Potency of *Vigna angularis* against ERα through *in silico* studies

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*Vigna angularis*, from the legume family, contains phytoestrogens. Phytoestrogens can activate estrogen receptors and are safer than estrogen. The study aims to analyze the potential of *Vigna angularis* as an activator of estrogen receptor-α (ERα) through *in silico* studies. The analysis of molecular docking used SAR (Structure Activity Relationship). A previous study has shown that *Vigna angularis* contains isoflavones such as Genestein (0.5%), daidzin (14.9%), glycitein (25.8%), formononetin (13%), and biochanin A (45.5%). The results of molecular docking to ERα show that genestein has a free energy binding value of -9.3 and the same amino acid structure, with a control level over estrogen of 66%. In conclusion, *in silico* studies have shown that genestein from *Vigna angularis* is the main component that activates the ERα.

**Key word**: *Vigna angularis*, phytoestrogen, and estrogen receptor-α

**INTRODUCTION**

The use of estrogen as a hormone replacement therapy (HRT) is known to cause many side effects, such as an increased risk of endometrial and ovarian cancer (Beral et al., 2005; Beral et al., 2007). This condition leads to an increase in the consumption of phytoestrogen supplements as HRT agents, as these are safer (Patisaul et al., 2010).

*Vigna angularis*, from the legume family, is known to contain isoflavones such as genestein (Lee et al., 2005). Genestein is the isoflavone with the highest bioactivity, because genestein has a structure that is similar to estrogen and has more hydroxyl groups than other types of isoflavones. Genestein has 2 hydroxyl groups at the 5th and 7th bond positions on the A ring and one hydroxyl group at the 4th position of the B ring (Ruffer and Kulling, 2006).

Estrogen is a steroid compound that works in the development and function of female reproductive organs. Estrogen consists of three compounds: estradiol, estrone and estriol. Estradiol is produced by the ovaries from testosterone in women during menarche until the pre-menopause stage. Estrone and estriol are produced by the supra-renal network from androstenedione. The potential of estradiol is stronger than that of estrone and estriol. Estradiol levels will decrease at menopause, associated with a loss of ovarian follicles, while estrone levels become more elevated (Fang et al., 2001).

To cause biological effects, estrogen binds to its receptors. There are two kinds of estrogen receptor: ERα and ERβ. Both include an intracellular receptor type and are antagonistic to each other. The two receptors are distributed differently in women (Lee et al., 2012).

Based on the above facts, this study aims to measure the activation potential of the active ingredient on ERα receptors through an *in silico* study.

**METHODS**

The experiment was carried out by the laboratory to test the potency of physicochemical compounds from *Vigna angularis* as activators of ERα through an *in silico* study. This research was conducted in the Biochemistry Laboratory of the Faculty of Medicine, University of Islam Malang and Bioinformatika of Brawijaya University of Malang.

**Procedure**

The active compound of *Vigna angularis* was determined based on the results of previous studies. *Vigna angularis* is known to contain flavonoids, phenolic compounds, tannins and alkaloids. Analysis of ethanol extracts from *Vigna angularis* using LCMS found the following active compounds: genestein, daidzein, biochanin A, formononetin, and glicitein (Damayanti et al., 2017).

**Potential analysis of *Vigna angularis* for the activation of ERα using computational methods**
3D Structure: The active compounds of *Vigna angularis* were downloaded from the PubChem server with a CID that has been previously recorded. Target proteins were screened using HITPICK. The ERα structure was obtained from PubChem with the ID 1a52. The activator reference estrogen (CID 5757) was taken from PubChem.

Next, the docking process was assessed using the PyRx program (autodock vina) (Dallakyan and Olson, 2015). Docking was specifically achieved with the compound as a ligand and with estrogen as the activator (CID 5757). Selected energy bindings with the smallest or most negative results showed the best complex conformation.

Docking results were stored and visualized using Ligandscout and PyMol (Delano, 2000). The docking results were visualized interactively to obtain interaction data between the two molecules (ERα and activator reference) using LigPlot (Wallace et al., 1995). The LigPlot results show the hydrophobic bonds and hydrogen bonds that occur in the complex. The activation potential is seen from the active site of the ERα that is bound by the active compound.

RESULTS

Target analyses and molecular docking of phytoestrogen to ERα

The results of potential protein measurements from *Vigna angularis* were computed as follows:

1. Genistein (CID 5280961): Potential target protein: estrogen receptor α/β. Accuracy: 100%

2. Biochanin (CID 5280373): Potential target protein: CYP19A1 (aromatase catalyzes the formation of aromatic C18 estrogens from C19 androgens). Accuracy: 100%

3. Glycitein (CID 5317750): Potential target protein: estrogen receptor β. Accuracy: 89.8%

4. Formononetin (CID 5281812): Potential target protein: estrogen receptor β. Accuracy: 89.8%

5. Daidzein (CID 5281708): Target potential for estrogen receptor β with 100% accuracy.

To determine whether the compound can act as an analogue compound of estrogen, it was necessary to compare interactions with those of estrogen binding to ERα.

Table 1. Comparison of the interactions of phytoestrogens with estrogen to ERα.

| LIGAND            | RECEPTOR                        | ENERGY  |
|-------------------|---------------------------------|---------|
| Glycitein (CID 5317750) | Estrogen Receptor α (ID: 1a52) | -8.4    |
| Biochanin (CID 5280373)   | Estrogen Receptor α (ID: 1a52) | -7.0    |
| Genistein (CID 5280961)   | Estrogen Receptor α (ID: 1a52) | -9.3    |
| Formononetin (CID 5280378) | Estrogen Receptor α (ID: 1a52) | -7.0    |
| Daidzein (CID 5281708)    | Estrogen Receptor α (ID: 1a52) | -9.2    |
| Estrogen (CID 5757)       | Estrogen Receptor α (ID: 1a52) | -10.6   |

Based on potential analyses, only genistein has 100% accuracy when binding ERα. Glycitein, formononetin and daidzein have greater accuracy against ERβ. Based on the molecular docking, estrogen as a control has a free energy binding value to ERα of -10.6. Genistein has the lowest free energy binding value against ERα of -9.3 compared to other isoflavones such as glycitein -8.4, biochanin A -7.0, formononetin -7.0, and daidzein -9.2.

The results of molecular docking interactions of some phytoestrogen compounds contained in *Vigna angularis* are as follows:
Figure 1. Interaction between glycitein (CID 5317750) with ERα (ID: 1a52).
Hydrogen bonds: **His524**, Gly521, and Arg394; hydrophobic bonds: **Leu525**, Met421, Ile424, Leu387, Leu391, Glu353, Ala350, Leu346, Phe404, and Leu384.

Figure 2. Interaction between Biochanin A (CID 5280373) and ERα (ID: 1a52).
Hydrogen bonds: **His524**, and Gly521; hydrophobic bonds: Leu384, **Leu525**, Leu387, Arg394, Glu353, **Met388**, **Leu391**, Ile424, and **Phe404**.

Figure 3. Interaction between genestein (CID 5280961) and ERα (ID: 1a52).
Hydrogen bonds: Gly521, **His524**, and Arg394; hydrophobic bonds: Glu353, **Phe404**, Leu346, **Leu525**, **Met388**, Leu384, **Leu391**, **Leu387**, and Leu394.

Figure 4. Interaction between formononetin (CID 5281812) and ERα (ID: 1a52). Hydrogen bonds: **His524**, and Gly521. Hydrophobic bonds: **Leu525**, Leu384, **Phe404**, Glu353, Arg394, **Met388**, **Leu391**, **Leu387**, Ile424, and Met421.
DISCUSSION

Vigna angularis (Willd.), also known as Azuki bean, Adzuki bean or red bean, is a leguminous plant, with dark red or brick-red seeds that are kidney-shaped and larger than green beans (Balai Materia Medika, 2015).

The results of a previous study have shown that Vigna angularis is known to contain flavonoids, phenolic compounds, tannins and alkaloids. Analysis of the ethanol extract of Vigna angularis using LCMS obtained the active compounds genestin (0.5%), daidzein (14.9%), biochanin A (45.5%), glycitein (25.8%) and Formononetin (13%) (Damayanti et al., 2017).

Phytoestrogens have antioxidant, anti-proliferative, anti-cancer, and anti-cholesterol activities. The isoflavones genestin and daidzein are the most well-known types of isoflavones that have an effect on health. In addition, there are other isoflavone groups found in bean crops such as formononetin and biochanin A (Boueä, 2003).

Phytoestrogens (isoflavones) have the ability to activate estrogen receptors 100-500 times lower than estrogens (17-β-estradiol) (Kuiper et al., 1998). Differences in the effects of phytoestrogens in some organs, especially reproductive organs, are influenced by differences in the distribution of receptors and differences in the ability of each type of phytoestrogen (Kuiper et al., 1998).

Potential analysis and molecular docking of phytoestrogen to estrogen receptor α

Computational approaches began to be widely used for the discovery of active ingredients. This process will shorten the time and cost necessary to test active ingredients prior to release on the market in the form of a drug (Srinivasa Rao and Srinivás, 2011). The active compound is predicted to have the ability to bind to the target protein and interact spontaneously.
if it has a free bond energy that is equal to or less than that of the control (Histo, et al., 2014) and is capable of binding to one of the same amino acid residues on the active site as the reference control or inhibitor (Zukrullah, et al., 2012). Hydrogen bond strength is lower than that of covalent bonds, but its existence is very important. An active compound is predicted to have a strong bond to the target receptor if it is able to bind through hydrogen bonding (Zukrullah, et al., 2012).

The results of the analysis of phytoestrogen compounds contained from Vigna angularis showed that genestein acts on ERα and ERβ with 100% accuracy, biochanin A works on CYP19A1 (aromatase catalyzes the formation of aromatic C18 estrogens from C19 androgens) with 100% accuracy, glycitein works on ERβ with 89.8% accuracy, formononetin acts on ERβ with 89.8% accuracy and daidzein (CID 5281708) acts on ERβ with 100% accuracy.

Based on molecular docking results it was found that genestein has the smallest free energy among the phytoestrogens, and has the ability to bind estrogen receptor α through hydrogen bonds on the same amino acid with a control level of 66% and hydrophobic bonds on the same amino acid with a control level of 62.5%. Although other phytoestrogens are able to bind to the same amino acids as controls, their affinity for ERα is very low and their free energy binding is higher than controls; therefore, it is assumed that it is not able to form a stable bond so does not activate ERα.

Phytoestrogens such as genestein and daidzein prefer to interact with ERβ than ERα (Pettersson and Gustafsson, 2001). However, the activity of ERα is more potent than that of ERβ, meaning that even small amounts have a quite significant effect (Foster, 2012). Given at 2.5mg/kgBW, Vigna angularis showed an increasing number of breast ducts and elevated expression ERα in breast tissue (Damayanti et al., 2017), increased uterine weight, wall thickness and the amount of ERα in the uterus of hypo-estrogenic rats. The administration of Vigna angularis at 2.5mg/kgBW is considered safe due to the absence of side effects in breast and uterine tissue (Riani, 2016; Damayanti et al., 2017).

Based on these facts it can be computationally predicted that genestein has the best ability to bind to estrogen receptor α compared to other isoflavones. This was characterized by a targeted work on estrogen receptor α with 100% accuracy and a free energy of -9.8. Also, it has the same amino acids bound by hydrogen bonds type of 66% compared to estrogen control.

References

1. Balai Materia Medika Kota Batu, 2015. Determinasi tanaman kacang merah Vigna angularis.
2. Beral V, Bull D, Reeves G Million women study collaborators. Endometrial cancer and hormone-replacement therapy in the million women study. Lancet. 2005;365:1543–51. [PubMed]
3. Beral V, Bull D, Green J, Reeves G Million Women Study Collaborators. Ovarian cancer and hormone replacement therapy in the million women study. Lancet. 2007;369:1703–10. [PubMed]
4. Bouët S.M., Wiese T.E., Nehls , Burow M.E, Elliott S., Carter C.H., –Wientjes, Shih B.Y., Mc Lachlan J.A, and Cleveland T.E., (2003) , Evaluation of the Estrogenic Effects of Legume Extracts Containing Phytoestrogens, J. Agric. Food Chem. 2003, 51, 2193-2199
5. Dini Sri Damayanti, Alfi Nur Fatimah, Ariani Ratri Dewi (2017), An Increasing Number of Breast Ducts and Estrogen Receptor α (ERα) on the Breast Tissue of Hypo-estrogen Rat Treated with Vigna angularis ethanol extract, Oral presentation, FOKI, Lombok, Indonesia
6. Dallakyan S, Olson AJ. Small-Molecule Library Screening by Docking with PyRx, Methods Mol Biol. 2015;1263:243-50
7. Delano W. L. (2000). The PyMOL Molecular Graphics System. http://www.pymol.org
8. Fang H, Tong W, Shi L, Blair R, Perkins R, et al., (2001). "Structure-activity relationships for a large diverse set of natural, synthetic, and environmental estrogens.". Chem Res Toxicol 14 (3): 280–94. PMID 11258977
9. Foster TC. Role of Estrogen Receptor land β expression and signaling on cognitive function during aging. Hippocampus.2012;22(4):656–69. [PubMed: 21538657]
10. Histo DM., Utomo EP, Himawan T, (2014), Kajian secara in silico terhadap potensi eugenol dan sitronelal sebagai pestisida nabati untuk pengendalian serangga Helopeltis antoni, Kimia Student Journal, Vol.2,No.2,pp. 562-568, Universitas Brawijaya Malang
11. Kuiper GG, Lemmen JG, Carlsson B, Corton JC, Safe SH, van der Saag PT, van der Burg B, Gustafsson JA. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology, 139(10):4252-63.

12. Lee HR, Kim TH, Choi KC. Functions and physiological roles of two types of estrogen receptors, ER-1 and ERβ, identified by estrogen receptor knockout mouse. Lab Anim Res. 2012 Jun;28(2):71-6. doi: 10.5625/lar.2012.28.2.71.

13. Patisaul HB, Jefferson W. The pros and cons of phytoestrogens. Frontiers in neuroendocrinology. 2010;31(4):400-419. doi:10.1016/j.fneur.2010.03.003.

14. Pettersson K, Gustafsson JA. Role of estrogen receptor beta in estrogenaction. Annu. Rev. Physiol 2001;63:165-92.

15. Riani Siti Ulfi, Reza Hakim, Dini Sri Damayanti. Efek Ekstrak Etanol Kacang Merah (Vigna Angularis) terhadap Berat Uterus, Ketebalan Dinding Uterus dan Jumlah Reseptor Estrogen α Uterus Tikus Novergicus Strain Wistar Betina Paska Bilateral Ovariektomi. SKRIPSI.

16. Fakultas Kedokteran Universitas Islam Malang, 2016.

17. Ruffer and Kulling, 2006. Antioxidant Activity of Isoflavones and Their Major Metabolites Using Different in Vitro Assays. J. Agric. Food Chem. 2006, 54, 2926–2931

18. Srinivasa Rao V and Srinivas K, ( 2011), Modern drug discovery process: An in silico approach Journal of Bioinformatics and Sequence Analysis, Vol. 2(5), pp. 89-94.

19. Wallace, R. A. Laskowski, J. M. Thornton. LIGPLOT: (1995) a program to generate -schematic diagrams of protein-ligand interactions. Protein engineering, Vol. 8, No. 2., pp. 127-134, doi:10.1093/protein/8.2.127

20. Zukhurullah M, Aswad M, dan subehan, (2014), Kajian beberapa senyawa antiinflamsi : Docking terhadap Siklooksigenase-2 secara in silico, Majalah Farmasi dan Famakologi, Vol.16, No.1, hal; 37-44