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Research paper

In Silico Study Of Pomegranate Peel Polyphenols As Breast Anticancer

Studi *In Silico* Polifenol Kulit Delima sebagai Antikanker Payudara

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ABSTRACT

Breast cancer is one of the causes of women's death. Estrogen-α receptors are one of the targets for breast cancer treatment because it plays a role in cancer cell proliferation. Several studies have stated that Flavonoid compounds have high activity in inhibiting the growth of breast cancer cells. This study aims to inhibit polyphenolic compounds in pomegranate peel (gallic acid, cafeic acid, ellagic acid, and chlorogenic acid) against estrogen receptors-α through molecular docking. The 3D structures of the polyphenolic compounds were obtained from the PubChem database and the estrogen-α receptors from the Protein Data Base. Molecular docking simulations were carried out using AutoDock Vina and supporting software such as Biovia Discovery Studio Client 4.1, AutoDockTools 1.5.6, PyMOL, and LigPlot. The results showed that the four polyphenolic compounds had a better potential to inhibit estrogen-α receptors than tamoxifen. The inhibitory potential is evidenced by the low affinity of ligand-protein binding energy (approximately -5.4 to -9.0 kcal/mol). The phenol group of polyphenolic compounds can strengthen the ligand-protein interactions through hydrogen bonds with the active site of ER-α proteins. Hydrophobic and π-π stacking interactions between polyphenolic and the active site of proteins also support the inhibition potential of polyphenolic compounds. The conclusion is that the polyphenolic compounds in pomegranate peel have the potential as breast anticancers.
Keywords: polyphenol, pomegranate peel, breast cancer, estrogen-α receptor, molecular docking

INTRODUCTION

Breast cancer is one of the causes of women's death (Siegel et al., 2022). The number of breast cancer sufferers is increasing and first ranks among cancer cases in Indonesia (Gautama, 2022). So far, breast cancer therapy has been carried out using various techniques such as chemotherapy, surgery, hormones, and drugs (Waks & Winer, 2019). Apart from causing various side effects, using this technique is less effective in preventing the proliferation of advanced breast cancer cells (Ko & Moon, 2015). Further research is needed regarding more effective breast cancer treatment techniques. One of the factors that triggers the development of breast cancer cells is the activity of estrogen and estrogen receptors (Sharma et al., 2018).

Estrogen plays an important role in the proliferation of breast epithelium. there was a significant increase in estrogen and estrogen receptor-α (ER-α) activity in breast cancer patients (Roy & Vadlamudi, 2012). Uncontrolled estrogen activity will increase abnormal cell proliferation and trigger the development of breast cancer cells (Li et al., 2022). In general, breast cancer patients who are diagnosed as positive for ER-α are given endocrine therapy such as tamoxifen, toremifen, and raloxifene. However, regular use of these drugs can trigger the development of endometrial cancer, and cause blood clots and pulmonary embolisms (Sharma et al., 2018). It is necessary to explore estrogen-α receptor inhibitor compounds that are safer and have higher effectiveness.

Several studies show that polyphenol compounds have high potential as anticancer (Oršolic, et al., 2022; Darwati, et al., 2021). Phenol groups of polyphenol compounds can increase their pharmacological activities such as antioxidant, anti-inflammatory, and antibacterial (Alwahibi, et al., 2020). Polyphenol compounds can inhibit the growth of cancer cells by modifying the expression of genes that play in carcinogenesis process (Vladu, et al., 2022). Polyphenolic compounds also slow the development of cancer cells by regulating cellular mechanisms and limiting the capacity of cancer cells to metastasize (Oyenihi, et al., 2019).

Pomegranate (Punica granatum L.) is rich in polyphenolic compounds. Pomegranate peel contains polyphenolic compounds such as gallic acid, caffeic acid, ellagic acid, and chlorogenic acid, as well as flavonoids such as apigenin, quercetin, pelargonidin, and cyanidin. (Singh et al., 2018). The active compounds in pomegranate peel have pharmacological activity as anticarcinogenic, antibacterial, and antioxidant (Nge, et al., 2019). Therefore, this study aims to test the activity of polyphenolic compounds as anti-breast cancer agents through their inhibition of the estrogen-α receptor by molecular docking.

METHODS

Tools and materials

This research uses AutoDockTools 1.5.6, AutoDock Vina, PyMOL, and LigPlot software. The 3D structure of estrogen-α receptor (ER-α) protein was obtained from Protein Data Bank (PDB) and the ligands used in this research were polyphenolic compounds from pomegranate peel (gallic acid, caffeic acid, chlorogenic acid, and ellagic acid) (Singh, 2018). The 3D structures of polyphenolic compounds were obtained from the PubChem data bank.

Ligand and Protein Preparation

The 3D structure of the estrogen-α receptor (ER-α) protein was downloaded from Protein Data Bank (PDB) with ID code: 5WGQ (Fanning, et al., 2018), while the 3D structures of the ligands were downloaded from the PubChem data bank, gallic acid (CID) code: 370), caffeic acid (CID: 689043), ellagic acid (CID: 5281855), and chlorogenic acid (CID: 1794427).

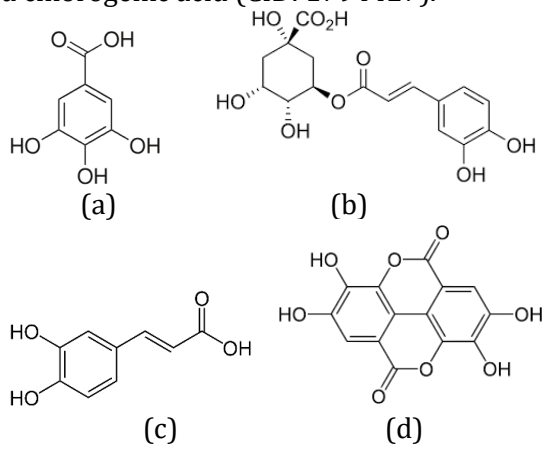


Figure 1. Structure of polyphenol compounds in pomegranate peel (a) gallic acid (b) chlorogenic acid (c) caffeic acid (d) ellagic acid

ER-α protein molecules were prepared using AutoDockTools 1.5.6 software. by cleaning proteins from water, ligands, and other compounds that are not needed in molecular docking. The protein structure is added with charges and polar hydrogen and then saved in PDBQT format. Polyphenol ligand structures were also prepared using AutoDockTools 1.5.6. by assigning charges, analyzing bond rotations, and re-saving the ligands in PDBQT format. In this preparation, the size of the grid box used in molecular docking was also determined by setting the active site of the protein using AutoDockTools 1.5.6.

Validation of Molecular Docking Methods

The molecular docking method was validated by redocking the native ligand with the ER-α protein using AutoDock Vina. The native ligand from redocking was compared with the original ligand from the PDB using PyMOL to produce a root mean square deviation (RMSD) value. The molecular docking method is valid if the RMSD value is smaller than 2.0 Å (Morris et al., 2008). The validity of

this method shows that the grid box size is appropriate and can be used for molecular docking simulations of polyphenolic compounds from pomegranate peel.

Molecular Docking

Anti-breast cancer activity of polyphenolic compounds was measured through molecular docking of the estrogen-α receptor using AutoDock Vina. The results of the molecular docking simulation were analyzed using PyMOL to see the position of the polyphenolic compounds in the active site of the ER-α protein. The interactions formed between polyphenolic compounds and ER-α were analyzed using LigPlot and Biovia Discovery Studio Visualizer software.

RESULTS AND DISCUSSION

The estrogen-α receptor is a protein that plays a role in various hormonal processes in a woman's body (Tan, et al., 2019). ER-α is widely used as a target in therapy for the treatment of breast and sexual cancer because it plays a direct role in the proliferation process of cancer cells (Sharma et al., 2018, Lei, et al., 2019). The structure of the estrogen receptor-α used in this study is the human estrogen-α receptor obtained from the PDB with code 5WGQ. The 3D protein structure used in molecular docking simulations is the chain A structure of the ER-α protein bound to the estradiol native ligand.

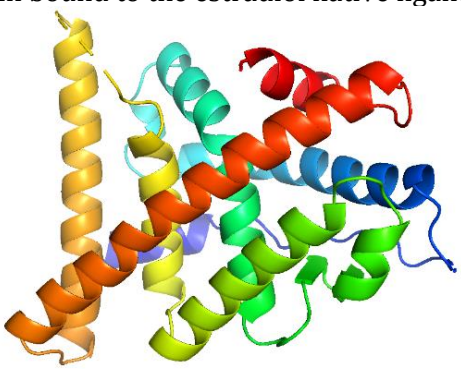


Figure 2. 3D structure of estrogen-α receptor protein (ID: 5WGQ)

Molecular docking validation was carried out through a redocking process between the original ligand (estradiol) and ER-α protein using AutoDock Vina. This is also used to determine the suitability of the size of the grid box used in the molecular docking process. Based on the validation results, it can be seen that the structures of the re-docked native ligand and the original native ligand have been squeezed and have an RMSD value of 0.636 Å. These results indicate that the grid box size is appropriate and the validity of molecular docking method has been proven.

Figure 3. Validation results of the molecular docking method (green: initial native ligand, red: redocking native ligand)

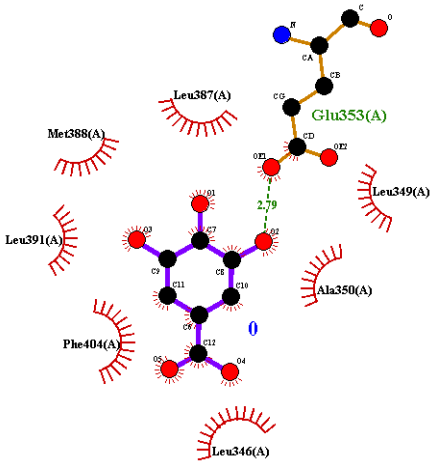
The pomegranate peel polyphenolic activity as anti-breast cancer can be measured based on their estrogen-α receptor inhibitory through molecular docking. The ability of ER-α inhibitory is analyzed through low binding energy and interactions between ligand compound and the active site of ER-α protein. The bond energy influences complex conformation stability, where the lower bond energy, the more stable the complex formed (Muttaqin et al., 2020). In Table 1 it can be seen that the four polyphenol compounds that have been tested have varying bond energies and all of them have negative values from -5.7 to -9.0 kcal/mol. The chlorogenic acid compound has the lowest bond energy (-9.0 kcal/mol), while the highest bond energy is the gallic acid (-5.4 kcal/mol). The binding energy of polyphenolic compounds is also lower than tamoxifen which has been used as an anti-breast cancer drug. This shows that the complex between polyphenolic compounds and the active site of the ER-α protein is more stable than tamoxifen. Therefore, pomegranate peel polyphenolic has a higher ER-α inhibitory potential compared to tamoxifen.

The potential of polyphenolic compounds as breast anticancer is also supported by ligand interaction with the active site of the estrogen-α receptor. Ligand-protein interactions such as hydrogen bonds, hydrophobic interactions, and π-π stacking can strengthen the complex formed between the ligand and ER-α (Stanzione et al., 2021). Based on the LigPlot analysis (Figure 5), it can be seen that the four polyphenolic compounds have quite good interactions compared to tamoxifen. Overall, polyphenols can interact by hydrogen bonds to amino acid residues on the ER-α active site. Chlorogenic acid have the most hydrogen bonds with ER-α active site residue (Leu387, Arg394, Glu353, His524 and Gly521).

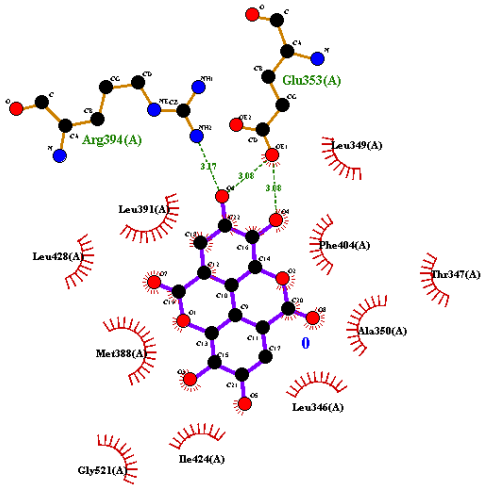
Tabel 1. Results of molecular docking of pomegranate peel polyphenolic against the estrogen-α receptor

Compounds	Energy (kcal/mol)	Hydrogen bonds	Interactions hydrophobic
Gallic acid	-5,7	Glu353 (2,97 Å)	Leu387, Leu391, Met388, Phe404, Leu346, Ala350, Leu 349
Caffeic acid	-6,3	Glu353 (2,92 Å), Arg394 (3,16 Å)	Ala350, Leu346, Leu349, Leu525, Leu387, Leu384
Ellagic acid	-9,0	Arg394 (3,17 Å), Glu353 (3,08 Å), Glu353 (3,08 Å)	Leu391, Leu428, Met388, Gly521, Ile424, Leu349, Phe404, Thr347, Ala350, Leu346
Chlorogenic acid	-8,6	Leu387 (3,22 Å), Arg394 (3,33 Å), Glu353 (2,87 Å), Glu353 (2,72 Å), His524 (3,01 Å) Gly521 (3,16 Å) Gly521 (2,91 Å)	Leu391, Leu349, Leu384, Trp383, Leu525, Phe404, Ala350, Met421, Met388, Ile421
Tamoxifen	-4,9	-	Arg394, Leu391, Glu353, Leu387, Leu349, Phe404, Thr347, Ala350, Leu346, Leu525, His524, Met343, Met421, Leu384, Gly521, Ile424, Met388, Leu428

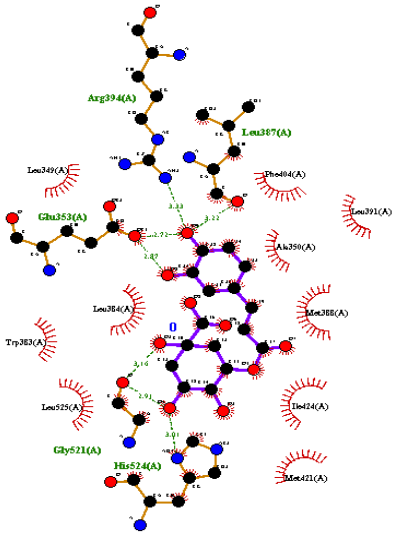
Caffeic acid and ellagic acid can interact via hydrogen bonds with two amino acid residues Glu353 and Arg394, while gallic acid only interacts with the amino acid residue Glu353. Even though the polyphenol compounds have carboxylic acid and ester groups, the hydrogen bonds formed between the ligand and the ER-α protein are only through the phenol group. The phenol group can act as a hydrogen bond donor or acceptor with the active site of estrogen-α receptor. Based on the results of the hydrogen bond interaction between polyphenol compounds and ER-α protein, it can be said that the phenol group in polyphenol compounds plays an important role in increasing its inhibitory activity against the estrogen-α receptor.



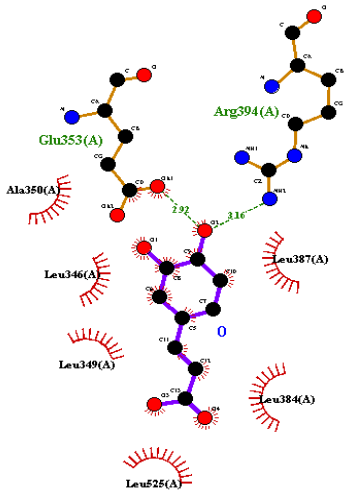
Gallic acid



Ellagic acid



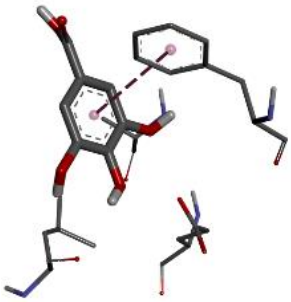
Chlorogenic acid



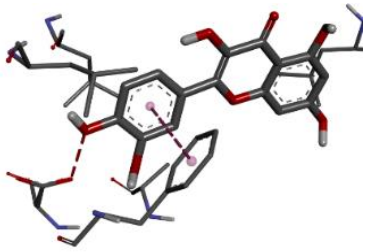
Caffeic acid

Figure 4. Interaction of polyphenolic compounds with ER-α active site

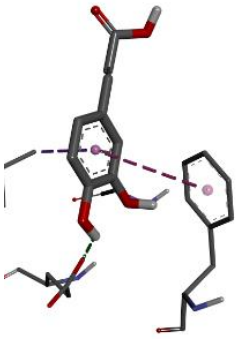
Hydrophobic interactions such as van der Waals and π-π stacking also affect the complex stability between ligand and protein. It can be seen that the four polyphenolic compounds are able to interact hydrophobically with six to ten amino acid residues of the ER-α active site (Figure 4). The inhibitory ability of polyphenol compounds is also supported by π-π stacking interactions between polyphenol aromatic rings and Phe404 amino acid (Figure 5). The anticancer potential of pomegranate peel polyphenolics is also supported by in vitro tests of several pomegranate peel extracts against cancer cells. Polyphenols can induce apoptosis and inhibit the proliferation of cervical cancer cells (Teniente et al., 2023). Ethanol extract from pomegranate peel which is rich of polyphenols also has high toxicity against colorectal cell proliferation (Habchi et al., 2023). Based on the molecular docking results, it can be said that the pomegranate peel polyphenolic compounds have the potential to inhibit estrogen-α receptors. Therefore, the pomegranate peel polyphenolic compounds have the potential to be used as breast anticancer candidates.



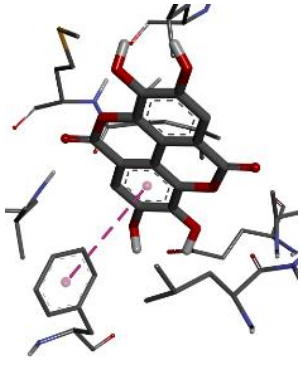
Gallic acid



Chlorogenic acid



Caffeic acid



Ellagic acid

Figure 5. π-π stacking interaction of polyphenolic compounds with ER-α

CONCLUSION

The molecular docking results of polyphenolic compounds against the estrogen-α receptor show that pomegranate peel polyphenolic compounds have the potential to anti-breast cancer. besides having a lower binding energy affinity than tamoxifen (-5.7 to -9,0 kcal/mol), pomegranate peel polyphenolic compounds also bind well to the estrogen-α receptor active site through hydrogen bonds, hydrophobic interactions, and π-π stacking. In vitro and in vivo testing of these polyphenolic compounds is very necessary for further validation of in silico test results.

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