

## Potency of *Vigna angularis* against ER $\alpha$ 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- $\alpha$  (ER $\alpha$ ) 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%), daidzein (14.9%), glycitein (25.8%), formononetin (13%), and biochanin A (45.5%). The results of molecular docking to ER $\alpha$  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 ER $\alpha$ .

Key word: *Vigna angularis*, phytoestrogen, and estrogen receptor- $\alpha$

### 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 5<sup>th</sup> and 7<sup>th</sup> bond positions on the A ring and one hydroxyl group at the 4<sup>th</sup> 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: ER $\alpha$  and ER $\beta$ . 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 ER $\alpha$  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 ER $\alpha$  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 glycitein (Damayanti et al., 2017).

**Potential analysis of *Vigna angularis* for the activation of ER $\alpha$  using computational methods**

**3D Structure:** The active compounds of *Vigna angularis* were downloaded from the Pub

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

Chemserver (<https://pubchem.ncbi.nlm.nih.gov>) with a CID that has been previously recorded. Target proteins were screened using HITPICK. The ER $\alpha$  structure was obtained from (<https://pubchem.ncbi.nlm.nih.gov>) with the ID 1a52. The activator reference estrogen (CID 5757) was taken from <https://pubchem.ncbi.nlm.nih.gov>. 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 (ER $\alpha$  and activator refence) 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 ER $\alpha$  that is bound by the active compound.

## RESULTS

### Target analyses and molecular docking of phytoestrogen to ER $\alpha$

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

1. Genestein (CID 5280961): Potential target protein: estrogen receptor  $\alpha/\beta$ . 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  $\beta$ . Accuracy: 89.8%

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

5. Daidzein (CID 5281708): Target potential for estrogen receptor  $\beta$  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 ER $\alpha$ .

Table 1. Comparison of the interactions of phytoestrogens with estrogen to ER $\alpha$ .

Based on potential analyses, only genestein has 100% accuracy when binding ER $\alpha$ . Glycitein, formononetin and daidzein have greater accuracy against ER $\beta$ . Based on the molecular docking, estrogen as a control has a free energy binding value to ER $\alpha$  of -10.6. Genestein has the lowest free energy binding value against ER $\alpha$  of -9.3 compared to other isoflavones such as glicitein -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:

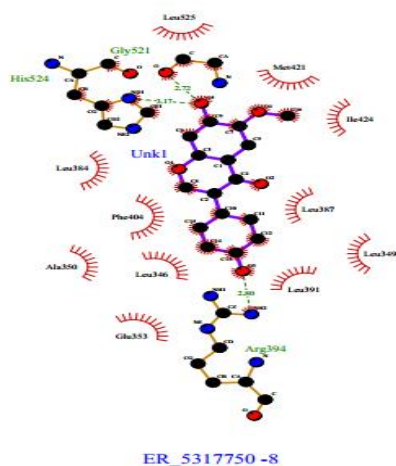


Figure 1. Interaction between glycitein (CID 5317750) with ER $\alpha$  (ID: 1a52).

Hydrogen bonds: **His524**, Gly521, and **Arg394**; hydrophobic bonds: **Leu525**, **Met421**, **Ile424**, **Leu387**, **Leu349**, **Leu391**, **Glu353**, **Ala350**, **Leu346**, **Phe404**, and **Leu384**

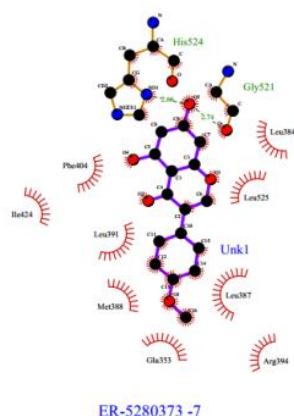


Figure 2. Interaction between Biochanin A (CID 5280373) and ER $\alpha$  (ID: 1a52).

Hydrogen bonds: **His524**, and Gly521; hydrophobic bonds: **Leu384**, **Leu525**, **Leu387**, **Arg394**, **Glu353**, **Met388**, **Leu391**, **Ile424**, and **Phe404**

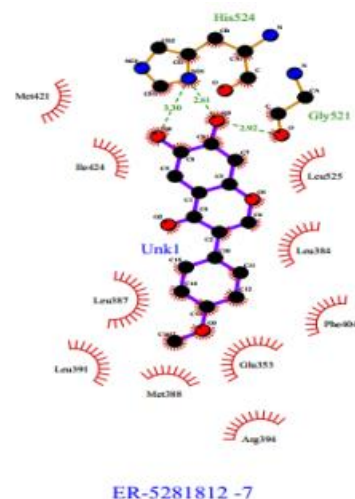


Figure 4. Interaction between formononetin (CID 5281812) and ER $\alpha$  (ID: 1a52). Hydrogen bonds: **His524**, and Gly521. Hydrophobic bonds: **Leu525**, **Leu384**, **Phe404**, **Glu353**, **Arg394**, **Met388**, **Leu391**, **Leu387**, **Ile424**, and **Met421**.

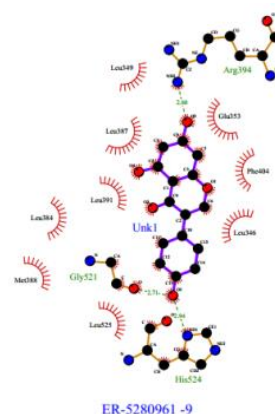


Figure 3. Interaction between genestein (CID 5280961) and ER $\alpha$  (ID: 1a52).

Hydrogen bonds: Gly521, His524, and Arg394; hydrophobic bonds: **Glu353**, **Phe404**, **Leu346**, **Leu525**, **Met388**, **Leu384**, **Leu391**, **Leu387**, and **Leu394**

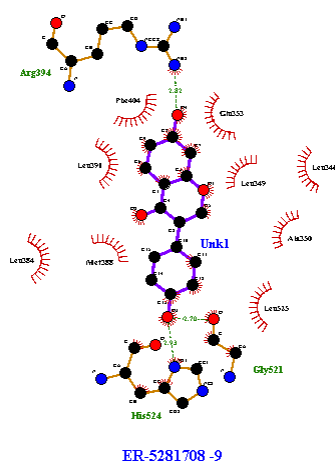


Figure 5. Interaction between Daidzein (CID 5281708) and ER $\alpha$ . Hydrogen bonds: **Arg 394**, **His 524**, and **Gly 521**; hydrophobic bonds: **Phe404**, **Leu391**, **Leu384**, **Met388**, **Glu353**, **Leu349**, **Leu346**, **Ala350**, and **Leu525**

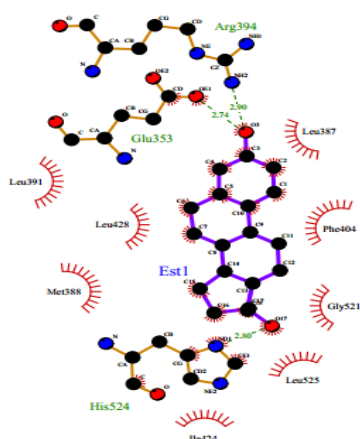


Figure 6. Interaction between estrogen and ER $\alpha$  (ID: 1a52).

Hydrogen bonds: **Arg394**, **Glu353**, and **His524**; hydrophobic bonds: **Leu387**, **Phe404**, **Gly521**, **Leu525**, **Ile424**, **Met388**, **Leu428**, and **Leu391**

Molecular interactions show estrogen bound to the active site of ER $\alpha$  at the amino acid molecules Arg394, Glu353, and His524, bonded through hydrogen bonds and hydrophobically bound amino acid molecules (Leu387, Phe404, Gly521, Leu525, Ile424, Met388, Leu428, and Leu391). Genestein has the ability to bind to ER $\alpha$  through hydrogen bonds on the same amino acid, with a control level of 66% and hydrophobic bonds on the same amino acids with a control level of 62.5%. Glycitein has the ability to bind ER $\alpha$  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 75%. Biochanin A has the ability to bind ER $\alpha$  through hydrogen bonds on the same amino acid with a control level of 33% and hydrophobic bonds on the same amino acid with a control level of 75%. Formononetin has the ability to bind ER $\alpha$  through hydrogen bonds on the same amino acid with a control level of 33% and hydrophobic bonds on the same amino acid with a control level of 75%. Daidzein has the ability to bind ER $\alpha$  through hydrogen bonds on the same amino acid with a control level of 33% and hydrophobic bonds on the same amino acid with a control level of 50%.

## 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 has 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 genestein (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 genestein 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- $\beta$ -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 $\alpha$

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 Srinivas, 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 (Zukhrullah, 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 (Zukhrullah, et al., 2012).

The results of the analysis of phytoestrogen compounds contained from *Vigna angularis* showed that genestein acts on ER $\alpha$  and ER $\beta$  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 ER $\beta$  with 89.8% accuracy, formononetin acts on ER $\beta$  with 89.8% accuracy and daidzein (CID 5281708) acts on ER $\beta$  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  $\alpha$  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 ER $\alpha$  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 ER $\alpha$ .

Phytoestrogens such as genestein and daidzein prefer to interact with ER $\beta$  than ER $\alpha$  (Pettersson and Gustafsson, 2001). However, the activity of ER $\alpha$  is more potent than that of ER $\beta$ , 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 ER $\alpha$  in breast tissue (Damayanti et al., 2017), increased uterine weight, wall thickness and the amount of ER $\alpha$  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  $\alpha$  compared to other isoflavones. This was characterized by a targeted work on estrogen receptor  $\alpha$  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.

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