Virtual screening and molecular docking of Indonesian phytoconstituents as potential inhibitors of peroxisome proliferator-activated receptor gamma in polycystic ovary syndrome

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Abstract. Polycystic ovary syndrome (PCOS) is the most common endocrinology disorder affecting women of reproductive age, characterized by irregular menstruation, androgen overproduction and insulin resistance. Peroxisome proliferator-activated receptor-gamma (PPARG) is one of the essential modulators of ovarian steroid hormone synthesis and lipid metabolism. Precision medicine and targeted therapy have emerged as practical therapeutic approaches, focusing on specific molecules/pathways to minimize side effects and improve outcomes. This study aimed to identify potential Indonesian phytoconstituents that inhibit PPARG both from a pharmacokinetic and pharmacodynamic perspective based on the in silico approach. Data of 6776 phytoconstituents from the Indonesian Medicinal Plant Database were screened for compounds with Pa>0.3 as "Insulin Sensitivity", "Lipid metabolism", and "Anti-inflammation" using PASSOnline. We performed pharmacokinetic profile prediction using SwissADME based on Lipinski criteria. Molecular docking was carried out with PPARG as the target proteins, utilizing PyMol, Pyrx, and Discovery Studio to evaluate top-hit compounds with the highest binding affinity. Through in silico screening and molecular docking, Quercitol exhibited superior binding with the protein target compared to the control ligands and metformin. Quercitol also met the Lipinski criteria, showing favourable bioavailability as a drug candidate. Hence, these findings will provide a theoretical basis for further studies as regards drugs targeting PPARG in PCOS.

1 Introduction

Polycystic ovary syndrome (PCOS) is the most frequent endocrinology condition that influences women of reproductive age all over the world [1]. The prevalence of the patient is typically characterized by irregular menstruation, ovarian and adrenal androgen

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overproduction, and insulin resistance [2]. According to the Rotterdam criteria, it is necessary to have two of the following: anovulation or oligoovulation, hyperandrogenism, or an increased ovarian volume (≥10 mL), and/or polycystic ovary morphology, which is characterized by the presence of numerous cystic follicles in either one or both ovaries, as observed through ultrasound [3]. About 50 to 70% of PCOS women have insulin resistance, which plays a vital role in the pathophysiology of the condition [3, 4]. When insulin levels in the circulatory system exceed normal ranges, it is thought to result in excessive anovulation and increased androgen production due to elevated circulating insulin levels in the ovary [4].

The principal aim of the pharmacological therapeutic approach for females with PCOS who are unable to conceive is to regulate hyperandrogenism and menstrual cycle abnormalities, as well as to enhance quality of life and address comorbidities. The first line of treatment for hyperandrogenism and/or irregular menstruation is a combination oral contraceptive pill or progestin. Concurrently, metformin, classified as a biguanide drug, may be used alongside a combination of oral contraceptives and lifestyle modifications to improve metabolic outcomes, menstrual period irregularities, and body weight control [5]. Metformin is a potentially successful allopathic dual medication primarily utilized for diabetic care. However, it has also been recommended for the treatment of PCOS as a result of its diverse therapeutic properties. Metformin, an insulin sensitizer, may stimulate weight loss, restore ovulation, enhance the pregnancy rate, and prevent the number of complications associated with pregnancy [6]. Nevertheless, there have been substantial concerns regarding its harmful effects, which encompass diarrhoea, vomiting, stomachaches, nausea, and ketoacidosis. Moreover, the metformin group experienced a significantly higher percentage of women who discontinued treatment as a result of adverse effects [7].

Peroxisome proliferator-activated receptors (PPARs) are expressed in the ovaries and have a significant impact on fertility [8]. Peroxisome proliferator-activated receptors (PPARG) are essential lipid metabolism regulators, cell cycle regulation, ovarian steroid hormone synthesis and metabolism, and follicular development [9]. PPARG dysregulation has been linked with severe insulin resistance, which is a typical metabolic outcome in PCOS patients. PPARG expression is stimulated during folliculogenesis, leading to the development of large follicle stages, and is subsequently downregulated after the luteinizing hormone (LH) surge [8]. Recent investigations have demonstrated that PPARG levels have a favourable metabolic effect on hyperinsulinemia, signalling that hyperandrogenemia may contribute to the onset of PCOS [9]. PPARG activity in adipose tissue (AT) regulates glucose and lipid metabolism. PPARG controls adipokines, including adiponectin, TNF-α, MCP-1, and resistin. Decreased free fatty acid concentrations in the bloodstream and modified adipokine profiles enhance insulin sensitivity. This improvement is facilitated by a reduction in hepatic glucose synthesis (gluconeogenesis), increased absorption of glucose in adipose tissue and skeletal muscles, and stimulation of insulin production in the pancreas. Activation of PPARG suppresses macrophage infiltration into adipose tissue and generates an anti-inflammatory M2 macrophage [10]. These findings suggested that prospective PCOS therapeutic targets and medications could be associated with the metabolic characteristics of PPARG and PCOS.

Recently, systems-level and biological backgrounds have enhanced our comprehension of drug-protein interactions, and diverse protein interaction networks have been employed to predict drug processes, investigate disease genes, and elucidate pathogenesis [11]. Precision medicine has become progressively crucial in disease management, and targeted therapy represents a significant shift in the treatment of diseases. In contrast to conventional medication, which induces side effects, targeted therapy concentrates on specific molecules or mechanisms implicated in illness growth and progression. This precision minimizes disturbance to other important goals, such as fertility problems, which patients need, leading to improved outcomes. Therefore, looking for new potential treatment targets for this

condition is necessary. Indonesia is well-known for its enormous natural resource reserves, which can be utilized to investigate new drug candidates. In this study, we performed on 6776 bioactive natural phytocompounds and evaluated the three best compounds with the capability to inhibit PPARG proteins using in silico or bioinformatic methods.

2 Methods

Virtual screening was conducted on 6776 active compounds retrieved from Indonesia's phytoconstituents database (http://herbaldb.farmasi.ui.ac.id/v3/). Subsequently, we predicted the pharmacokinetic profile, including molecular weight, donor and acceptor hydrogen bonds, and MlogP. The assessment of drug-likeness with Lipinski criteria was analyzed using the SWISSADME webserver (http://www.swissadme.ch/). A total of 241 candidate compounds were obtained, and these were further analyzed for their "Insulin Sensitivity", "Lipid metabolism" and "Anti-inflammation" potency prediction using PASSOnline, with criteria for Probability of Active (Pa) > 0.3. Three compounds were selected for a molecular docking test with PPARG as the target proteins to evaluate the top-hit compounds with the highest binding affinity. The molecular docking procedure was executed using PyRx 0.95 software, PyMol, and Discovery Studio, which revealed interaction sites and binding affinity between ligands and protein targets. The ligand binding affinity scores were then compared with control ligands to assess the interactions of amino acid residues.

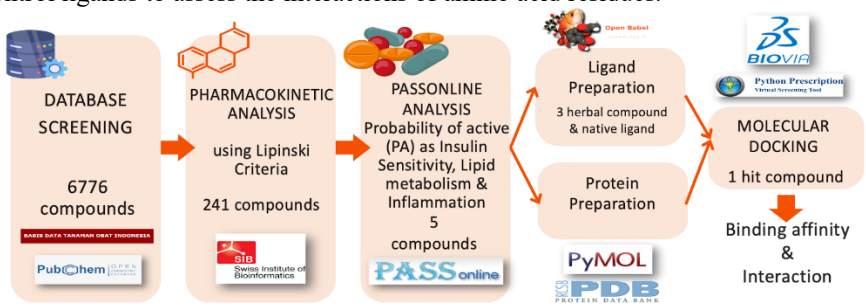


Fig. 1. Virtual screening and molecular docking using silico approaches.

3 Results

3.1 Natural Phytocompound Profile

Among 241 compounds that fulfilled Lipinski criteria, three compounds met the Pa criteria. The results of molecular docking show that quercitol, artocarpin, and barbinervic acid, demonstrated remarkable binding affinity with both PPARG proteins. Quercitol exhibited superior binding with the protein target compared to the control ligands. In contrast, artocarpin and barbinervic acid had slightly lower affinity compared to the control ligands.

3.2 Molecular Docking Ligand-Protein Targets

Molecular docking results reveal binding affinity interactions and amino acid residues between ligands and proteins. A more negative value indicates a stronger bond. The following table presents the results of the molecular docking test:

Table 1. Dimension, active site, and grid box center of the molecular docking.

Protein Target	Native Ligand	Active Site	Dimension (A)	Docking Center Grid
PPAR Gamma PDB ID (7AWC)	Rosiglitazone	Cys285 Arg288 Ser289 His323 Leu330 Val339 Ile341 Met348 Leu353 Met364 His449 Tyr473	X: 5.9 Y: 11.8 Z: 9.3	X: 41.721 Y: 4.345 Z: 82.395

Table 2. Binding affinity and type of interaction.

Ligand	Binding Affinity (Kcal/mol)	Residue	Chemistry Bond	Distance
Quercitol	-5.3	Cys285	Unfavorable	2.97
		His323	Hydrogen Bond	2.06
		Tyr327	Hydrogen Bond	2.14
Metformin	-5.0	UNK1	Unfavorable	1.98
		Cys285	Hydrogen Bond	2.81
			Hydrogen Bond	2.86
		Tyr473	Hydrogen Bond	2.29
			Carbon Hydrogen Bond	3.64

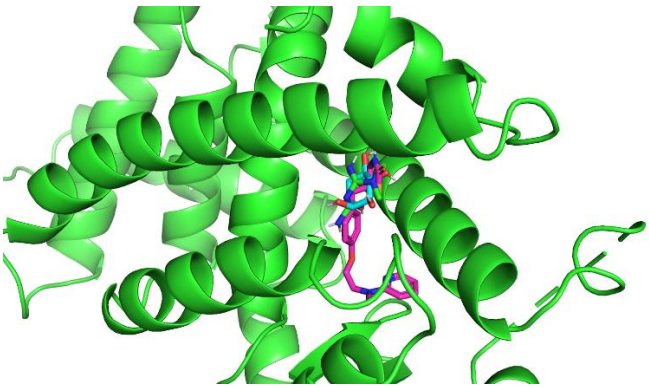
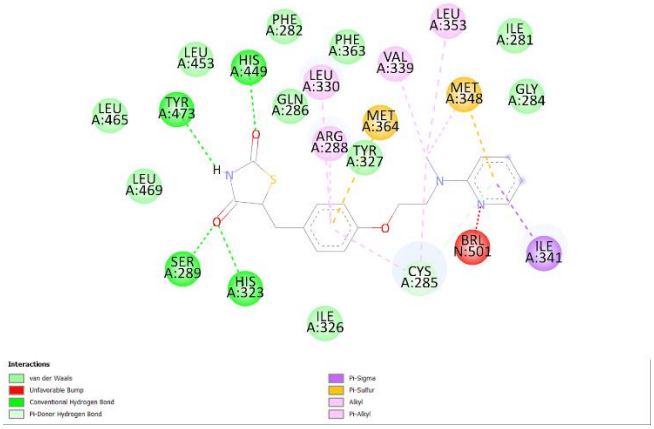
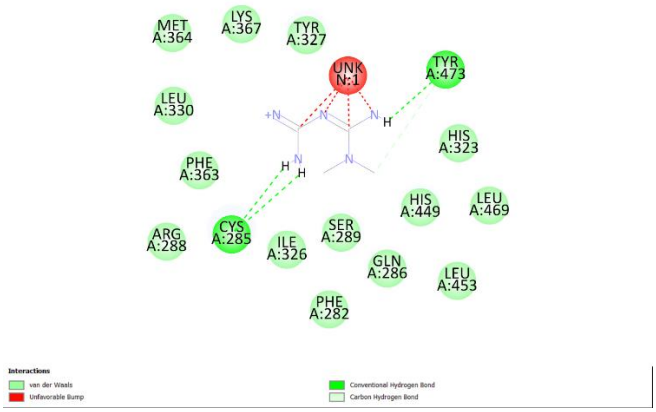


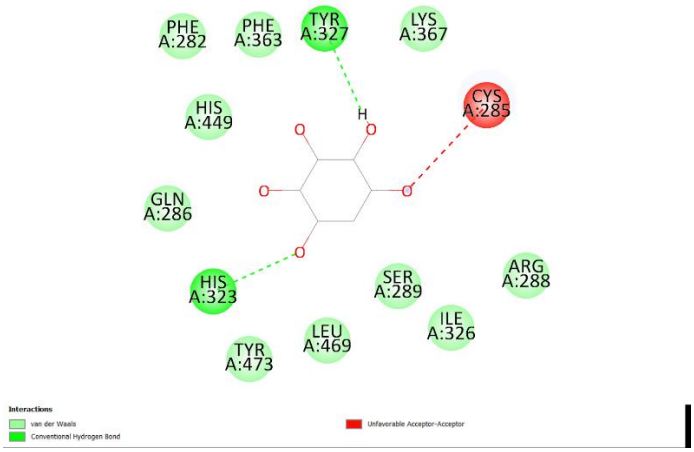
Fig. 2. PPAR Gamma (green) with Risoglitazone (pink), metformin (blue), and Quercitrol (Magenta) Colour illustrations.



(A)



(B)



(C)

Fig. 3. Ligand interaction with the amino acid residue of Protein PPAR Gamma. (A) Rosiglitazone–PPAR gamma. (B) Metformin–PPAR Gamma. (C) Quercitrol–PPAR Gamma.

4 Discussion

The results of molecular docking and previous experimental studies indicate that the binding between Quercitol and metformin occurs at the receptor of the PPAR gamma protein. The amino acid sequence at the PPAR gamma protein receptor includes Ile262, Gln286, Arg288, Ser289, His323, Ser342, Glu343, His449, and Tyr473 [12]. The binding of the native ligand, rosiglitazone, to these amino acids is both orthosteric and allosteric. The binding between the native ligand and the amino acid Arg288 is allosteric. It forms a salt bridge, leading to the stabilization of the Ligand-Binding Domain (LBD) in the PPARG protein. Meanwhile, the binding of the native ligand with the amino acids Ile262, Gln286, Ser289, His323, Glu343, His449, and Tyr473 is orthosteric within the Ligand Binding Domain [13].

The molecular docking test between the Quercitol and metformin compounds shows that ligand-amino acid binding occurs in the LGB of the PPARG protein. The Quercitol ligand binds to the amino acid residues Cys285, His323, and Tyr327, forming one unfavourable acceptor-acceptor bond and two conventional hydrogen bonds. The Metformin ligand binds to the amino acid residues Cys285 and Tyr473, forming three conventional hydrogen bonds and one carbon-hydrogen bond. In the binding affinity test, the quercitrol ligand exhibited a higher binding affinity than the metformin ligand. Several factors that influence binding affinity include the bond distance between the ligand and residues of amino acids in hydrogen bonds, van der Waals forces, and electrostatic forces [14,15].

Peroxisome proliferator-activated receptors (PPARs) function are nuclear hormone receptors, which are activated by fatty acids. The three main isoforms of PPARs, alpha, beta/delta, and gamma, each have distinct metabolic regulation actions, tissue distribution, and ligand-binding characteristics. Apart from insulin sensitivity, the PPARG isoform has the highest expression in adipocytes and is essential for lipoprotein metabolism, lipid production, and adipogenesis [16,17]. PPAR isotypes are reported to influence metabolic syndrome significantly. Metabolic syndrome, which consists of central adiposity, insulin resistance, hypertension, and atherogenic dyslipidemia, can be present in up to 43% of adult women with PCOS [18]. The PPARG isoform, in addition to its role in insulin sensitivity, is predominantly expressed in adipocytes and plays a significant role in adipogenesis, lipid production, and lipoprotein metabolism [17]. A variety of natural factors may influence PPARG expression in PCOS. In a randomized controlled experiment involving 53 women with PCOS, Shokrpour et al. discovered that supplementing with myoinositol markedly increased the expression of the PPARG gene [19].

Quercitol has several synonyms, including D-1-deoxy-muco-inositol, 5-deoxyinositol, D-chiro-Inositol, and Acorn sugar. This compound can be found in *Allium ascalonicum* (Shallot), *Nuphar lutea* (yellow water lily), and *Gardenia jasminoides* [20]. Inositols have been proposed as insulin sensitizers, with two main compounds, including myo-inositol (MYO) and d-chiro-inositol (DCI). DCI is produced via an insulin-dependent mechanism that converts myo-inositol [21]. Over the past ten years, research has shown that impaired insulin sensitivity, or insulin resistance, is present in people with polycystic ovary syndrome (PCOS), even in those who are neither overweight nor obese. A particular biological adaptation known as insulin resistance causes compensatory hyperinsulinemia in roughly 70–80 percent of women who have PCOS and central obesity, as well as 15–30 percent of women who are slim and have been confirmed with PCOS [22]. Patients with hyperinsulinemia associated with PCOS have been treated with insulin-sensitizing medications such as troglitazone, pioglitazone, and metformin. Since metformin frequently causes adverse effects, innovative, integrative methods that make use of new potential compounds, such as quercetin, have been suggested to treat insulin resistance modulated by PPARG.

5 Conclusion

Based on the findings from this study concerning pharmacokinetic profiles and molecular docking, the quercitol compound had a higher potential to inhibit PPARG compared to metformin. Quercitol also met the Lipinski criteria. Therefore, these compounds have a favourable bioavailability as a drug candidate. Hence, these findings will provide a theoretical basis for further studies regarding drugs targeting PPARG in PCOS. Nonetheless, for more precise results, further investigation enhancing molecular dynamics and in vitro and in vivo testing is needed.

References

1. Y. Liu, Y. Qin, B. Wu, H. Peng, M. Li, H. Luo, L. Liu. DNA methylation in polycystic ovary syndrome: Emerging evidence and challenges. Reprod Toxicol. 2022 Aug; **111**: 11-19. Epub (2022)
2. D. Baldani, L. Skrgatic, R. Ougouag. Polycystic Ovary Syndrome: Important Underrecognised Cardiometabolic Risk Factor in Reproductive-Age Women. Int J Endocrinol (2015)
3. W. Wolf, R. Wattick, O. Kinkade, M. Olfert. Geographical Prevalence of Polycystic Ovary Syndrome as Determined by Region and Race/Ethnicity. Int J of Environmental Research and Public Health, **15**(11), 2589 (2018)
4. H. Zhao, J. Zhang, X. Cheng, *et al*. Insulin resistance in polycystic ovary syndrome across various tissues: an updated review of pathogenesis, evaluation, and treatment. J Ovarian Res, **16**, 9 (2023)
5. H. Sadeghi, I. Adeli, D. Calina, A. Docea, T. Mousavi, M. Daniali, S. Nikfar, A. Tsatsakis, M. Abdollahi. Polycystic Ovary Syndrome: A Comprehensive Review of Pathogenesis, Management, and Drug Repurposing. Int. J. Mol. Sci., **23**, 583 (2022)
6. D. Wishart, C. Knox, A. Guo, S. Shrivastava, M. Hassanali, P. Stothard, Z. Chang, J. Woolsey. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic Acids Res, **34**, D668–D672 (2006)
7. G. Attia, M. Almouteri, F. Alnakhli. Role of Metformin in Polycystic Ovary Syndrome (PCOS)-Related Infertility. Cureus, **15**(8), e44493 (2023)
8. C.-Y. Shi, J.-J. Xu, C. Li, J.-L. Yu, Y.-T. Wu, H.-F. Huang. A PPARG Splice Variant in Granulosa Cells Is Associated with Polycystic Ovary Syndrome. J. Clin. Med., **11**, 7285. (2022)
9. K.-K. Ryan, B. Li, B.-E. Grayson, E.-K. Matter, S.-C. Woods, R.-J. Seeley. A role for central nervous system PPAR-γ in the regulation of energy balance. Nat Med, **17**, 623-626 (2011)
10. M. Ahmadian, J. Suh, N. Hah, C. Liddle, A. Atkins, M. Downes, R. Evans. PPARγ signaling and metabolism: the good, the bad and the future. Nature Medicine, **99**(5), 557–566 (2013)
11. B. Ramly, N. Afqah, Z.-A. Mohamed. Protein–protein interaction network analysis reveals several diseases highly associated with polycystic ovary syndrome. Int J Mol Sci., **20**, 2959 (2019)
12. S. Willems, L. Gellrich, A. Chaikuad, S. Kluge, O. Werz, J. Heering. Endogenous vitamin E metabolites mediate allosteric PPARγ activation with unprecedented co-regulatory interactions. Cell Chem Biol, **28**(10)**,** 1489-1500 (2021)
13. A. Najmi, M.-S. Alam, N. Thangavel, M. Taha, A.-M. Meraya, M. Albratty M. Synthesis, molecular docking, and in vivo antidiabetic evaluation of new benzylidene-2,4-thiazolidinediones as partial PPAR-γ agonists. Sci Rep, **13**(1), 19869 (2023)

14. T. Pantsar, A. Poso. Binding Affinity via Docking: Fact and Fiction. Mol Basel Switz, **23**(8), 1899 (2018)
15. J.-B Bruning, M. Chalmers, S. Prasad, S. Busby, T. Kamenecka, Y. He Y, et al. Partial Agonists Activate PPARγ Using a Helix 12 Independent Mechanism. Structure, **15**(10), 1258–71 (2007)
16. L. Han, W.-J Shen, S. Bittner, F. Kraemer, S. Azhar. PPARs: Regulators of metabolism and as therapeutic targets in cardiovascular disease. Part II: PPAR-beta/delta and PPAR-gamma. Future Cardiol, **13**, 279–296 (2017)
17. A. Christofides, E. Konstantinidou, C. Jani, V. Boussiotis. The role of peroxisome proliferator-activated receptors (PPAR) in immune responses. Metabolism, **114**, 154338 (2021)
18. W. Chen W, Y. Pang. Metabolic Syndrome and PCOS: Pathogenesis and the Role of Metabolites. Metabolites, **11**, 869 (2021)
19. M. Shokrpour, F. Foroozanfard, Ebrahimi, Z. Vahedpoor Z, E. Aghadavod, A. Ghaderi, Z. Asemi. Comparison of myo-inositol and metformin on glycemic control, lipid profiles, and gene expression related to insulin and lipid metabolism in women with polycystic ovary syndrome: A randomized controlled clinical trial. Gynecol. Endocrinol, **35**, 406–411 (2019)
20. A. Rutz, J. Bisson, P. Allard. The LOTUS Initiative for Open Natural Products Research: frozen dataset union wikidata (with metadata). Zenodo (2023)
21. A.-D. Genazzani. Inositol as putative integrative treatment for PCOS. Reproductive BioMedicine Online, **33**(6) (2016)
22. B. Fauser, B. Tarlatzis, R.-W. Rebar, R.-S. Legro, A.-H. Balen, R. Lobo, E. Carmina, J. Chang, B.-O. Yildiz, J.S. Laven, J. Boivin. Consensus on women's health aspects of polycystic ovary syndrome (PCOS): the Amsterdam ESHRE/ASRM-Sponsored 3rd PCOS Consensus Workshop Group. Fertility and sterility, **97**(1), 28-38 (2012).