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EFFECT OF CLITORIA TERNATEA ON FOLLICULOGENESIS POLYCISTIC OVARY SYNDROME: IN SILICO STUDY ON LUTEINIZINNG HORMONE RECEPTOR

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ABSTRACT

Polycystic Ovary Syndrome (PCOS) is a complex hormonal disorder involving dysregulation of luteinizing hormone (LH) and its receptor, leading to menstrual irregularities and anovulation. This study evaluates the potential of Clitoria ternatea, a traditional medicinal plant, in modulating LH receptor activity through in silico analysis. Methods: Active compounds from Clitoria ternatea, including flavonols (kaempferol, isorhamnetin), flavones (baicalein, luteolin, apigenin), phenolic acids (chlorogenic, protocatechuic, gallic), and epicathechin, were identified via PubChem. Lipinski's rule and LD50 classifications were used to assess drug-like properties and toxicity. Bioactivity was predicted using PASS Online, SwissTarget Prediction, PharmMapper, and SuperPred. The LH receptor's 3D structure was modeled using Swiss Model and validated with Procheck and Errat Check. Molecular docking studies using PyRx assessed binding affinities between the compounds, spironolactone, flutamide, and the LH receptor. Results: Docking results revealed strong binding affinities of Clitoria ternatea compounds with the LH receptor, particularly phenolic acids and flavonoids, showing comparable or better interactions than spironolactone and flutamide. These interactions suggest a potential role in restoring hormonal balance and ovulatory function. The study highlights the therapeutic potential of Clitoria ternatea for PCOS management. Its compounds demonstrate significant LH receptor interactions, offering a promising basis for further research. Conclusion: Clitoria ternatea shows promise as a natural therapeutic candidate for PCOS. Future in vitro, in vivo, and clinical trials are needed to validate these findings

Keywords*: Clitoria Ternatea, Luteinizing Hormone Receptor, Polycistic Ovary Syndrome*

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is one of the most common hormonal abnormalities occurring in women around the world and has a serious impact on women's reproductive health. One of the main characteristics of PCOS is changes in the menstrual cycle and disruption in the ovulation process(Goldrat & Delbaere, 2018). To understand and address this disorder, many studies have been conducted to identify the role of hormones and receptors in the regulation of folliculogenesis, a very important process in ovulation and ovarian follicle development. Luteinizing hormone (LH) is one of the hormones that plays a central role in ovarian follicle regulation. LH binds to the LH receptors in the ovary follicles to trigger ovulation. In women with PCOS, changes occur in the regulation of LH and its receptors, which can affect ovarian follicle processes. Increased LH levels occurring in PCOS can lead to hyperandrogenism, irregular follicular growth, and the inability to ovulate normally(Esparza et al., 2020; Stevenson et al., 2022).

One of the medicinal plants that is increasingly attracting attention in the treatment of PCOS is Clitoria ternatea. It has been traditionally used in treating a variety of health conditions, and recent research shows the potential of this plant as a drug candidate in the management of PCOS. The Clitoria ternatea contains active compounds that can

interact with the hormonal system in the body(Adisakwattana et al., 2020; Taufik & Ainiyah, 2021). Inflammatory conditions and oxidative stress can play a role in the development of PCOS(Shaaban et al., 2019). This plant can help reduce the inflammation and oxidative damage associated with PCOS(Deb Barma et al., 2022). Insulin existence is a common characteristic in women with PCOS and can contribute to metabolic problems(Purwar & Nagpure, 2022). Clitoria ternatea has shown potential for increasing insulin sensitivity, which can help control blood sugar levels(Kalaiselvi et al., 2021). Obesity is often associated with PCOS and can worsen its symptoms(Shaaban et al., 2019). Some studies suggest that Clitoria ternatea can help with weight management through its effects on metabolism and feeling full(Wang et al., 2022). Clitoria ternatea has adaptogenic properties that can help reduce stress and improve mental well-being(Margret et al., 2019; Mittal et al., 2021). Clitoria ternatea has proven to offer potential as an exciting drug candidate in the management of PCOS.

The in-silico research on Clitoria ternatea as a PCOS drug has given promising results in understanding the molecular interactions between the compounds in these plants and the biological targets associated with PCOS. The results of this study could pave the way for further experimental research, including clinical trials in humans. With the development of computer technology and increasingly sophisticated molecule simulation methods, the in-silico study can be a powerful tool in identifying and testing potential drug candidates in PCOS treatment. Thus, silica research about Clitoria ternatea offers hope as an important alternative to develop better therapies for women suffering from PCOS, helping them achieve optimal reproductive health(Tanchuk et al., 2016).

METHODS

Through a study of the literature, Clitoria ternatea compounds are gathered, and their efficacy as therapeutic candidates is evaluated. The compound is examined for medication compatibility and toxicity based on the Lipinski and LD50 classifications (Table.1). The test compounds included kaempferol and isorhamnetin, flavanols (epicathechin), flavones (baicalein, luteolin, and apigenin), and phenolic acids (chlorogenic, protocatechuic, and gallic) from the Clitoria ternatea(Makasana et al., 2017).

Table 1. Table of Drug Similarities (Druglikeness) and Toxicity of Clitoria Ternatea Compounds

Compounds		*Druglikeness* (Lipinski)	LD50 (mg/kg)	Toxicity Class
Flavonol	Kaempferol	Yes	3919	5
	Quercetin	Yes	159	3
	Myricetin	Yes	159	3
	Isorhamnetin	Yes	5000	5
Anthocyanin	Ternatin	Yes	5000	5
	Petunidin	Yes	5000	5
	Peonidin	Yes	5000	5
	Delphinidin	Yes	5000	5
	Malvidin	Yes	5000	5
	Cyanidin	Yes	5000	5
Flavonols	Epicatechin	Yes	10000	6
Flavones	Scutellarin	No	5000	5
	Baicalein	Yes	3919	5
	Luteolin	Yes	3919	5
	Apigenin	Yes	2500	5
	Chlorogenic	Yes	5000	5
	Protocatechuic	Yes	2000	4
	Gallic	Yes	2000	4
	Anthraquinone	Yes	5000	5

PubChem ID, canonical SMILES, and 3D chemical structures of Clitoria ternatea were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov). Analysis of the biological activity of compounds utilizing PassOnline (http://www.way2drug.com/passonline) for natural compound potential The SwissTarget Prediction database (http://www.swisstargetprediction.ch), the Pharm Mapper database (https://www.lilab-ecust.cn), and the SuperPred database (https://prediction.charite.de) were used to forecast the protein targets. Using the organism "Homo sapiens" and the string-db database (https://string-db.org), it will be possible to explore the bioactive components and potential processes of Clitoria ternatea on PCOS. Target protein structure is modelled in three dimensions using the Swiss model (https://swissmodel.expasy.org). Using Saves-V6.0 (http://saves.mbi.ucla.edu),

structures and molecular models have been validated and improved in quality. Procheck and Errat Check PyMOL is used to see the protein model's structure in three dimensions. The molecular binding affinity was investigated with PyRx(Trott & Olson, 2010). The binding affinity of phenols found in the petals of Clitoria ternatea, such as flavonoid, phenolic acid, and anthraquinone, was investigated using the protein LH receptor. The binding affinity of spironolactone and flutamide (all of which are used to treat hyperandrogenism) with the LH receptor was also investigated to compare the efficacy of phenol in the treatment of PCOS.

RESULT

The strength of the link between the ligand and the target is measured by binding affinity scores, which also indicate how strong or weak the contact is. Understanding the potential of molecular treatment, drug development, and the creation of novel compounds requires knowledge of the binding affinity score(Tanchuk et al., 2016). Binding affinity score of Clitoria ternatea compound sees table. 2, and binding affinity score control drug sees table. 3.

Table 2. Binding affinity score compounds of Clitoria ternatea flowers on LH Receptor

Compounds	Binding affinity score (rmsd/ub & rmsd/lb=0.0)
Kaempferol	-8.2
Isorhamnetin	-6.6
Ternatin	-6.3
Petunidin	-6.7
Peonidin	-6.7
Delphinidin	-7.0
Malvidin	-6.7
Cyanidin	-6.5
Epicatechin	-6.8
Baicalein	-7.1
Luteolin	-7.2
Apigenin	-6.8
Chlorogenic	-6.3
Protocatechuic	-5.5
Gallic	-6.1
Anthraquinone	-6.8

Table 3. Binding affinity score of Control drugs on LH Receptor

Compounds	Binding affinity score (rmsd/ub & rmsd/lb=0.0)
Cyproterone Acetate	-7.1
Flutamide	-6.5
Spironolactone	-7.4

Kaempferol exhibits a binding affinity score of -8.2, indicating a strong interaction with the LH receptor. This suggests that Kaempferol may potentially modulate LH receptor activity. Isorhamnetin with a binding affinity score of -6.6, Isorhamnetin also shows a notable affinity for the LH receptor. This could imply a role in influencing LH receptor function. Ternatin displays a binding affinity score of -6.3, indicating a moderate interaction with the LH receptor. Its potential impact on LH receptor activity warrants further investigation. Petunidin, Peonidin, Malvidin, Cyanidin compounds share similar binding affinity scores around -6.7, suggesting a comparable level of interaction with the LH receptor. Delphinidin with a binding affinity score of -7.0, Delphinidin demonstrates a relatively stronger interaction with the LH receptor, making it a potential candidate for LH receptor modulation. Epicatechin, Apigenin compounds exhibit moderate binding affinities of -6.8, indicating a potential influence on LH receptor activity. Baicalein shows a binding affinity score of -7.1, suggesting a relatively strong interaction with the LH receptor. This compound may be explored for its impact on LH receptor modulation. Luteolin has a binding affinity score of -7.2, indicating a strong interaction with the LH receptor. This suggests its potential significance in LH receptor-related processes. Chlorogenic with a binding affinity score of -6.3, Chlorogenic exhibits a moderate interaction with the LH receptor, warranting further investigation into its functional implications. Protocatechuic, Gallic compounds have binding affinity scores of -5.5 and -6.1, respectively. While Protocatechuic shows a lower affinity, Gallic exhibits a moderate affinity, suggesting

potential roles in LH receptor modulation. Anthraquinone has a binding affinity score of -6.8, indicating a moderate interaction with the LH receptor. Further studies may elucidate its impact on LH receptor function.
Cyproterone Acetate, this control drug shows a binding affinity score of -7.1, indicating a strong interaction with the LH receptor. Cyproterone Acetate is known for its antiandrogenic properties, and its affinity for the LH receptor aligns with its endocrine regulatory effects. Flutamide exhibits a binding affinity score of -6.5, suggesting a moderate interaction with the LH receptor. As an antiandrogen, its binding affinity may contribute to its therapeutic effects. Spironolactone with a binding affinity score of -7.4, Spironolactone demonstrates a strong interaction with the LH receptor. This aligns with its antiandrogenic and anti-mineralocorticoid properties, indicating potential endocrine regulatory effects.
The binding affinity scores provide insights into the potential interactions between Clitoria ternatea flower compounds and the LH receptor, 2D molecular docking see Figure 1-19. Compounds like Kaempferol, Delphinidin, Luteolin, and Baicalein exhibit strong affinities, suggesting their potential as LH receptor modulators. Further experimental validation is essential to confirm these in-silico findings and explore the functional implications of these interactions. Additionally, comparing these scores with control drugs such as Cyproterone Acetate and Spironolactone provides context and highlights the potential pharmacological relevance of Clitoria ternatea compounds in endocrine regulation.

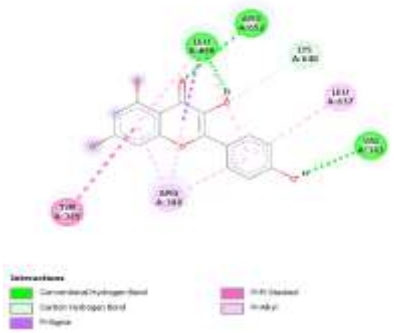

Figure 1. Molecular Docking LHR-Kaempferol

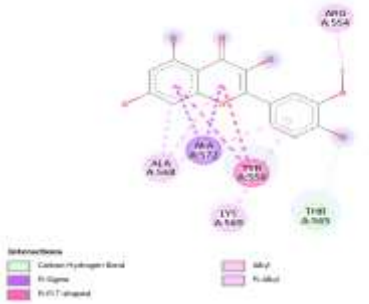

Figure 2. Molecular Docking LHR -Isorhamnetin

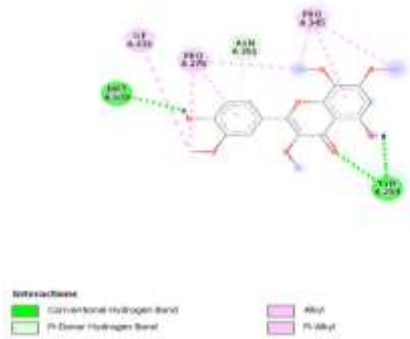

Figure 3. Molecular Docking LHR -Ternatin

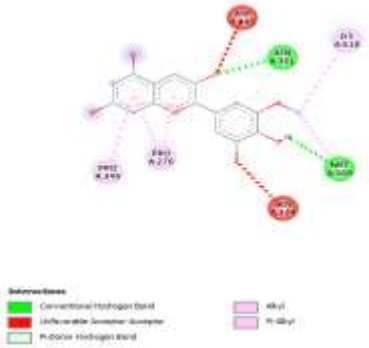

Figure 4. Molecular Docking LHR -Petunidin

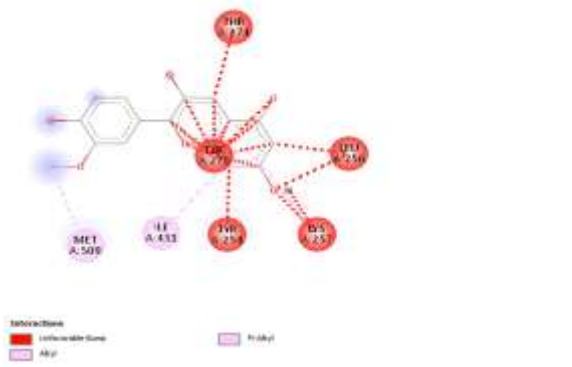

Figure 5. Molecular Docking LHR -Peonidin

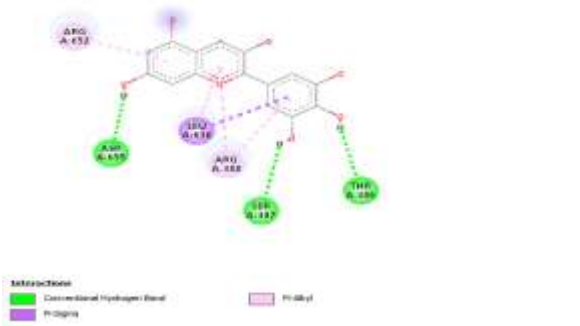

Figure 6. Molecular Docking LHR -Delphinidin

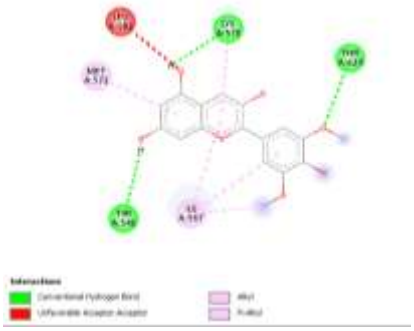

Figure 7. Molecular Docking LHR -Malvidin

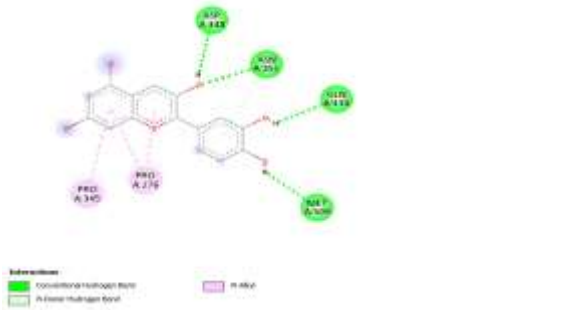

Figure 8. Molecular Docking LHR -Cyanidin

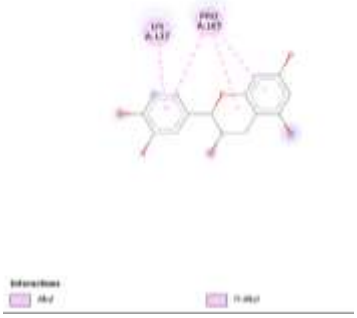

Figure 9. Molecular Docking LHR -Epicatechin

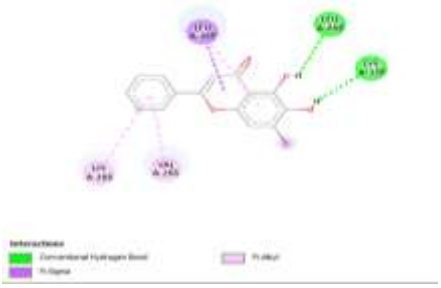

Figure 10. Molecular Docking LHR -Baicalein

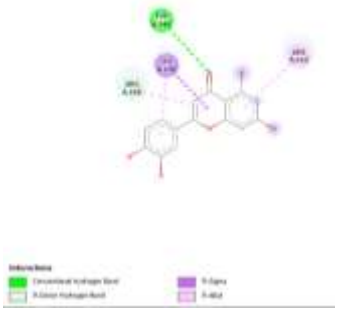

Figure 11. Molecular Docking LHR -Luteolin

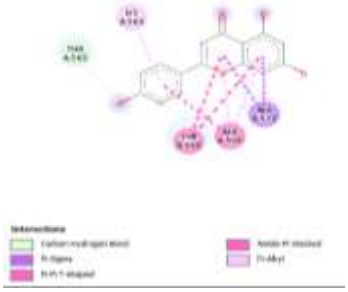

Figure 12. Molecular Docking LHR -Apigenin

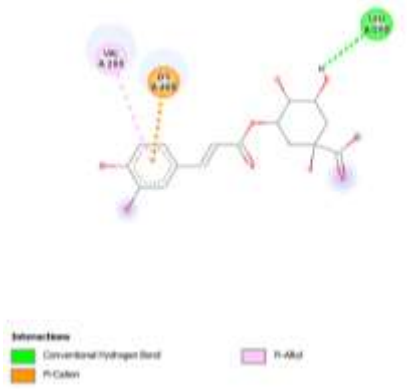

Figure 13. Molecular Docking LHR - Chlrogenic

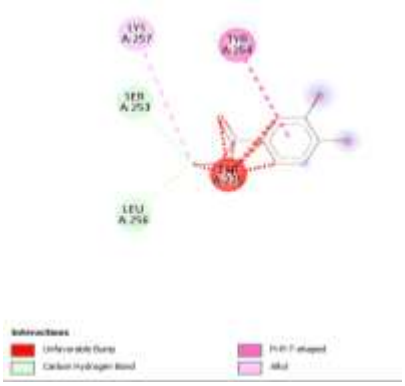

Figure 14. Molecular Docking LHR - Protocatechuic

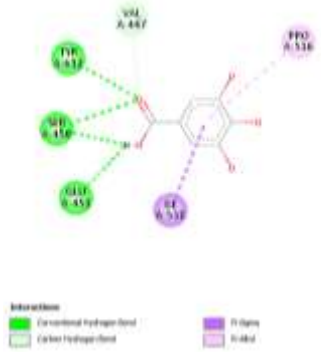

Figure 15. Molecular Docking LHR -Gallic

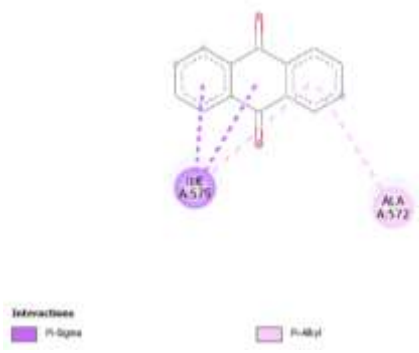

Figure 16. Molecular Docking LHR - Anthraquinone

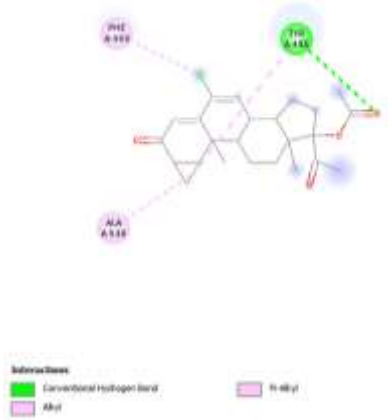

Figure 17. Molecular Docking LHR- Cyproterone A

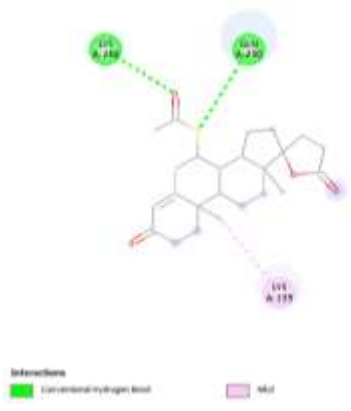

Figure 18. Molecular Docking LHR - Spironolactone

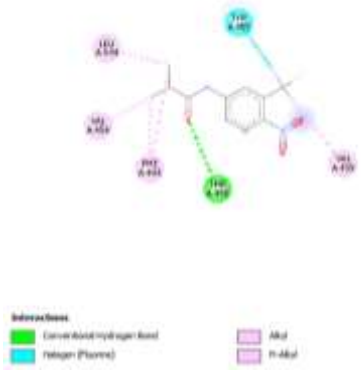

Figure 19. Molecular Docking LHR -Flutamide

DISCUSSION

Luteinizing hormone receptors (LH) play an important role in regulating hormonal imbalances and the symptoms associated with this condition. PCOS is a complex hormonal disorder that often involves disorders in the regulation of LH and its receptors. One of the main characteristics of PCOS is hyperstimulation of LH, which means an increase in the level of the hormone LH in the blood. This occurs due to an imbalance between the gonadotropin-releasing hormone (GnRH) produced by the hypothalamus and the response of gonadotrophins (LH and FSH) by the pituitary gland(Saadia, 2020). LH binds to the luteinizing hormone (LHR) receptors located on the surface of ovarian cells. This interaction activates an intracellular signal path that sends a signal into the cell and induces a variety of responses(Negrón & Radovick, 2020; Stevenson et al., 2022).

Binding affinity suggests that the compounds in Clitoria ternatea have the potential to interact strongly with the LH receptor, which can have important implications for the regulation of menstrual and ovulation cycles in women with PCOS. The high binding affinity between the clitoria ternatea compound and LH receptor may indicate the potential of these compounds in regulating LH activity and, consequently, menstrual cycles and ovulation. This can provide a basis for the development of new therapies or supplements that can help manage the symptoms of PCOS associated with abnormal LH(Ashma & Esther Rani, 2022; Tanchuk et al., 2016).

The molecular docking results showed that the entire ligand of the tested Clitoria ternatea compound has a binding affinity and a good bond between the LH receptor protein and the ligand. Compared to the control drug Spironolactone, the compound Kaempferol has a greater binding affinity to the LH receptor protein. The compounds Kaempferol, Baicalein, and Delphinidin have greater binding and are equivalent to the drug control cyproterone acetate. In addition, other compounds that are equivalent to flutamide control drugs except ternatin, chlorogenic, protocatechuic, and gallic have lower binding affinity values than control drugs. Petunidin, Peonidin, Malvidin, and protocatechuic compounds have an inconsistency of interaction between proteins and ligands, characterized by the presence of unfavorable bonds between bulbs as well as unfavorable acceptor-acceptors. These bonds can cause a decrease in the ligand's affinity to proteins, alter the specificity of ligands to proteins, and reduce the biological activity of ligand molecules, which can affect their ability to produce the desired biological response(Tanchuk et al., 2016; Wu et al., 2023).

Understanding the underlying processes of PCOS can be improved by carefully examining the chemical interactions between the substances in Clitoria ternatea and the LH receptor. This can assist in identifying molecular pathways that can be altered to control LH and treat PCOS, which can serve as the starting point for additional research such as in vitro studies and human clinical trials. This is required to verify the components in Clitoria ternate's actions on the LH receptor and to evaluate their palpable effects on PCOS patients(Zian et al., 2022). The outcome of the molecular docking simulation is only a forecast of how the drug will interact with the receptor; it still must be confirmed by biological testing and more experimental research. Results may vary depending on the programmed and docking parameters chosen. The outcomes of molecular docking should be connected to broader biological effects. It is important to conduct biological experiments, either in vitro or in vivo, to examine Clitoria ternatea's possible impacts on hormone regulation and PCOS symptoms.

CONCLUSION

PCOS is a complex hormonal disorder that often involves disorders in the regulation of LH and its receptors. This interaction activates an intracellular signal path that sends a signal into the cell and induces a variety of responses. Binding affinity suggests that the

compounds in Clitoria ternatea have the potential to interact strongly with the LH receptor, which can have important implications for the regulation of menstrual and ovulation cycles in women with PCOS.

The molecular docking results showed that the entire ligand of the tested Clitoria ternatea compound has a binding affinity and a good bond between the LH receptor protein and the ligand. Understanding the underlying processes of PCOS can be improved by carefully examining the chemical interactions between the substances in Clitoria ternatea and the LH receptor. This can assist in identifying molecular pathways that can be altered to control LH and treat PCOS, which can serve as the starting point for additional research such as in vitro-in vivo studies and human clinical trials.

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