

***In Silico* biological evaluation of propolis for modulating Ghrelin, Leptin and CYP17A1 gene pathways in Polycystic Ovary Syndrome**

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ABSTRACT: The study focused on identifying the potential phytochemicals from the propolis, a beehive byproduct, as a modulator in Ghrelin, Leptin and CYP17A1 gene pathways. From ADMET and molecular docking analysis, Quercetin and Chrysin show good binding interactions with target receptors and obey drug-like properties compared to the standard drug Lorcaserin. The Molecular dynamics simulation of protein-ligand complexes revealed Quercetin-binding proteins are slightly lower RMSD indicating a stable association of Quercetin with the Ghrelin receptor. © 2025 Association for Advancement of Entomology

KEY WORDS: Docking, molecular dynamics, toxicity, molecules screening analysis

INTRODUCTION

Propolis is a beehive byproduct that has been used as a natural remedy for centuries. It has antimicrobial, anti-inflammatory and antioxidant properties and has many health benefits. Propolis is widely used to treat ulcers and swelling in the mouth, cold sores, and diabetes. Additionally, it is used to treat wound healing, genital herpes, burns, and canker sores. (Ashmitha *et al.*, 2024). Aside from these potentially beneficial substances, propolis also includes minerals like magnesium and potassium, as well as amino acids, vitamins A, C and E (Cirasino *et al.*, 1987). Specifically, propolis contains polyphenols called flavonoids. In addition to its flavonoid concentration, it possesses anti-allergy, immunomodulatory, anti-cancer, anti-fungal, and anti-ageing properties (Castaldo *et al.*, 2002).

Propolis has been demonstrated in studies to control ovarian hormone function as well as promote digestive system development and function. Polyphenols are antioxidants that fight disease and damage in the body (Sapmaz *et al.*, 2022). In this study, we aimed to identify the potential bioactive molecules in propolis to target the Ghrelin, Leptin, and CYP17A1 gene receptors using ADMET, molecular docking, and molecular dynamics simulation approaches. It's a new avenue way to control PCOS issues.

MATERIALS AND METHODS

ADMET analysis: The structure of compounds present in propolis was obtained from PubChem and While conducting docking research, it is crucial to assess the drug-likeness property using the Qik

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prop module to forecast their druggability property, such as the Absorption, Distribution, Metabolism Excretion, and Toxicity (ADMET). The CNS, blood-barrier coefficient, human blood absorption, and Lipinski's rules of three and five were among the significant characteristics that were examined. The bioactive chemicals that demonstrated practical outcomes were selected for the ADMET, and the docking rationale has been tabulated with the preferred docking poses (Puratchikody *et al.*, 2019).

Molecular docking: The 3D structure of obesity-responsible proteins was retrieved from the Protein Data Bank (www.rcsb.org). The protein structure was initially created using the BIOVIA DS visualiser's Prepare protein setup before the docking tests. Preparing the protein is an optimisation procedure that addresses charge anomalies, atomic and bond lengths, and structure. The protein structure was cleared of water molecules and the first inhibitor. Using Maestro v10.2's protein residue template, hydrogens were inserted again. After that, the optimisation phase was used to produce stable conformation and docking analysis was performed by converting to PDBQT format. The bio constituents Quercetin, Chrysin, Quercetin pentamethyl ether, 2, 4,4-Trimethyl, Ethyl alpha-d-glucopyranoside, Hexacosane, Capric acid, Carprofen, Tetracontane identified from Propolis were retrieved from PubChem chemical databases. The ligands were imported to the workspace Schrodinger software and preparation was done.

Molecular dynamics: M D simulations were performed using the Desmond package on the OPLS3e force field. As mentioned in other works, the simulation process and additional experimental data were used precisely (Balasubramaniyan *et al.*, 2018). At 100 ps intervals during the 100 ns runtime of the experiment, trajectory snapshots were taken. M D trajectories were examined using the Desmond Simulation Interaction Diagram (SID) to predict the binding configuration and stability of the ligand.

RESULTS AND DISCUSSION

ADMET analysis: Each component of ADMET

addresses a fundamental aspect of a drug's absorption through the human body. Absorption determines how effectively a drug enters the bloodstream from its site of administration. Distribution examines the extent and manner in which the drug spreads to various tissues and organs. Metabolism involves the biochemical modifications the drug undergoes, primarily in the liver, influencing its activity and duration of effect. Excretion covers the pathways and efficiency of drug elimination from the body, while toxicity assesses the potential adverse effects, ensuring safety at therapeutic doses. Optimizing these parameters helps in predicting the *in vivo* behavior of drug candidates, thereby reducing the risk of late-stage failures in clinical trials.

In parallel, drug-like properties are essential for ensuring that a compound not only exhibits the desired biological activity but also possesses favorable characteristics for development into a viable drug. Key properties consist of polar surface area, hydrogen bonding ability, lipophilicity, and molecule size which influence a drug's solubility, permeability, and overall pharmacokinetic profile. A balance between these properties is crucial; for instance, a compound needs to be lipophilic enough to cross cell membranes yet not so lipophilic that it becomes insoluble in aqueous environments. Similarly, appropriate molecular size and limited rotatable bonds enhance the drug's bioavailability and stability. By rigorously evaluating and optimizing these properties alongside ADMET criteria, researchers can more effectively identify and refine drug candidates with the highest potential for success in clinical development and eventual therapeutic use.

From the GCMS analysis of Propolis, 44 compounds were identified. These compounds were subjected to the ADMET analysis to predict the drug-likeness properties. Quikprop 4.4 in the Schrodinger and Swiss ADME suite was used to anticipate the activities of biochemical compounds. Drug likeness properties were determined based on Molecular weight, CNS activity, metabolic processes, human oral absorption, percent human oral absorption, brain/blood partition coefficient, dipole moment,

Table 1. Drug-like properties of the selected 13 propolis compounds

Compound	Molecular weight	Q Plog BB	CNS	Human oral absorption %	Human oral absorption %	Lipinski rule
1-[2-(2-Acetoxy-ethoxy)-5-methyl-phenyl]-3-trimethylsilyloxy-2-aza-1,3-butadiene	335.474	-0.786	-1	1	100	0
2,4,4-Trimethyl-5-(1,1,3,3-tetramethylisoindol-2-yl) oxypentan-2-ol	319.486	0.331	1	3	100	0
Trimethylsilyl 3- (trimethylsilyl amino) propanoate	233.457	-0.147	0	3	100	0
Chrysin	254.242	-0.876	-1	3	87.1	0
Ethyl alpha-d-glucopyranoside	208.211	-1.293	-2	2	60.7	0
Quercetin	372.374	-0.075	0	3	100	0
Capric acid	172.26	0.142	-2	3	94	0
Carprofen	284.484	-0.195	-1	2	91.3	1
Tetracontane	563.096	-0.107	2	1	82.7	2
Hexacosane	366.718	1.128	0	2	87.6	1

volume, and total solvent accessible surface (SASA) are among the factors that influence the Lipinski's rule of five and the Jorgensen's rule of three. Out of the 44 compounds, 13 compounds show good drug-like properties. The predicted properties have been evaluated and the drug likeness properties of screened 13 Propolis compounds. These molecules obey the standard drug-like properties (Table 1).

Molecular docking: *In silico* molecular docking, analysis is performed against various enzymes that induce PCOS, including Ghrelin, Leptin, and CYP17A. In this docking study, the selected 13 compounds from the ADMET analysis were subjected to further docking studies. From the docking result, the Quercetin and Chrysin molecules show a good binding score compared to the standard drug Lorcaserin (Table 2).

Quercetin with the Ghrelin receptor: In these docking results, the good binding interaction molecules of Quercetin and chrysin with the respective receptor were mentioned and discussed. The 3D and 2D interactions of the Quercetin with

the Ghrelin receptor is -9.1 Kcal/mol. The binding score of the Quercetin with The OH group of the Quercetin forms one strong Hydrogen bond with the His 210 residue with 2.39Å bond length (Fig. 1). Further, the aromatic benzene groups are involved in the Alkyl and Pi-Alkyl interactions.

Chrysin with Leptin: The Chrysin molecules show a good binding score with the Leptin receptor. The biding score of the Chrysin with Leptin is -7.1 Kcal/mol. In these binding interactions, the two methoxy and ketone groups forms the three strong hydrogen bond with the Val 123, Ser127, Gln130. The bond length of these interactions is 2.37 Å, 2.08 Å and

Table 2. Molecular docking score of compounds in propolis

Ligand	Ghrelin	Leptin	CYP17A
Quercetin	-9.1	-7.0	-8.3
Chrysin	-8.3	-7.1	-8.2
Lorcaserin	-4.3	-4.8	-6.5

2.57 Å respectively. The Ile 24 amino acid interacted with Quercetin by Pi-Sigma interactions (Fig. 2).

Quercetin with CYP17: The Quercetin molecules show a good binding score compared to the other molecules (-8.3Kcal/mol). In this docking analysis, the active site amino acids of Gly297 and Asp 298 form two strong hydrogen bonds with the methoxy groups and the bond length is 2.4 Å and 2.87 Å respectively. The other active site amino acids Gly301, Ala113, Val482 form Ile 205 Alkyl and Pi-Alkyl interactions with the Quercetin molecules (Fig. 3).

Standard drug Lorcaserin: The Lorcaserin Standard drug shows a binding score of - 4.8Kcal/mol. In this docking analysis, the active site amino acids of Leu 13 in Leptin with aromatic benzyl group and bond length of 4.8 Å with Pi-sigma interaction (Fig. 4a). The Lorcaserin Standard drug shows a binding score of -6.5Kcal/mol. In this docking analysis, the active site amino acids of Leu 66, Phe 42, Pro 44, and PHE 53 with aromatic benzyl group and bond lengths of 4.4 Å, 4.5 Å, 4.7 Å, 5.0 Å, 4.3 Å. The aromatic benzyl group of Lorcaserin form alkyl, Pi-pi stacked, Pi alkyl interactions with the respective amino acids (Fig. 4b). In this docking analysis, the active site amino acids of Asp 3, Phe 4, Ser 6, and Glu 9 with aromatic benzyl group, strong hydrogen bond, and chloride bond with binding lengths of 4.4 Å, 4.5 Å, 4.7 Å, 5.0 Å, 4.3 Å. The aromatic benzyl group and conventional hydrogen bonds of Lorcaserin form Pi-pi, and Carbon hydrogen bonds with the respective amino acids (Fig. 4c).

Molecular dynamic simulation: To analyse the stability of the protein, a molecular dynamic simulation was performed using the best docking score obtained from the Quercetin-Ghrelin complex. The stability of ligands in the aqueous environment of the chosen target's active site and the role of significant amino acids in the interaction between ligand and protein can both be investigated using molecular dynamics (MD) modelling, which is a highly helpful method. When it comes to the Ghrelin receptor, the quercetin molecule has a better docking

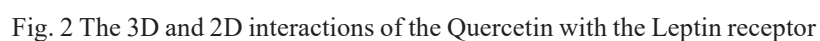
score than the target receptors in obesity.

The RMSD parameter and the protein-ligand interactions were used to analyse the MD trajectories. The stability and flexibility of molecular docked complexes are discussed in these works. This investigation was carried out following the MM-GBSA computation. The protein-ligand interaction is discussed in this work, and the protein-ligand complex's RMSD fluctuation and variation can be evaluated. If the complex was found to be within the 4Å range, it was considered stable.

MD simulation of Quercetin with the Ghrelin complex for 100 ns in order to assess the stability of the complexes and observe potential ligand binding patterns. The protein complex with the backbone of the quercetin molecule exhibited a shift in RMSD from 0 to 2.34 Å over the first 60 ns (Fig. 5). Additionally, throughout the simulation, the protein configuration's RMSD value increased until, after 58 ns, it stabilised at 2.53 Å. More stability is maintained by the Quercetin molecule in the protein-ligand complex, as evidenced by the average RMSD of the crystal structure complex with the reference molecule of roughly 2.53 Å. Throughout the simulation, the RMSD's variation did not go above 2.4 Å. Additionally, the average 2.61 Å low RMSD values show that the quercetin ligands have established a stable complex during the MD simulation and have attained good equilibrium in the Ghrelin protein's active region.

The Ghrelin complex with Quercetin RMSF graphs is displayed in (Fig. 6). The Ghrelin complex with Quercetin had a mean RMSF value of 1.0 Å. The less flexible residues in the active region are confirmed by the smaller RMSF differential between the ligand and receptor. Based on these findings, it was deduced that quercetin and ghrelin bind together to form stable complexes that do not alter the locations of the active site residues. Although there are minor variations in some residues of Leu 138, Arg178, Arg129, Ala232, and Val181, the overall stability of the complex under study is unaffected by these variations.

Propolis is one of the most significant compounds



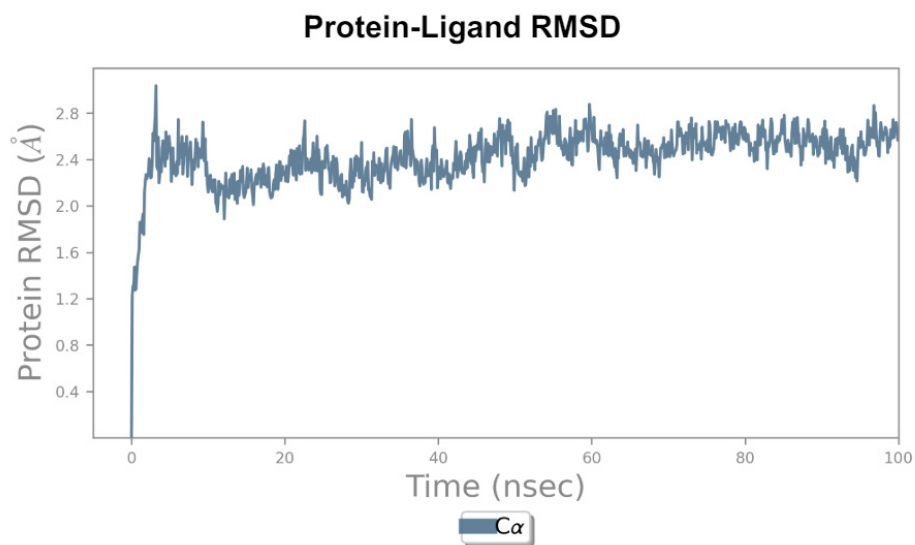


Fig 5. Root mean squared deviation of Quercetin with the Ghrelin complex for 100 ns

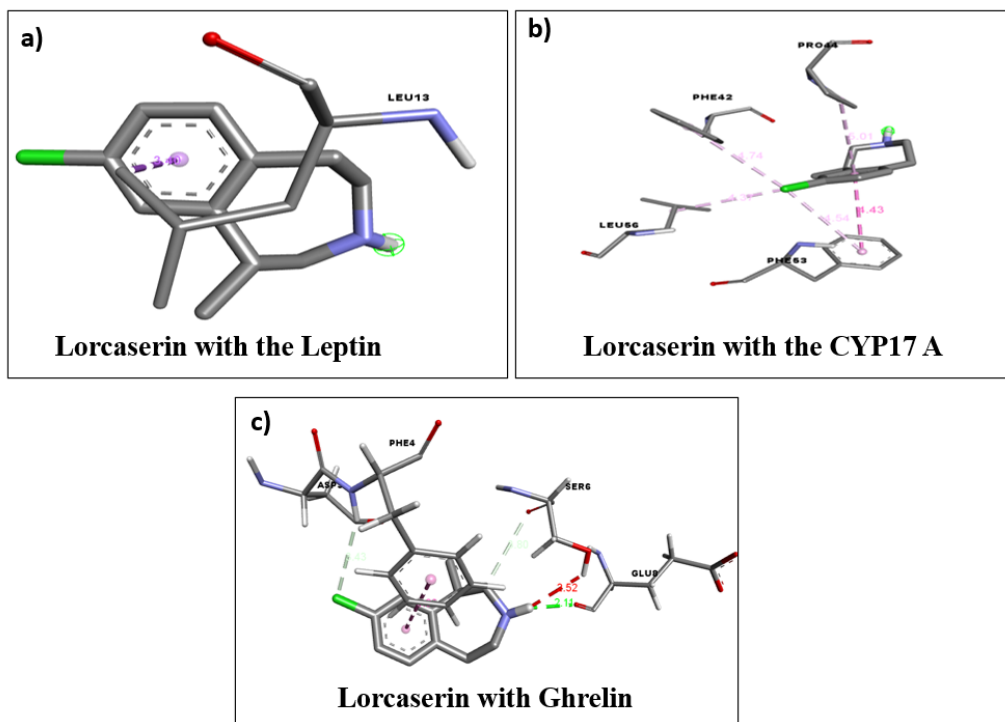


Fig. 4 The 3D binding interactions of Lorcaserin with a) Leptin, b) CYP17A and c) Ghrelin receptor

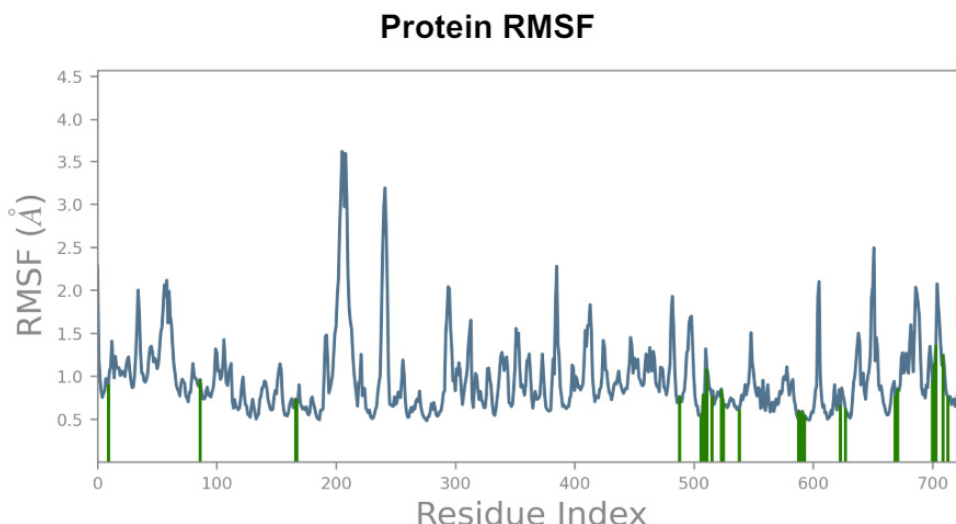


Fig. 6 Root mean squared fluctuation of Quercetin with the Ghrelin complex for 100 ns

with a wide range of biological functions that is produced by various species of honeybees. In this study, ADMET, molecular docking, and molecular dynamics simulations on propolis phytochemicals. Based on the ADMET analysis, screened the best drug-like properties contained in phytochemicals. From the molecular docking analysis, Quercetin and Chrysin ligands as an excellent candidates as inhibitors in Ghrelin, Leptin and CYP17A1 gene receptors comparable with other ligands and standard drugs. The molecular dynamics simulation demonstrates that Quercetin can also serve as good candidates as inhibitors and maintain the ligand-protein (Ghrelin) stability. Further research should be conducted to verify the potency and safety of Quercetin and Chrysin ligands in the treatment of PCOS.

REFERENCES

- Ashmitha E., Aarthi N. and Harathi B. (2024) Investigating the Therapeutic Potential of Propolis Extract Against Diabetes-associated Enzymes. *African Journal of Biological Sciences* 6(5):1289–1299.
- Balasubramaniyan S., Irfan N., Umamaheswari A. and Puratchikody A. (2018) Design and virtual screening of novel fluoroquinolone analogs as effective mutant DNA GyrA inhibitors against urinary tract infection-causing fluoroquinolone resistant *Escherichia coli*. *RSC advances* 8(42), 2362–23647.
- Stener-Victorin, E., Teede, H., Norman, R. J., Legro, R., Goodarzi, M. O., Dokras, A., and Piltonen, T. T. (2024) Polycystic ovary syndrome. *Nature Reviews Disease Primers* 10(1): 2–35.
- Castaldo S. and Capasso F. (2002) Propolis, an old remedy used in modern medicine. *Fitoterapia* 73: 1–6.
- Daghestani M.H., Daghestani M., Daghistani M., El-Mazny A., Bjørklund G, Chirumbolo S. and Warsy A. (2018) A study of ghrelin and leptin levels and their relationship to metabolic profiles in obese and lean Saudi women with polycystic ovary syndrome (PCOS). *Lipids in health and disease* 17: 1–9.
- Doi S.A., Al-Zaid M., Towers P.A., Scott C.J. and Al-Shoumer K.A. (2005) Ovarian steroids modulate neuroendocrine dysfunction in polycystic ovary syndrome. *Journal of Endocrinological Investigation* 28: 88–892.
- Glintborg D., Andersen M., Hagen C., Frystyk J., Hulstrøm V., Flyvbjerg A. and Hermann A.P. (2006) Evaluation of metabolic risk markers in polycystic ovary syndrome (PCOS). Adiponectin, ghrelin, leptin and body composition in hirsute PCOS patients and controls. *European journal of endocrinology* 155(2): 337–345.
- Panda P.K., Rane R., Ravichandran R., Singh S. and

- Panchal H. (2016) Genetics of PCOS: A systematic bioinformatics approach to unveil the proteins responsible for PCOS. *Genomics data* 8: 52–60.
- Puratchikody A., Irfan N. and Balasubramaniyan S. (2019) Conceptual design of hybrid PCSK9 lead inhibitors against coronary artery disease. *Biocatalysis and Agricultural Biotechnology* 17: 427–440.
- Qin K.N. and Rosenfield R.L. (1998) Role of cytochrome P450c17 in polycystic ovary syndrome. *Molecular and cellular endocrinology* 145(1-2): 111–121.
- Rezgoun M.L., El Khour D., Bendaoud H. and Chellat D. (2024) CYP17A1 (rs74357) polymorphism and polycystic ovary syndrome risk: a systemic review and meta-analysis. *Acta Biomedica Atenei Parmensis* 94(4): e2023167.
- Sapmaz T., Sevgin K., Topkaraoglu S., Tekayev M., Gumuskaya F., Efendic F. and Irkorucu O. (2022) Propolis protects ovarian follicular reserve and maintains the ovary against polycystic ovary syndrome (PCOS) by attenuating degeneration of zona pellucida and fibrous tissue. *Biochemical and Biophysical Research Communications* 636: 97–103.
- Shaw L.J., Bairey Merz C.N., Azziz R., Stanczyk F.Z., Sopko G, Braunstein G.D., Kelsey S.F., Kip K.E., Cooper-Dehoff R.M., Johnson B.D., Vaccarino V., Reis S.E., Bittner V., Hodgson T.K., Rogers W. and Pepine C.J. (2008) Postmenopausal women with a history of irregular menses and elevated androgen measurements at high risk for worsening cardiovascular event-free survival: results from the National Institutes of Health-National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation. *The Journal of Clinical Endocrinology & Metabolism* 93: 1276–1284.

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