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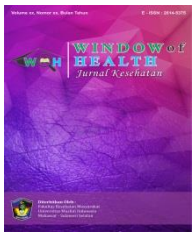

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In Silico of AMHRII Receptor Binding and Toxicity Prediction of Clitoria ternatea

[C]Erna Yovi Kurniawati[1], Endang Mahati [2], Zaw Myo Hein[3]
[1]Midwifery Profession Program, Faculty of Health Science, Alma Ata University, Indonesia
[2]Department of Pharmacology and Therapy, Medical Faculty, Diponegoro University, Indonesia
[3]Department of Basic Medical Sciences, College of Medicine, Ajman University, UAE
Email of Corresponding Author ([C]): yovi.raharjanto@gmail.com
yovi.raharjanto@gmail.com[1], endangmahati@fk.undip.ac.id[2], z.hein@ajman.ac.ae[3]

ABSTRACT

Clitoria ternatea compounds show significant potential as herbal drug candidates for polycystic ovary syndrome (PCOS) through improving anti-Müllerian hormone (AMH) parameters. This study aims to evaluate the potential of these compounds in binding to AMHRII receptors using an in silico approach. Several compounds, baicalein, isorhamnetin, and malvidin, showed high binding affinity towards AMHRII receptors, approaching or even surpassing some control drugs. These compounds have favourable toxicity profiles, with high LD50s, suggesting low toxicity and high safety potential for therapeutic use. Some compounds, such as isorhamnetin, termination, petunidin, malvidin, cyanidin, luteolin, and protocatechuic acid, have an unfavourable number of hydrogen donors, as does gallic acid, which has an unfavourable number of hydrogen acceptors. The development of technologies that improve bioavailability and pharmacokinetics can overcome these challenges. Further studies in vivo are needed to confirm the effectiveness and safety of these compounds in potential clinical therapies and develop innovative herbal therapies for Clitoria ternatea-based PCOS.

Keyword : Clitoria ternatea, PCOS, AMHRII, pharmacokinetics, bioavailability

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INTRODUCTION

Clitoria ternatea contains active compounds such as flavonoids, alkaloids, saponins, and tannins, which have antioxidant and anti-inflammatory properties. Clitoria ternatea affects the gut microbiota and improves hormonal balance.[1] Clitoria ternatea extract can inhibit the production of pro-inflammatory cytokines, reduce oxidative stress, and protect against liver and kidney damage; it can also help regulate lipid metabolism, lower cholesterol levels, and improve insulin sensitivity.[2,3] Its pharmacokinetics make Clitoria ternatea a promising natural component and potential herbal ingredient for polycystic ovary syndrome.[4] Clitoria ternatea compounds have been shown to have strong binding with FSH receptors in the enhancement of folliculogenesis of polycystic ovary syndrome.[5]

AMH (Anti-Müllerian Hormone) is a significant diagnostic marker for polycystic ovary syndrome (PCOS). This receptor is critical in understanding and diagnosing PCOS, as it plays a vital role in folliculogenesis and ovarian function. Elevated AMH levels are often associated with PCOS, making AMHRII an essential parameter in identifying and managing this condition.[6,7] Studying the interaction of natural compounds from Clitoria ternatea with AMHRII receptors may provide new insights into potential treatments for PCOS.

This study investigated the impact of Clitoria ternatea on AMHRII receptors, which are critical for reproductive regulation through molecular docking techniques. Understanding the pharmacokinetic and toxicity profile of Clitoria ternatea is crucial for its development as a potential drug. Pharmacokinetic analysis is essential as it affects the pharmacological and toxic effects of Clitoria ternatea extracts. In silico methods using Swiss ADME and Protox II offer a faster and more cost-effective way to understand natural compounds' pharmacokinetic and toxicity properties.[8]

This study aims to predict the potential pharmacological effects of Clitoria ternatea, understand its interaction with the human body, its distribution within the body, and possible toxic effects using an in silico approach.[8] These findings will shed further light on the potential applications of Clitoria ternatea as a therapeutic agent.[4] This research can serve as a basis for further study and advancement in the pharmaceutical industry and the use of traditional knowledge in herbal medicine.

METHOD

Clitoria ternatea compounds were collected based on a literature review of previous studies.[4,9,10] Clitoria ternatea compounds were tested for drug similarity (Lipinski's rule of five) and toxicity classification (LD50) using ProTox II (https://tox-new.charite.de/protox_II) by inputting canonical SMILES compounds.

The binding affinity of Clitoria ternatea flower compounds on AMHRII was further analyzed using software through the steps mentioned in Figure 1. Control drugs used as comparisons include Metformin, Spironolactone, Siproterone Acetate, and Flutamide. Binding affinity analysis using PyRx, the centre point of the docking coordinates set was X: 125.562, Y: 134.711, Z: 168.4683, with search space dimensions of X = 73.8431 Å, Y = 57.9777 Å, and Z = 128.5658 Å[11,12].

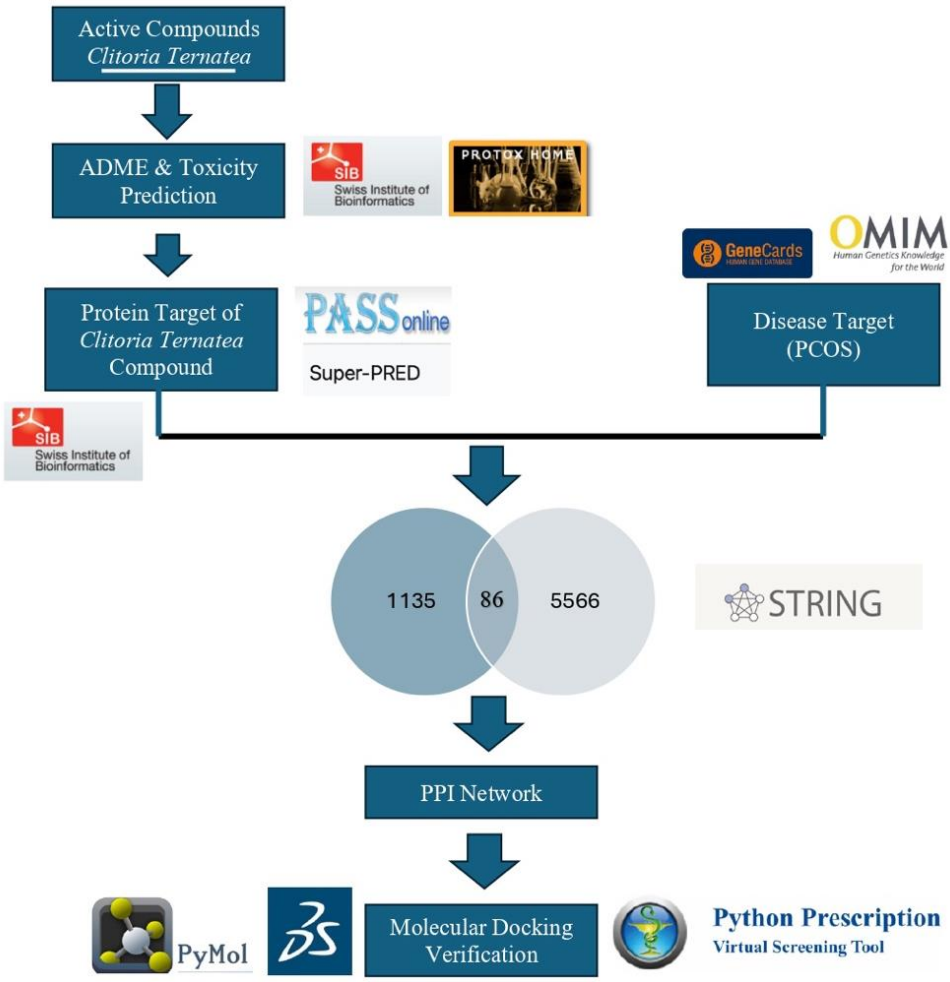


Figure 1. Workflow Clitoria Ternatea on AMHRII In Silico Study

RESULT

Table 1. Drug-likeness and Toxicity in Silico Prediction 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

Compounds	*Druglikeness* (Lipinski)	LD50 (mg/kg)	Toxicity Class
Chlorogenic	Yes	5000	5
Protocatechuic	Yes	2000	4
Gallic	Yes	2000	4
Anthraquinone	Yes	5000	5

Kaempferol showed drug similarity according to Lipinski's rule with an LD50 of 3919 mg/kg, placing it in toxicity class 5, indicating low toxicity. Quercetin and myricetin had a lower LD50 of 159 mg/kg with moderate toxicity. Isorhamnetin meets the drug similarity criteria with an LD50 of 5000 mg/kg, similar to kaempferol, indicating low toxicity. Anthocyanins, including ternatin, petunidin, peonidin, delphinidin, malvidin, and cyanidin, all showed drug similarity with an LD50 of 5000 mg/kg, having low toxicity and potentially safe for use in herbal formulations.[8]

Epicatechin among flavanols showed drug similarity with a very high LD50 of 10000 mg/kg, indicating very low toxicity and high safety. Scutellarin did not meet the criteria of drug similarity but had an LD50 of 5000 mg/kg, indicating low toxicity that still needs further investigation. Baicalein and luteolin showed drug similarity with an LD50 of 3919 mg/kg, indicating low toxicity. Apigenin met the drug similarity criteria with an LD50 of 2500 mg/kg, indicating low toxicity. Chlorogenic acid met the drug similarity criteria with an LD50 of 5000 mg/kg, indicating low toxicity. Protocatechuic acid and gallic acid showed drug similarity with an LD50 of 2000 mg/kg, showing moderate to low toxicity. Anthraquinones showed drug similarity with an LD50 of 5000 mg/kg, indicating low toxicity.[8]

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

Compounds	Binding affinity score (rmsd/ub & rmsd/lb=0.0)
Kaempferol	-7.1
Isorhamnetin	-7.3
Ternatin	-7.0
Petunidin	-6.9
Peonidin	-6.7
Delphinidin	-6.8
Malvidin	-7.3
Cyanidin	-6.8
Epicatechin	-7.2
Baicalein	-7.4
Luteolin	-7.1
Apigenin	-7.2
Chlorogenic	-6.8
Protocatechuic	-4.8
Gallic	-5.3
Anthraquinone	-6.4

Table 3. Binding affinity score of control drugs on AMHRII

Compounds	Binding affinity score (rmsd/ub & rmsd/lb=0.0)
Metformin	-4.6
Cyproterone Acetate	-6.9
Flutamide	-6.3
Spironolactone	-8.1

The tested compounds include flavonols (kaempferol and isorhamnetin), anthocyanidins (ternatin, petunidin, peonidin, delphinidin, malvidin, and cyanidin), flavonols (epicatechin), flavones (baicalein, luteolin, and apigenin), and phenolic acids (chlorogenic, protocatechuic, and gallic) derived from Clitoria ternatea. Scutellarin was not included in the test due to its poor drug similarity status, while quercetin and myricetin were not included in the trial due to their moderate toxicity classification.[8]

The binding affinity scores of Clitoria ternatea compounds to AMHRII receptors showed potency variations. Baicalein (-7.4), isorhamnetin (-7.3), and malvidin (-7.3) showed the highest binding affinity scores, signifying strong binding to AMHRII. These compounds had binding affinities that were close to or even higher compared to some control drugs. For instance, the binding affinity score of spironolactone was -8.1, which was the highest score among all the compounds tested, both from Clitoria ternatea and the control drugs.

Kaempferol (-7.1), epicatechin (-7.2), luteolin (-7.1), and apigenin (-7.2) also showed high binding affinity, almost comparable to isorhamnetin and malvidin, more robust compared to flutamide (-6.3) and nearly equivalent to cyproterone acetate (-6.9). Ternatin (-7.0), petunidin (-6.9), delphinidin (-6.8), cyanidin (-6.8), chlorogenic acid (-6.8), anthraquinone (-6.4), and peonidin (-6.7) showed fairly good binding affinity, exhibiting competitive binding affinity scores, almost equivalent to cyproterone acetate (-6.9) and higher compared to flutamide (-6.3). In contrast, protocatechuic acid (-4.8) and gallic acid (-5.3) showed the lowest binding affinity scores, lower compared to metformin (-4.6), indicating weaker binding potential with AMHRII.metformin (-4.6), yang menunjukkan potensi pengikatan yang lebih lemah dengan AMHRII.

The docking results were visually analysed using Discovery Studio software to examine the correlation between the ligand and AMHRII[12]. Figure 2-21 shows a graphical representation of these results.

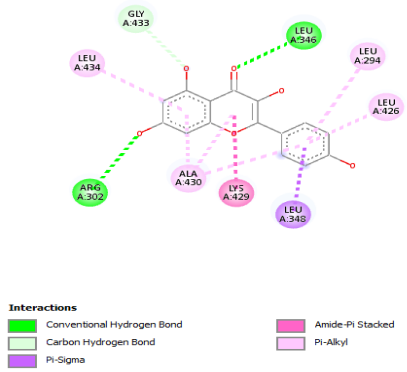


Figure 2. Molecular Docking AMHRII-Kaempferol

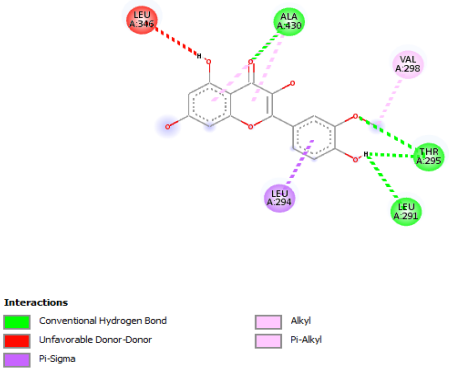


Figure 3. Molecular Docking AMHRII-Isorhamnetin

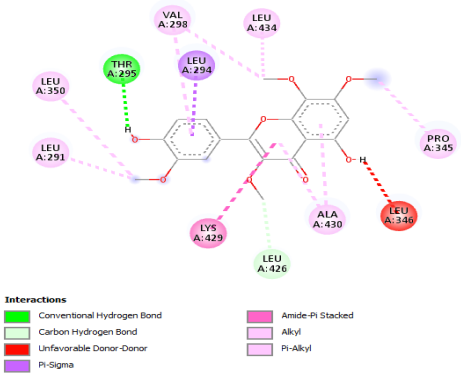


Figure 4. Molecular Docking AMHRII-Ternatin

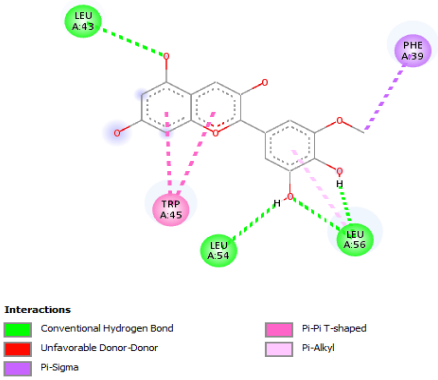


Figure 5. Molecular Docking AMHRII-Petunidin

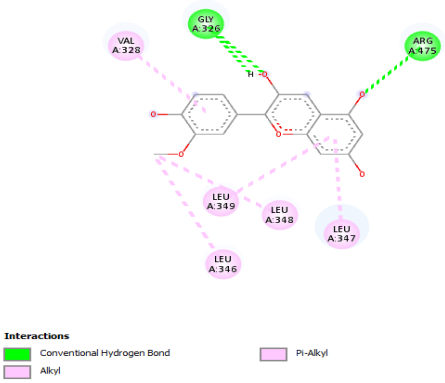


Figure 6. Molecular Docking AMHRII-Peonidin

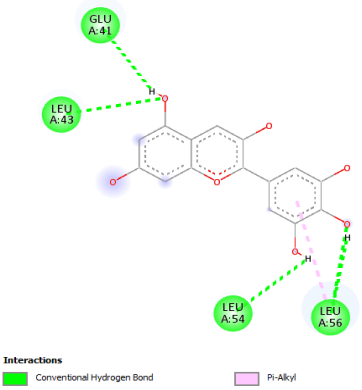


Figure 7. Molecular Docking AMHRII-Delphinidin

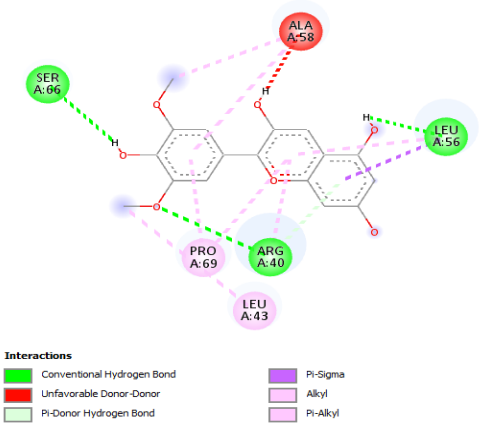


Figure 8. Molecular Docking AMHRII-Malvidin

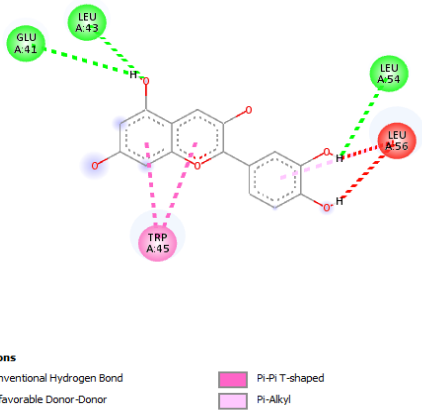


Figure 9. Molecular Docking AMHRII-Cyanidin

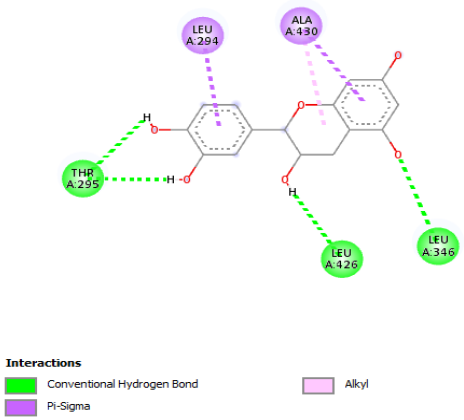


Figure 10. Molecular Docking AMHRII-Epicatechin

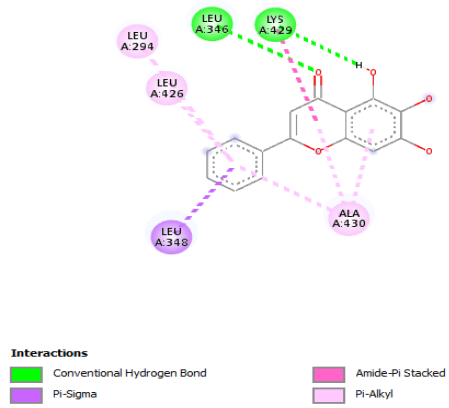


Figure 11. Molecular Docking AMHRII-Baicalein

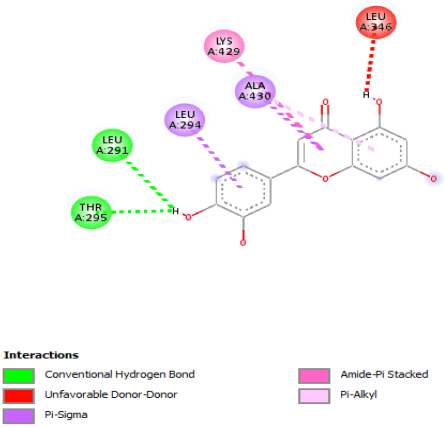


Figure 12. Molecular Docking AMHRII-Luteolin

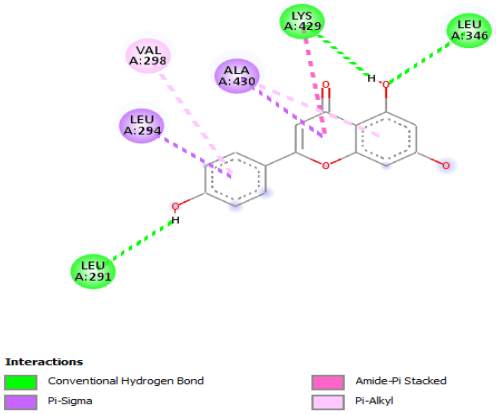


Figure 13. Molecular Docking AMHRII-Apigenin

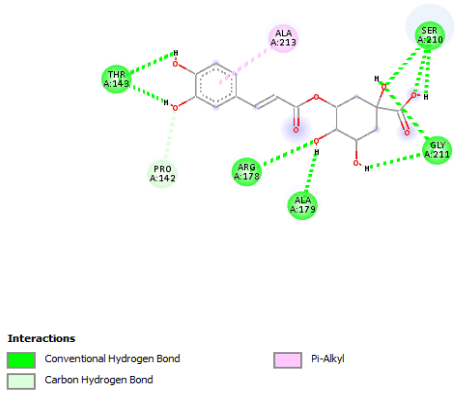


Figure 14. Molecular Docking AMHRII-Chlorogenic

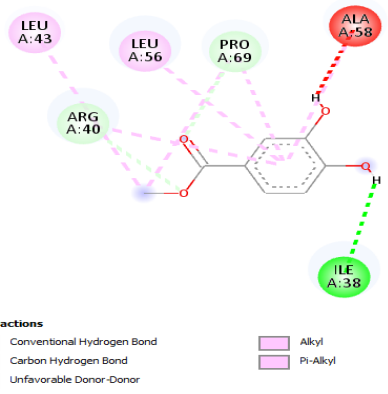
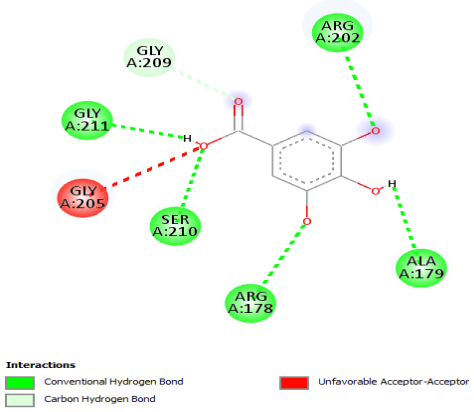


Figure 15. Molecular Docking AMHRII-Protocatechuic



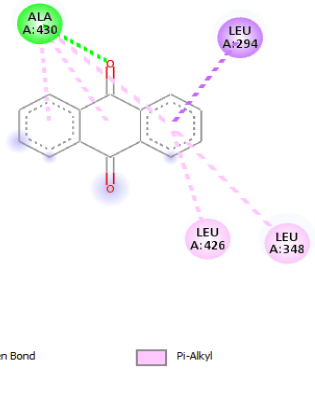


Figure 16. Molecular Docking AMHRII-Gallic

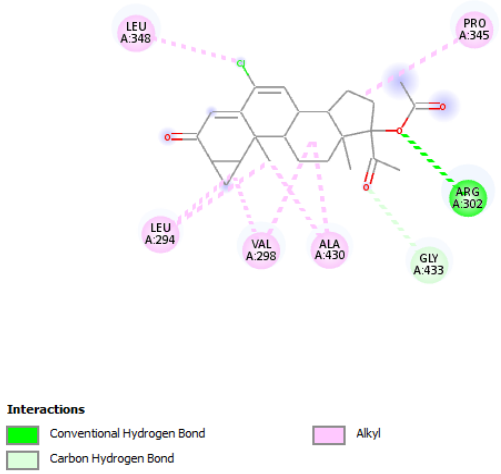


Figure 17. Molecular Docking AMHRII-Anthraquinone

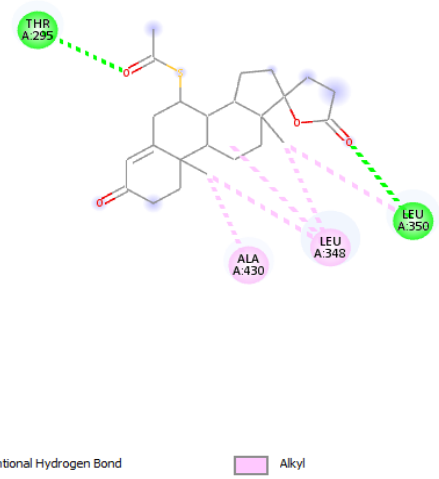


Figure 18. Molecular Docking AMHRII-Cyproterone

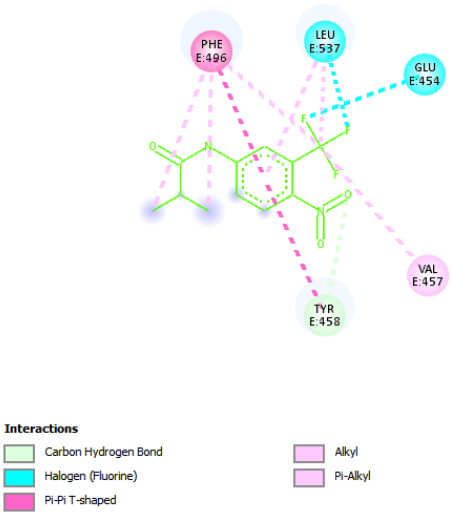


Figure 19. Molecular Docking AMHRII-Spironolactone

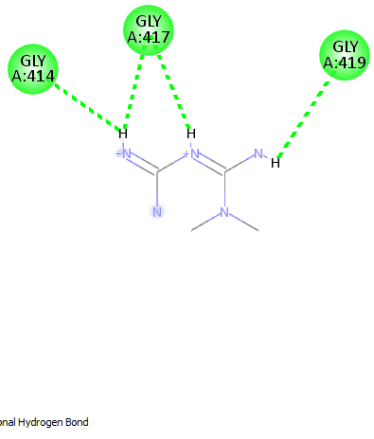


Figure 20. Molecular Docking AMHRII-Flutamide

Figure 21. Molecular Docking AMHRII-Metformin

Isorhamnetin, ternatin, petunidin, malvidin, cyanidin, luteolin, and protocatechuic have an unfavourable number of hydrogen donors, while gallic acid has an unfavourable number of hydrogen acceptors, which may affect their bioavailability and pharmacological effectiveness, affecting their interaction with biological targets. Hydrogen donors play an essential role in forming hydrogen bonds necessary for stability and binding affinity with receptors. Too many or too few hydrogen donors can lead to low solubility and difficulty penetrating cell membranes, ultimately decreasing bioavailability. Hydrogen acceptors are essential for the formation of stable hydrogen bonds with receptors. An unfavourable number of hydrogen acceptors can lead to weak binding and low stability, reducing the compound's biological effectiveness.[5,11]

DISCUSSION

Based on the results of in silico analysis, compounds derived from Clitoria ternatea showed significant potential to be herbal drug candidates in the treatment of Polycystic Ovary Syndrome (PCOS) through the improvement of Anti-Müllerian Hormone (AMH) parameters. AMH is a vital biomarker used in diagnosing and monitoring PCOS,[7] so the ability of these compounds to interact with the AMHRII receptor provides a strong basis for developing new therapies. Several compounds from Clitoria ternatea, such as baicalein (-7.4), isorhamnetin (-7.3), and malvidin (-7.3), showed high binding affinity to the AMHRII receptor. These high binding affinity scores indicate that these compounds have the potential to regulate AMH function, which may help in the normalization of menstrual cycles and improvement of fertility in patients with PCOS. In addition, compounds such as kaempferol (-7.1), epicatechin (-7.2), luteolin (-7.1), and apigenin (-7.2) also showed competitive binding affinity, adding to the list of potential compounds for the herbal treatment of PCOS.[4]

Favourable toxicity profiles have also supported the safety of these compounds, indicating that using these compounds in therapeutic doses is likely safe for humans. In addition, anthocyanins such as ternatin, petunidin, peonidin, delphinidin, malvidin, and cyanidin also have LD50 values of 5000 mg/kg, indicating low toxicity and potential safety for long-term use.[8] Nonetheless, some compounds have an unfavourable number of hydrogen donors or acceptors, such as isorhamnetin, ternatin, petunidin, malvidin, cyanidin, luteolin, and protocatechuic acid, which have an unfavourable number of hydrogen donors, as well as gallic acid, which has an unfavourable number of hydrogen acceptors. These factors may affect these compounds' bioavailability and pharmacological effectiveness, even though they exhibit high binding affinities, necessitating the development of herbal medicines with technologies that can improve their bioavailability and pharmacokinetics.[5,11]

Clitoria ternatea compounds can affect SMAD and MAPK pathways through interaction with AMHRII in improving folliculogenesis in PCOS women[7]. SMAD and MAPK pathways are essential to regulate ovarian follicular growth and development. Activation of these pathways may exert beneficial modulating effects on AMHRII activity, which may improve ovarian function and ameliorate the impaired folliculogenesis in PCOS.[13]

Clitoria ternatea compounds can increase AMHRII receptor activity through the SMAD pathway and activate specific SMAD proteins, such as SMAD2 and SMAD3. SMAD activation then forms a complex with SMAD4 and translocates to the cell nucleus as a transcription factor, allowing it to regulate the expression of target genes involved in granulosa cell proliferation, differentiation, and apoptosis. Increased activity of the SMAD pathway may optimize the process of ovarian follicle formation and development, which is critical in regulating the menstrual cycle and fertility.[13]

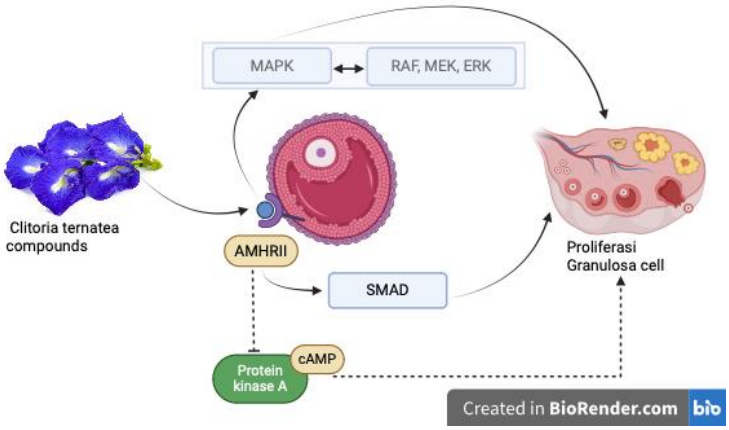


Figure 21. Mekanisme Senyawa Clitoria ternatea terhadap AMHRII

Clitoria ternatea compounds affect the MAPK pathway. Through this pathway, active compounds in Clitoria ternatea can interact with AMHRII and activate a series of kinases, including RAF, MEK, and ERK kinases. Activation of ERK, a significant endpoint in the MAPK pathway, regulates the transcription of target genes involved in various cellular processes, including cell proliferation and differentiation.Optimizing this MAPK pathway, Clitoria ternatea may promote ovarian follicle formation and development and reduce disorders associated with PCOS.[13,14] AMHRII inhibits the cAMP signalling pathway through interaction with AMH, leading to decreased PKA activity; this occurs because AMHRII inhibits the enzyme adenylate cyclase, which is responsible for converting ATP (adenosine triphosphate) into cAMP. Inhibition of adenylate cyclase by AMHRII reduces the production of cAMP in the granulosa cells.[13]

Decreased cAMP production decreases PKA activity, which may reduce granulosa cell proliferation. AMHRII affects the opposite signaling pathway to FSHR, inhibiting ovarian granulosa cell proliferation.[13] In the context of folliculogenesis, this inhibition is essential to regulate the number and development of ovarian follicles and prevent excessive activity that can disrupt the hormonal balance in the female reproductive cycle.[13,14] Clitoria ternatea compounds showed promising potential as herbal drug candidates for treating PCOS by improving AMH parameters. Further, in vivo studies are needed to confirm the effectiveness and safety of these compounds in potential clinical treatment for developing innovative herbal therapies for PCOS.

CONCLUSIONS AND RECOMMENDATIONS

Clitoria ternatea compounds have potential as herbal drug candidates for PCOS through enhancing AMH parameters. Compounds such as baicalein, isorhamnetin, and malvidin have almost equivalent or higher binding affinity compared to some control drugs, signifying great potential in regulating AMH function, which is crucial in normalizing the menstrual cycle and improving fertility in PCOS patients. The low toxicity profile of this compound also supports its safety for long-term use.

Further, in vivo studies are needed to confirm the effectiveness and safety of these compounds in potential clinical therapies.

Innovative Clitoria ternatea-based herbal therapies for PCOS can be developed by utilizing technologies that improve the bioavailability and pharmacokinetics of compounds with unfavourable numbers of hydrogen donors and acceptors.

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