El-Hayah
JURNAL BIOLOGI
Journal Homepage: http://ejournal.uin-malang.ac.id/index.php/bio/index
e-ISSN: 2460-7207, p-ISSN: 2086-0064

Original research article

Phytoestrogen Activity of Alkaloid Compounds of Butterfly Pea (Clitoria ternatea) Using In Silico Analysis

Fitriyah[1], Anggia Noor Ramadhani[2]

[1]*Biology Study Program, Science and Technology Faculty Universitas Islam Negeri Maulana Malik Ibrahim Malang, Jl. Gajayana No.50, Kota Malang, Jawa Timur 65144, Indonesia*
[2]*Inbio Indonesia*

[*]*Corresponding author*
Email: fitriyahfaizin@bio.uin-malang.ac.id
DOI: 10.18860/elha.v9i4.25516

Article Info

Article history:
Received 30 November 2023
Received in revised form 16 January 2024
Accepted 28 February 2024

Key Word:
Alkaloid
Cllitoria ternatea
Fitoestrogen

Abstract

Estrogen is a hormone involved in the development of sexual and reproductive characteristic, particullary in females. Estrogen is not only important for reproduction, but also in other systems such as the cardiovascular system, neuroendocrine system, immune system and muscoskeletal system in both men and women. The absence of estrogen during the menopause phase is a key factor contributing in the onset of cardiovascular disease. Phytoestrogens are polyphenolic and non-steroidal compounds that have a structure and biological activity similar to 17β-estradiol in humans. Most phytoestrogens are found in flavonoids, isoflavones and also alkaloids whose esterogenic potential has not been widely explored. Butterfly Pea (Clitoria ternatea) is a type of wild creeping plant from the Fabaceae family which contains number of active ingredients, including alkaloids. This research aims to determine the active compounds derived from Butterfly Pea (Clitoria ternatea) alkaloids which have the best potential as phytoestrogens using the in silico methods. A number of ligands which are active compounds derived from alkaloids in Butterfly Pea are linked to estrogen alpha as a receptor using molecular docking. The result of testing six alkaloid derivative compounds from Butterfly Pea indicate that Cephalotaxinone is the most effective candidate for phytoestrogenic use.

1. INTRODUCTION

Estrogen is an important sex steroid hormone that functions primarily in the female reproductive system as well as various systems such as the cardiovascular, nervous, immune and musculoskeletal systems **[1; 2]**. Estrogen is synthesized mainly in the ovaries, although the adrenal glands and adipose tissue also contribute to its production. Estrogen has one of the main functions in regulating the estrous or menstrual cycle in women **[3]**. The main mechanism of action of estrogen is through binding to estrogen receptors (ER) both α and β **[4; 5]**.

Decreased estrogen levels can be caused by various factors, especially menopause. The decrease estrogen during menopause is one of the factors indicating the onset of cardiovascular disease, which is characterized by variations in profile and accumulation of visceral fat **[6]**. Hormonal therapy that can be carried out during the menopause phase is by using exogenous estrogen using Estradiol (E2) **[7]**. However, long-term use of exogenous estradiol needs to be considered because it is a trigger factor for tumor development **[8]**.

Phytoestrogens are polyphenolic and non-steroidal compounds similar to human estrogen originating from plants and are structurally similar to 17β-Estradiol **[9][10]**. Most phytoestrogens are found in plant flavonoids and isoflavones, while alkaloids are very rarely developed as sources of phytoestrogens. The most common reason why alkaloids are not widely explored for their estrogenic properties is because alkaloids are generally considered active plant compounds that have the potential to be toxic. However, several pharmacological studies have shown that several types of plant alkaloids show low toxicity to humans and have potential as a source of new phytoestrogens, including the alkaloid type Erythroidine. Alkaloid. **[11]**.

Butterfly Pea (Clitoria ternatea) is a wild creeping plant belonging to the Fabaceae family, and it possesses various active component, including alkaloid **[12]**. Alkaloids have potential as a new source of phytoestrogens that work like estradiol in the human body. Alkaloids are contained in all parts of plants including roots, stems, leaves, flowers and seeds. Based on this background, it is necessary to study the potential of phytoestrogens derived from alkaloids of Butterfly Pea.

2. MATERIALS AND METHODS

This research based on the In silico method which was carried out in June – October 2021 at the Biology Study Program Laboratory, Faculty of Science and Technology, UIN Maulana Malik Ibrahim Malang..

Ligan Preparation s

Alkaloid derivative compounds that act as ligands are downloaded in 3D via pubChem (http://pubchem.ncbi.nlm.nih.gov) and then saved in SDF format (*sdf). Ligan were optimized in pyRx software.

Receptor Preparation

The 3D structure of ERα was downloaded from the Protein Data Bank (PDB) via http://www.rscb.org and saved in PDB format (*pdb). Receptor separated from the complex ligan and water molecule using PyMol 2.5.1.

ADME Test

The ADME test is carried out to assess the ability of active ingredients to be absorbed, distributed, metabolized and excreted in the body via the SwissADME online software.

Tanimoto Similarity Calculation

Tanimoto Similarity calculations utilizing RDKit were employed for conducting a similarity analysis. This analysis makes it possible to determine the similarity of the structural fingerprints of alkaloid of Butterfly Pea and Estradiol as a comparison.

Compounds that are not similar are not continued in the docking stage.

Molecular Docking and Visualization

Molecular docking was carried out to analyze the binding potential of ligands and receptors based on binding energy using pyRx autodock vina software. The docking results were visualized using Discovery Studio software to see the similarity of the docking area with the control ligand.

Data Analysis Method

The data obtained is presented in a qualitative descriptive manner and analyzed using several criteria as follows:

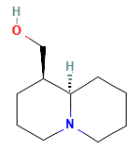
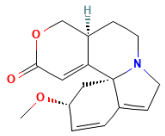
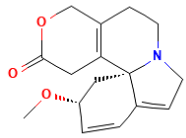
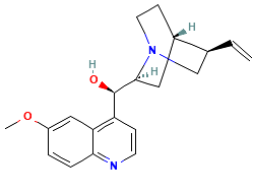
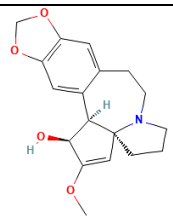
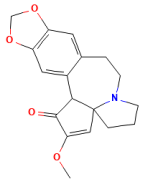
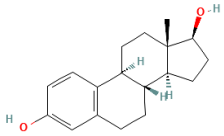
a) Drug similarity analysis shows that the compound has a good ability to be absorbed in the gastrointestinal tract and can be applied orally as indicated by compliance with ADME criteria.
b) Has a high value of structural similarity to control.
c) Has a low binding energy in the results of molecular docking analysis.
d) Binds precisely to the control drug binding area and interactions involving key residues.

3. RESULTS and DISCUSSION

Identification Ligan and Receptor

In silico analysis was carried out by preparing ligands consisting of: Lupinine (CID: 91462), alpha Erythroidine (CID: 441076), beta Erythroidine (CID: 10074), Quinine (CID: 3034034), Cephalotaxine (CID: 65305) and Cephalotaxinone (CID : 628613) with a comparison ligand in the form of Estradiol (CID: 5757) (Table 1). Ligand structures were downloaded from NCBI's PubChem Compound (https://www.ncbi.nlm.nih.gov/pccompound) . The receptor used is estrogen receptor alpha (PDB ID: 1X7R) which was downloaded from https://www.rcsb.org/.

Table 1. Ligan

No	Name of Ligan	Chemical Structure
1	Lupinine	
2	Alpha Erythroidine	
3	Beta Erythroidine	
4	Quinine	
5	Cephalotaxine	
6	Cephalotaxinone	
7	Estradiol	

Pharmacokinetic Analysis

Pharmacokinetic analysis to assess absorption ability in the gastrointestinal system is as presented in table 2. Compounds

that meet the requirements are then subjected to structural similarity analysis through tanimoto similarity analysis. Compounds that have structural similarities to estradiol will then be analyzed using molecular docking with the results as in table 3.

Table 2. The Result of ADME Test

Ligan	Pharmakokinetik		Druglikeness	
	GI Absorbtion	Log Kp (cm/s)	Lipinski	Bioavailability score
Lupinine	High	-6,45	Yes	0.55
Alpha Erythroidine	High	-7,68	Yes	0.55
Beta Erythroidine	High	-8.09	Yes	0.55
Quinine	High	-6,23	Yes	0.55
Cephalotaxine	High	-8,07	Yes	0.55
Cephalotaxinone	High	-6,66	Yes	0.55

Table 3. Tanimoto Similarity and Binding Affinity Score

No	Ligan	Tanimoto Similarty Score	Binding Affinity Score
1	Estradiol		-10.34
2	Lupinine	0.144	-6.58
3	Alpha Erythroidine	0.250	-8.37
4	Beta Erythroidine	0.239	-7.77
5	Quinine	0.282	-8.26
6	Cephalotaxine	0.362	-7.03
7	Cephalotaxinone	0.349	-8.33

The active compound with a structure most resembling estradiol and exhibiting the lowest binding energy will be visualized in three dimensions to assess if its binding position align precisely with the estradiol binding site, as illustrated in Figure 1.

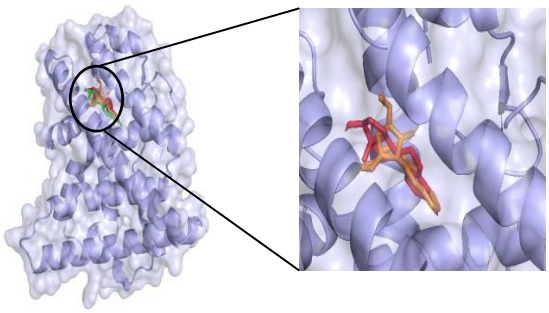


Figure 1.3D Visualizatiom of the molecular docking Ligand and Reseptor Estrogen Alpha. Orange: Cephalotaxinone, Red: Estradiol. Alkaloid can interact on the same side as estradiol

4. DISCUSSION

The results of the pharmacokinetic analysis show that the alkaloid compounds of the Butterfly Pea plant have the ability to be well absorbed in the gastrointestinal tract, which is indicated by the "High" category in the GI Absorption parameter. Meanwhile, the drug similarity results show that the alkaloid compounds are possible to be used as oral drugs as shown in the Lipinski parameters (Table 3). A bioavailability score of 0.55 indicates that the active compound has a bioavailability potential of > 10% **[13]**.

Interaction of Ligan and Receptor

Similarity analysis was carried out using Tanimoto Similarity calculations using RDKit running on Jupyter Notebook. The maximum Tanimoto Similarity score is 1. Through machine learning with the Tanimoto Coefficient approach, it is possible to determine the similarity of the structural fingerprints of the alkaloid compounds of Butterfly Pea and Estradiol as a comparison. Compounds that do not have similarities are not continued in the molecular docking stage.

Molecular docking was performed using Autodock in PyRx 0.9.5. The parameters used

are the Lamacrkian Genetic Algorithm (4.2). The target protein used is Estrogen Receptor Alpha X-Ray Diffraction with ID (PDB: 1X7R), namely Crystal structure of Estrogen Receptor Alpha in complex with Genistein as an agonist control. Genistein was chosen as an agonist because it is one of the main phytoestrogens. Binding with experimental ligand agonist genistein, used for grid box and control. Docking is carried out on the grid box as follows:

1) Grid Center: X: 15,857 Y: 32,236 Z: 21,496
2) Number of Points: X: 34 Y: 43 Z: 50
3) Spacing: 0.375 Angstrom (Å)

Based on the docking results, alpha erythroidine (-8.37) is the compound with the best binding affinity value, compared to other compounds. However, if you look at the Tanimoto similarity score, then cephalotaxinone is the best compound. Because it has a Tanimoto similarity score with estradiol (0.349) and a fairly high binding affinity (-8.33) **[14]**.

The molecular docking results are then analyzed for their bonds in the 2-dimensional structure to see the amino acids that bind to the docking results. This analysis was carried out using discovery studio software. The results of the analysis of the interaction of estradiol and cephalotaxinone with the estrogen receptor show that there are similar amino acids which are the key to binding, namely MET421 in the van der Walls bond and LEU 384 in the hydrophobic bond. MET421 and LEU384 are the binding cavity ligand positions in ESR1 **[15]**. Cephalotaxinone is able to interact on this side, such as the interaction of estrogen receptors with estradiol. So it is possible that cephalotaxonine can function as a phytoestrogen based on the results of the Tanimoto similarity and binding affinity prediction scores .

5. CONCLUSION

From the research results it can be concluded that of the six alkaloid compounds of the Butterfly Pea plant consisting of Lupinine, Alpha Erytrhroidine, Beta Erythroidine, Quinine, Cephalotaxine and Cephalotaxinone, Cephalotaxinone is the most promising candidate as a phytoestrogen because it has a structure and binding ability with values close to estradiol.

6. REFERENCES

1. Yoh, Kenta., Kazuhiro Ikeda, Kuniko Horie and Satoshi Inoue. 2023. Roles of Estrogen, Estrogen Receptors, and Estrogen-Related Receptors in Skeletal Muscle: Regulation of Mitochondrial Function. International Joirnal of Molecular Science 24: 1853
2. Hamilton, K. J., Arao, Y., & Korach, K. S. (2014). Estrogen hormone physiology: Reproductive findings from estrogen receptor mutant mice. Reproductive Biology, 14(1), 3–8. https://doi.org/10.1016/j.repbio.2013.12.002
3. Fuentes, Nathalie and Paticia Silveyra. 2019. Estrogen Receptor Signaling Mechanism. Adv Protein Chem Struct Biol. 2019 ; 116: 135–170. doi: 10.1016/bs.apcsb.2019.01.001
4. Couse, J. F., Lindzey, J., Grandien, K., Gustafsson, J.-Å., & Korach, K. S. (1997). Tissue Distribution and Quantitative Analysis of Estrogen Receptor-α (ERα) and Estrogen Receptor-β (ERβ) Messenger Ribonucleic Acid in the Wild-Type and ERα-Knockout Mouse. Endocrinology, 138(11), 4613–4621. https://doi.org/10.1210/endo.138.11.5496
5. Nilsson, S., Mäkelä, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., Enmark, E., Pettersson, K., Warner, M., & Gustafsson, J.-Å. (2001). Mechanisms of Estrogen Action. Physiological Reviews, 81(4), 1535–1565. https://doi.org/10.1152/physrev.2001.81.4.1535
6. Hemalatha, V. T., Julius, A., Kumar, S. P. K., Vijayalakshmi, L., & Narayanan, S. (2019). Estrogen deficiency during

menopause and its management: A current update. Drug Invention Today, 12(2), 7.
7. Fait, T. (2019). Menopause hormone therapy: Latest developments and clinical practice. Drugs in Context, 8, 1–9. https://doi.org/10.7573/dic.212551
8. Yue, W., Wang, J.-P., Li, Y., Fan, P., Liu, G., Zhang, N., Conaway, M., Wang, H., Korach, K. S., Bocchinfuso, W., & Santen, R. (2010). Effects of estrogen on breast cancer development: Role of estrogen receptor independent mechanisms. International Journal of Cancer, 127(8), 1748–1757. https://doi.org/10.1002/ijc.25207
9. Nikolić, I., Savić-Gajić, I., Tačić, A., & Savić, I. (2017). Classification and biological activity of phytoestrogens: A review. Advanced Technologies, 6(2), 96–106. https://doi.org/10.5937/savteh1702096N
10. Poluzzi, Elisabetta., Carlo Piccinni., Emanuel Raschi, Angela Rampa, Maurizio Recanatini and Fabrizio DE Ponti. 2014. Phytiestrogen in Postmenopause: The State of the Art from a Chemical, Pharmacological and Regulatory Perspective. Current Medical Chemistry 21: 417-346
11. Djiogue, S., Halabalaki, M., Njamen, D., Kretzschmar, G., Lambrinidis, G., Hoepping, J., Raffaelli, F., Mikros, E., Skaltsounis, A.-L., & Vollmer, G. (2014). Erythroidine Alkaloids: A Novel Class of Phytoestrogens. Planta Medica, 80(11), 861–869. https://doi.org/10.1055/s-0034-1382861
12. Afrianto, Whisnu Febry , Fadila Tamnge, Laeli Nur Hasanah. 2020. Review: A relation between ethnobotany and bioprospecting of edible flower Butterfly Pea (Clitoria ternatea) in Indonesia. ASIAN JOURNAL OF ETHNOBIOLOGY Volume 3, Number 2, November 2020 E-ISSN: 2580-4510 Pages: 51-61
13. Zhu, J., Wang, J., Yu, H., Li, Y., & Hou, T. (2011). Recent Developments of In Silico Predictions of Oral Bioavailability. Combinatorial Chemistry & High Throughput Screening, 14(5), 362–374.
14. Manas ES, Xu ZB, Unwalla RJ, Somers WS. Understanding the selectivity of genistein for human estrogen receptor-beta using X-ray crystallography and computational methods. Structure. 2004 Dec;12(12):2197-207. doi: 10.1016/j.str.2004.09.015. PMID: 15576033
15. Paterni I, Granchi C, Katzenellenbogen JA, Minutolo F. Estrogen receptors alpha (ERα) and beta (ERβ): subtype-selective ligands and clinical potential. Steroids. 2014;90:13-29. doi:10.1016/j.steroids.2014.06.0