti-breast cancer activity of isoflavone compounds in soybean (*Glycine max* L) against estrogen receptor alpha. *In Silico* test was conducted on isoflavone compounds in soybean, which consisted of 12 isoforms including Daidzein, Daidzin, Genistein, Glycitein, Genistin, Glycitin, Acetyl Daidzin, Acetyl Genistin, Acetyl glycitin, Malonyl Daidzin, Malonyl Genistin, and Malonyl Glycitin. The results showed that four compounds passed the Lipinski's rule test and achieved strong binding affinity namely Daidzein, Genistein, Glycitein, and Acetyl Daidzin with values of -8.47, -8.5, -8.6, and -7.09 respectively. These compounds also formed hydrogen bonds in the interactions with macromolecules. Specifically, Daidzein, Glycitein, and Acetyl Daidzin formed three hydrogen bonds each, while Genistein formed five hydrogen bonds. Based on the results, soybean has anti-breast cancer activity as shown by *In Silico* test on the estrogen receptor alpha.

Keywords: Soybean, in Silico, Isoflavon Compounds, Anti-Cancer

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Introduction

Breast cancer ensues when abnormal cells develop uncontrollably in breast tissue.[1], affecting both women and, rarely, men. It develops in the glandular tissue (lobes), ducts, or fatty tissue of the breast. According to the Global Cancer Statistics (Globocan) in 2020, 396,914 total cancer cases were recorded in Indonesia across all genders, with breast cancer ranking highest at 16.6% or accounting for 65,858 cases [(6)]. Breast cancer accounts for the second highest cancer mortality rate after lung cancer.[9] The death cases reached 22,430, implying that 9.6% of sufferers died.[2] In this context, chemotherapy and radiotherapy have significant side effects, hence, patients tend to explore alternative and complementary therapies that can improve life quality.[3]

This has, in no small measure, contributed immensely to patronizing herbal medical practitioners as a means of alternative complementary medicine for breast cancer patients, especially in the continent of Indonesia. The finding and development of drugs from natural sources, especially medicinal plants, have been an age-long practice.

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Due to the significant side effects of current therapies, there is a need to consider the development of new strategies for breast cancer treatment.[22] Molecular docking is one of the widely used strategies in discovering new drug therapies. In the simplest definition, molecular cancers are caused by ER-positive cells.[5;7] Estrogen consists of two subtypes namely, estrogen receptor alpha (ERα) and ER beta (ERβ). ERα is primarily present in the mammary gland, uterus, ovary (thecal cells), bone, male reproductive organs (testis and epididymis), prostate (stroma), liver, and adipose tissue. Conversely, ERβ is mainly found in the prostate (epithelium), bladder, ovary (granulosa cells), colon, adipose tissue, and immune system.[18] High expression of Rα is closely associated with the development of breast cancer cells. Due to the high percentage of breast cancer influenced by ER, one commonly used treatment regimen is hormonal drugs. [18]

In the field of natural pharmaceutical materials, certain plants contain phytoestrogen compounds. These compounds are plant-derived estrogen-like substances with a chemical system similar to 17 β-estradiol, a synthetic hormone drug used to treat hormone-related disorders, including breast cancer. Phenolic compounds categorized as phytoestrogens include isoflavones, coumestans, stilbenes as well as lignans.[4]

Materials and Methods

Ligand preparation

Twelve isoflavones have been specified in soybean (*glycitin max L*) , including three aglycones, namely daidzein, genistein, glycitein, and their corresponding glycosides daidzin, genistin, glycine, acetyl daidzin, acetyl genistin, acetyl glycitin, malonyl daidzin, malonyl genistin, and malonyl glycine as reported in the literature were used as ligands in the present investigation. The 2D structure data file (PDB) of selected compounds and reference drugs (Isoflavone and 4-Hydroxytamoxifen) was obtained from the PubChem database and optimized to 3D using MMFF94. The Autodock Tools Software

(Version 1.5.7, 2022) added hydrogen atoms and Gasteiger charges to the 3D structure and converted the PDB format to PDBQT format.

Protein Preparation
Estrogen Receptor-Alpha (Erα), the protein targeted in this study, was retrieved from the Protein Data Bank with code 3ERT and then saved in PDB format. Subsequently, the target protein was prepared by separating the ligand 4-Hydroxytamoxifen (tamoxifen), proven as hormonal therapy for breast cancer, using Discovery Studio Visualizer and AutoDock Tools. This process consisted of removing unnecessary components from the system, adding hydrogen atoms, applying the Kollman charge, and saving in pdb format.[24]

Docking Preparation
Virtual screening docking analysis of the target protein-ligand 4-Hydroxytamoxifen (tamoxifen) and isoflavone compounds from soybean (*Glycine max L*) conducted using AutoDock Tools Version 1.5.7, 2022). The configurations of AutoDock4 and AutoGrid4 in Notepad format were executed through the docking procedure using the command prompt. A customized grid box, output settings, and names of receptor and ligand were all included in the appropriate grid box setup. The docking results, visualized utilizing Discovery Studio Visualizer 2021 were saved in pdbqt format, containing binding affinity values and amino acid residue interactions.

Validation Methods
Validation of the method in this study used Autodock Tools software, where the approach used was to re-dock the ligand with the prepared protein. The method is said to be valid if the Root Mean Square Deviation (RMSD) shows a value of <2 Å.

Results and Discussion

The 2D structures of the twelve isoflavones compounds in soybean (*Glycine max L)* namely daidzein, glycitein, genistein, and their corresponding glycosides daidzin, genistin, glycitin, acetyl daidzin, acetyl genistin, acetyl glycitin, malonyl daidzin, malonyl genistin as well as malonyl glycitin were modeled and used as a target for the current docking studies against ERα which have been reported to be responsible for breast cancer. The 3D crystal structures of the target proteins, 3ERT from Estrogen Receptor-Alpha (Erα) including the native ligand 4-Hydroxytamoxifen (tamoxifen), are presented in figure 1 as visualized using Discovery Studio Visualizer 2021.

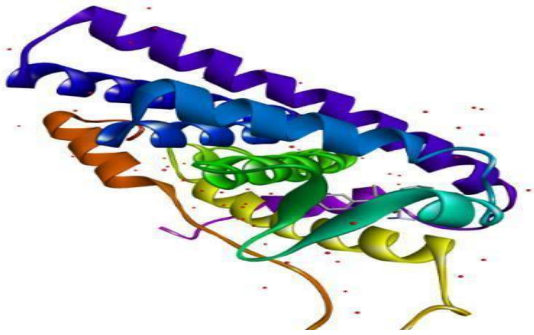

Figure 1: 3D Structure of target protein 3ERT in Discovery Studio Visualizer

Drug candidates that will be designed through molecular docking aim to find ligands that can interact with the target receptor, both agonist and antagonist interactions. However, this process does not ensure that the drug candidate can be made into an oral drug. Oral drugs have a long route to the target in the body which is commonly referred to as drug pharmacokinetics. To design an orally active drug, it must fulfill 'Lipinski's Rule of Five'. The Lipinski's rule is that a molecule can continue docking simulation if (1) molecular weight is less than 500 Da (2) log P value is less than 5, (3) the number of hydrogen bond donors is less than 5, (4) the number of hydrogen bond acceptors less than 10 (5) Molar refractivity between 40-130.[12]
The properties and characteristics of the test ligand to be used can be analyzed on the web page http://scfbioiitd.res.in/software/drugdesign/lipinski.jsp to determine whether the test ligand is able to meet the five Lipinski rules are presented in (Table 1). From the test results, there are 4 compounds that fulfill Lipinski's five rules and can be continued to the tethering process, namely Daidzein, Genistein, Glycitein, and acetyl Daidzin. In the current study, the use of Autodock Tools and flexible docking were utilized in precisely predicting the binding affinity and docking scores of isoflavones compounds from soybean (*Glycine max L)* and the native ligand 4-Hydroxytamoxifen (tamoxifen) against Estrogen Receptor-Alpha (Erα) (3ERT). All four compounds chosen in the current study were successfully docked with crystalline protein structures 3ERT, a target against breast cancer.

Table 1: Properties of test compounds based on lipinski test

Compounds	Molecular Weight (g/mol) <500	Log P <5	Hydrogen bond donors <5	Hydrogen bond acceptors <10	Molar Refractivity (40-130)
Daidzein	254	2,40	2	4	69,34
Genistein	270	2,11	3	5	71,00
Glycitein	284	2,72	2	5	75,70
Daidzin	416	-0,11	5	9	102,07
Genistin	432	-0,41	6	10	103,73
Glycitin	446	0,19	5	10	108,43
Acetyl Daidzin	430	0,84	4	9	106,66
Acetyl Genistin	474	-0,14	6	11	113,16
Acetyl Glycitin	488	0,76	4	11	117,97
Malonyl Daidzin	502	-0,09	5	12	118,20
Malonyl Genistin	518	-0,38	6	13	119.86
Malonyl Glycitin	532	0,22	5	13	124,55

The docking scores of the compounds and reference drugs were between -5.91 and -10.86 Kcal/mol, as presented in Table 1. Glycitein had the highest crucial affinity score of -8.60 Kcal/mol, closely followed by Genistein and Glycitin, with both having docking scores of -8.50 Kcal/mol. The least activity was observed in Malonyl Daidzin

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with -5.91 Kcal/mol, respectively (Table 2). In molecular docking, the lowest binding energy in Kcal/mol indicates a better ligand-receptor binding affinity. Therefore, the compound with the most reduced binding energy was interpreted as the most effective compound in suppressing the protein target, as the reduced binding energy implies greater affinity. The observed differences in the binding affinities could be attributed to the interaction between the residual amino acids and the compounds.

The 3D and 2D molecular interactions of the amino acid residues of 3ERT with twelve isoflavones are presented in (Table 2), and reference drugs 4-Hydroxytamoxifen (tamoxifen) are presented in Figure 3. The molecular docking results of the compounds depict the interaction of compounds with several interesting amino acids. The compound interacted with the amino acid residues through several forces such as conventional hydrogen bonding, carbon-hydrogen bonds, π-sulfur, alkyl, π-alkyl, unfavorable donor-donor as well as van der Waals. The two major interacting forces involved in docking these selected compounds and reference drugs are hydrogen bonds (conventional and carbon-hydrogen bonds) and π-alkyl. Interactions involving hydrogen bonds have been reported to be stronger than other bonds. The main drivers of cell-to-cell communication and dysregulation result from molecular interactions between small molecules and proteins, which have been implicated in many diseases such as cancer. Consequently, the bonding interactions between the test compounds and receptors could have played a substantial role in the suppression of the attractive charge as well as the enzymes.

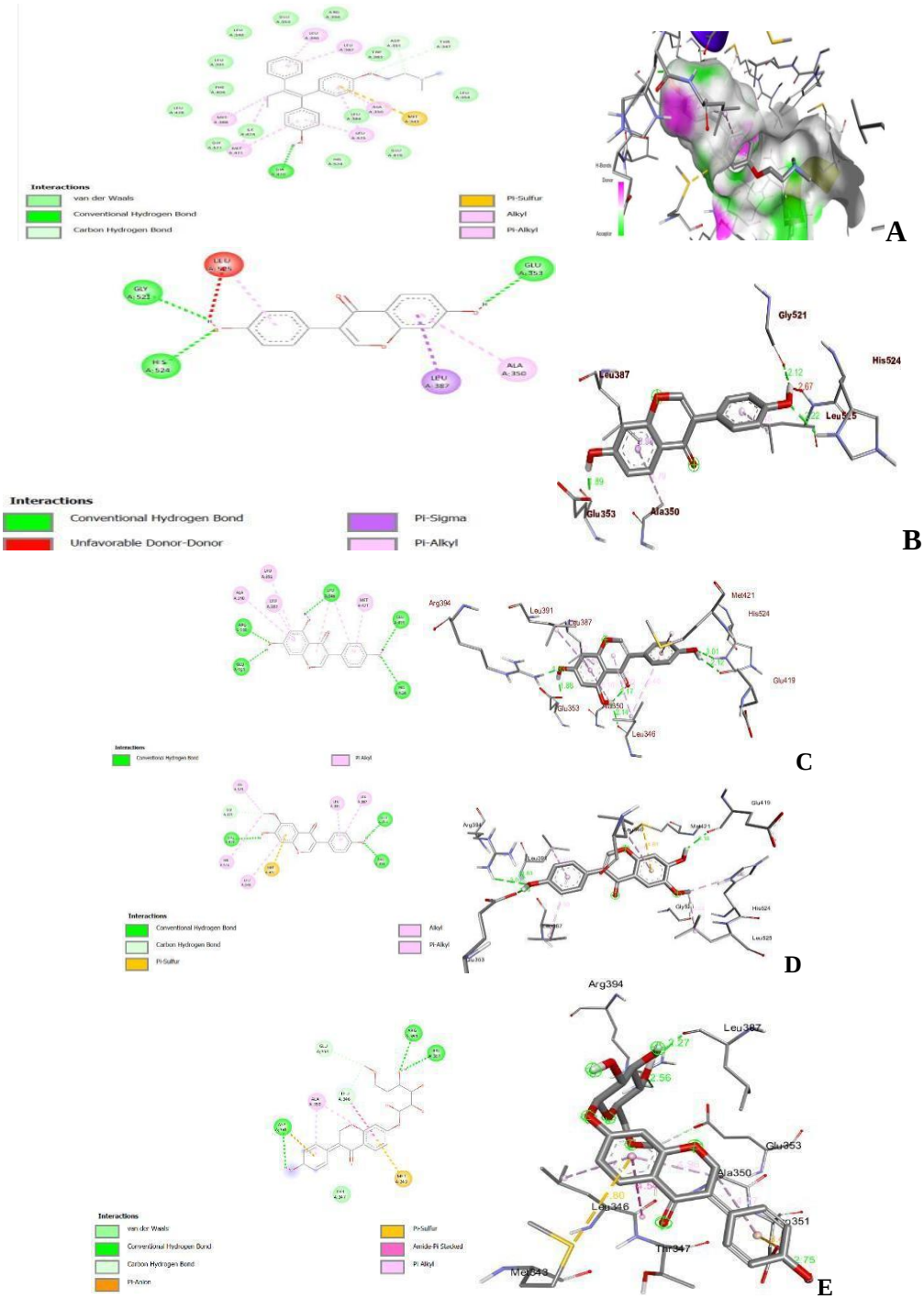

Figure 2: 2D (left) and 3D (right) interaction with the target protein (3ERT) docked using Autodock Tools, Visualized in Discovery Studio

(A) 4-hydroxytamoxifen (tamoxifen), (B) Daidzein, (C) Genistein, (D) Glycitein (E)

The twelve isoflavone compounds have more than one hydrogen bond in their interaction with the estrogen receptor alpha. This contrast to the reference drug 4-hydroxytamoxifen (tamoxifen), which has only one hydrogen bond with Gly-420. This hydrogen bond indicates a good conformation in the interaction between the isoflavone compounds and the estrogen receptor alpha, hence a good biological response can be produced [(30)]. The interaction that occurs between the ligand and the enzyme is not only in the form of hydrogen bonds, but there are also other non-convalent interactions that can increase the affinity of the inhibitor to the receptor. The docking results show that some of the bonds in the Glycitein compound are very similar to the reference drug tamoxifen. The glycine compound interacts with the amino acid Met-421 to form a π-sulphur bond, similar with the reference drug Met-342, which helps to increase the binding energy of the glycine compound. Bond distance is an advanced parameter to validate molecular tethering results. Bond length is the distance between two bonded atoms in a molecule. In general, the weaker the bond length, the weaker the bond strength[(31)]. From the molecular docking results, it was found that the hydrogen bonds in isoflavone compounds in soybeans have a distance of ≤3 Å. Strong hydrogen bond distance ranges below 3.3 Å. The angle of the bond shape is also important in determining the power of the hydrogen bond. The tighter the hydrogen bond is to the correct geometry, the more powerful the bond

Table 2: Molecular Docking Results

Compounds	KI (nM)	ΔG (kcal/mol)	Amino Acid	HBond	RMSD (Å)
4-hydroxytamoxifen	10.93	-10.86	Gly-420	1	1.162
Daidzein	623.2	-8.47	His-524	2	2.21
			Gly-521		2.12
Genistein	591.58	-8.5	Leu-387	5	2.14
			Glu-353		1.87
			Arg-394		1.83
			Glu-419		2.11
			His-524		3.00
Glycitein	496.11	-8.6	Glu-419	3	2.16
			Arg-394		1.82
			Glu-353		1.78
Acetyl Daidzin	6.38	-7.09	Asp-351	3	2.74
			Arg-394		2.55
			Leu-387		2.27

Conclusion

In conclusion, isoflavone compounds found in soybean, particularly Glycitein, showed strong ligand-receptor interactions and binding affinity with Erα. This suggested the potential ability as antagonists, offering promising therapeutic options for breast cancer treatment.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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