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Phytochemical Screening and Molecular Docking Studies of *Croton bonplandianus* Baill. Leaf Extract against Polycystic Ovary Syndrome (PCOS)-Related Targets

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Abstract

Polycystic ovary syndrome (PCOS) is a common hormonal disorder affecting women of reproductive age and is associated with infertility, insulin resistance, and metabolic abnormalities. The present study aimed to evaluate the anti-PCOS potential of the ethanolic leaf extract of *Croton bonplandianus* Baill. Preliminary phytochemical screening confirmed the presence of several bioactive compounds, including alkaloids, flavonoids, phenolics, tannins, saponins, and phytosterols. GC–MS analysis identified important phytoconstituents, which were further subjected to molecular docking against selected PCOS-related protein targets, including androgen receptor, insulin receptor, follicle-stimulating hormone receptor, luteinizing hormone receptor, and aromatase enzyme. Among the tested compounds, ergostane derivative, α-tocopherol acetate, and phytol showed strong binding affinity toward the target proteins. These findings suggest that *Croton bonplandianus* possesses promising phytochemicals that may be useful in the management of PCOS. Further in vivo studies are required to validate these results.

Keywords

Croton bonplandianus; Polycystic ovary syndrome (PCOS); Phytochemical screening; GC–MS; Molecular docking; Phytoconstituents.

INTRODUCTION:

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders affecting women of reproductive age, characterized by hyperandrogenism, ovulatory dysfunction, and polycystic ovarian morphology. According to the Rotterdam criteria, diagnosis requires the presence of at least two of these features.(1) In addition to reproductive abnormalities, PCOS is associated with metabolic disturbances including insulin resistance, obesity, dyslipidaemia, and impaired glucose metabolism, increasing the risk of type 2 diabetes mellitus and cardiovascular diseases.(2)

Globally, PCOS affects approximately 6–20% of reproductive-aged women, with prevalence varying according to diagnostic criteria and population characteristics.(3) The condition commonly presents with menstrual irregularities, infertility, acne, and hirsutism, and is frequently accompanied by obesity, metabolic syndrome, anxiety, and depression, significantly affecting quality of life.(4) In India, prevalence rates range

from 9% to 22.5%, particularly among adolescents and young women, largely attributed to urbanization, sedentary lifestyles, unhealthy dietary habits, and rising obesity rates.(5,6)

The etiology of PCOS is multifactorial, involving genetic, endocrine, environmental, and metabolic factors. Insulin resistance plays a central role by promoting ovarian androgen production and disrupting normal follicular development and ovulation.(7)Furthermore, dysregulation of the hypothalamic–pituitary–ovarian axis, chronic low-grade inflammation, and oxidative stress contribute to disease progression and symptom severity.(8,9)

Current treatment strategies include hormonal contraceptives, insulin-sensitizing agents such as metformin, ovulation-inducing drugs, and anti-androgen therapies.(10) However, these treatments mainly target symptoms and may be associated with adverse effects, highlighting the need for safer and more comprehensive therapeutic approaches.(11)

Medicinal plants have gained considerable attention as potential alternatives or adjuncts in PCOS management. Traditional medical systems such as Ayurveda, Siddha, and Unani utilize plant-based remedies rich in bioactive compounds, including flavonoids, alkaloids, terpenoids, and phenolics.(12) These phytoconstituents may improve insulin sensitivity, reduce oxidative stress, regulate hormonal imbalance, and support normal ovarian function. Consequently, medicinal plants represent a promising area of research for the development of effective and safer therapies for PCOS.(13)

Croton bonplandianus Baill. was first described in *Adansonia* 4:339 (1864) and is currently an accepted species. The species is native to a region extending from southern Bolivia to Uruguay, where it occurs as a subshrub or shrub in seasonally dry tropical habitats. Its natural distribution includes northeastern and northwestern Argentina, Bolivia, southern and west-central Brazil, Paraguay, and Uruguay. Outside its native range, it has been introduced to several countries and regions, including Bangladesh, Borneo, Cambodia, the Comoros, the Eastern and Western Himalayas, the Gulf States, India, Kenya, Laos, Malaya, Maryland, Mauritius, Myanmar, Nepal, the Nicobar Islands, Pakistan, Rodrigues, Réunion, Sri Lanka, Sulawesi, Taiwan, and Thailand

The species commonly occurs as a weed along roadsides, riverbanks, and wastelands, often forming dense populations. Its flowers attract a variety of pollinators and other insects, thereby contributing to local biodiversity. It also serves as an important host plant for jewel bugs and plays a valuable ecological role in maintaining insect populations and ecosystem.(14)

In view of the rising incidence of Polycystic Ovary Syndrome (PCOS) and the need for novel therapeutic agents, the present study aimed to perform phytochemical screening of the leaf extract to identify its bioactive constituents and subsequently assess their potential anti-PCOS activity through molecular docking studies against selected PCOS-related molecular targets.

MATERIALS AND METHODS:

COLLECTION OF PLANT MATERIALS

Fresh leaves of *Croton bonplandianus* were collected from a suitable geographical location. The plant material was authenticated at the Siddha Central Research Institute (CCRS), Chennai, Ministry of AYUSH, Government of India. The sample was identified as *Croton bonplandianus* Baill. (Leaf) and assigned authentication code C18032604B. The certificate of authentication was obtained and retained for reference. The fresh leaves were collected between January 2026 –February 2026. The collected leaves were further cleaned and shade dried. The dried materials were coarsely powdered by the means of mechanical grinder and sieved using sieve No.20. The sieved powder material was used for extraction process.

PREPARATION OF ETHANOLIC EXTRACT OF *Croton bonplandianus Baill leaves* (CBEL)

The powdered plant material was first defatted with petroleum ether and further 150 gm was subjected to continuous hot extraction method using ethanol with Soxhlet apparatus. The obtained alcoholic filtrate was evaporated using electrical water bath to obtain solid residue. The obtained ethanolic extract of ***Croton bonplandianus Baill*** leaves was subjected to preliminary phytochemical studies.

PRELIMINARY PHYTOCHEMICAL STUDIES:[15]

1. Detection of Alkaloids

a) Mayer's test:

The extract was treated with few drops of Mayer's reagent [Potassium mercuric iodide solution]. Formation of cream coloured precipitate indicates the presence of alkaloid.

b) Dragendroff's test:

The extract was treated with few drops of Dragendroff's reagent [Potassium bismuth iodide solution]. Formation of reddish-brown precipitate indicates the presence of alkaloids.

c) Wager's test:

The extract was treated with few drops of Wager's reagent [Solution of iodine in potassium iodide]. Formation of brown precipitate indicates the presence of alkaloids.

d) Hager's test:

The extract was treated with few drops of Hager's reagent [Saturated solution of Picric acid]. Formation of yellow colour precipitate indicates the presence of alkaloids

2.Test for carbohydrates

a) Molisch's test:

The extract was treated with few drops of alcoholic alpha naphthol, then added few drops of concentrated sulfuric acid slowly through the sides of the test tube. Formation of purple to violet colour ring at the junction of test tube indicates the presence of carbohydrates.

b) Barfoed's test:

The extract was treated with few drops of barfoed's reagent and heated for 2 minutes. Formation of red precipitate indicates the presence of carbohydrates.

c) Seliwanoff's test:

The extract was treated with 3ml of Seliwanoff's reagent and heated on water bath for 1minute. Formation of rose red colour (ketoses) indicates the presence of carbohydrates.

3. Detection of reducing sugars

a) Benedict's test:

The extract was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled on water bath. Formation of reddish-brown precipitate indicates the presence of reducing sugar.

b) Fehling's test:

The extract was treated with Fehling's solution A and B, heated for few minutes. Formation of brick red precipitate indicates the presence of reducing sugar.

4.Detection of glycosides

a) Borntrager test:

The extract was treated with 5-10ml of dilute HCl, boiled on water bath for 10mins and filtered. The filtrate was extracted with benzene or CCl_4 and added equal amount of ammonia solution to filtrate and shaken. Formation of pink or red colour in ammoniacal layer due to presence of anthraquinone moiety indicates the presence of glycosides.

b) Modified Borntrager test:

The extract was treated with 5ml of dilute HCl followed by 5ml ferric chloride. Boiled for 10mins and filtered. The filtrate was extracted with benzene or CCl4 and added equal amount of ammonia solution to filtrate and shaken. Formation of pink or red colour in ammoniacal layer due to presence of anthraquinone moiety indicates the presence of glycosides.

5.Detection of proteins and amino acids

a) Biuret test:

The extract was treated with 1ml of 10% sodium hydroxide, 1ml of 1% copper sulphate solution. Formation of violet colour indicates the presence of proteins and amino acids.

b) Ninhydrin test:

The extract was boiled with 0.2% solution of Ninhydrin (Indane 1,2,3 trione hydrate). Formation of violet colour indicates the presence of proteins and amino acids.

c) Xanthoprotein test:

The extract was treated with 2ml of concentrated nitric acid. Formation of orange colour indicates the presence of proteins and amino acids.

5.Detection of Flavonoids

a) Alkaline reagent Test:

The extract was treated with 10 ml of ammonium hydroxide. Formation of yellow fluorescence indicates the presence of flavonoids.

b) Ferric chloride test:

The extract was treated with few drops of 10% ferric chloride solution. Formation of Green precipitate indicates the presence of flavonoids.

c) Lead acetate test:

1 ml of the extract filtrate was taken in a test tube. 3–5 drops of 0.1% lead acetate solution were added, The formation of a gelatinous precipitate indicated the presence of tannins.

6.Detection of phenolic compounds

a) Iodine test:

The extract was treated with few drops of diluted iodine solution. Formation of transient red colour indicates the presence of phenolic compounds.

b) Ferric chloride test:

The extract was treated with 1ml of water and boiled for few minutes then it is filtered. The filtrate was treated with 5% ferric chloride solution. Formation of bluish black colour indicates the presence of phenolic compound.

7.Detection of Tannins

a) Gelatine test:

The extract was treated with 2ml of 1% gelatine and 10% of sodium chloride. Formation of white precipitate indicates the presence of Tannins

b) 10% sodium hydroxide test:

The plant extract was treated with 4 ml of 10% of sodium hydroxide solution and shaken well. Formation of emulsion indicates the presence of tannins.

8.**Detection of saponins**

a) Foam test:

The extract was treated with 10 ml of water and boiled for few minutes then it is filtered. The filtrate shaken well and noted for the stable foam.

9.Detection of Phytosterols

a) Salkowski Test:

The extract was treated with 2ml of chloroform, then added few drops of concentrated sulphuric acid, shaken and allowed to stand for some time. Formation of yellow colour ring at the junction which turns red after 2mins indicates the presence of phytosterols.

b) Libermann Burchard Test:

The extract was treated with 2ml of chloroform, small amount of acetic anhydride and 1ml of concentrated sulphuric acid. Change of colour from red to bluish green ring at the junction indicates the presence of phytosterols.

10.Detection of Quinones

a) Alcoholic KOH test:

To 1ml of plant extract, added few ml of alcoholic potassium hydroxide. Formation of red to blue colour indicates the presence of quinones.

b) Concentrated HCl test:

The extract was treated with few ml of concentrated HCl. Formation of green colour indicates the presence of quinones.

11.Detection of fixed oils and fats

a) Stain test:

Little quantity of plant extract was pressed in between two filter paper. Appearance of oil stain indicates the presence of fixed oils and fats.

Mass spectroscopy (GC–MS) analysis of *Croton bonplandianus* ethanolic extract

In the present study, GC–MS analysis was carried out to determine the chemical composition of the *Croton bonplandianus* ethanolic extract. The analysis enabled the identification of several phytoconstituents based on their retention times and mass spectral data by comparison with the NIST library. The identified compounds provide valuable insights into the phytochemical profile of the plant and help explain its reported therapeutic properties. Furthermore, the GC–MS findings serve as a scientific basis for selecting promising bioactive compounds for subsequent computational and pharmacological studies, including molecular docking and *in-vivo* investigations.

MOLECULAR DOCKING:

Molecular docking is a computational *in silico* approach used to predict the interaction between a ligand and a target protein, resulting in the formation of a stable ligand–protein complex. This technique identifies the most energetically favorable orientation of a ligand within the active site of a protein and estimates the binding affinity to evaluate the strength and stability of the interaction. As a key component of Structure-Based Drug Design (SBDD), molecular docking plays a crucial role in understanding the molecular basis of ligand–receptor recognition and interaction.

Protein–ligand docking specifically models the binding of a ligand to the active site of a receptor protein, predicting the optimal binding conformation based on structural and chemical complementarity. The resulting docking score provides an estimate of the ligand's binding potential and the stability of the formed complex. Additionally, molecular docking offers detailed insights into binding energy, free energy of interaction, hydrogen-bond formation, hydrophobic interactions, and other non-covalent forces that contribute to complex stabilization. These findings are extensively applied in computer-aided drug discovery to identify promising lead compounds and to elucidate ligand–receptor interactions at the molecular level.[16]

Software Used for Molecular Docking

The molecular docking study was carried out using the following software packages:

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- **BIOVIA Discovery Studio Visualizer:** Utilized for the visualization and preparation of protein and ligand structures, as well as for the analysis and interpretation of docking results. It was also used to examine ligand–protein interactions, including hydrogen bonds, hydrophobic interactions, and other non-covalent contacts.
- **AutoDock Tools (ADT):** Used for receptor and ligand preparation by adding polar hydrogen atoms, assigning Kollman and Gasteiger charges, defining the docking grid parameters, and generating the required

input files for molecular docking. The software facilitated the prediction of binding affinities, binding poses, and interaction profiles between the selected ligands and target proteins.

Procedure

Protein Preparation

The three-dimensional (3D) crystal structures of the target proteins were retrieved from the Protein Data Bank (PDB). Proteins with a resolution of ≤1.7 Å were preferentially selected to ensure structural accuracy for docking studies. The downloaded protein structures were imported into BIOVIA Discovery Studio Visualizer for preprocessing. All heteroatoms, including water molecules, co-crystallized ligands, and other non-essential molecules, were removed from the protein structure. The cleaned protein was subsequently saved in PDB format for further docking analysis.

Ligand Preparation

The three-dimensional structures of the selected phytoconstituents were obtained from the PubChem database in SDF format. The downloaded ligand structures were imported into BIOVIA Discovery Studio Visualizer, verified for structural integrity, and converted into PDB format for subsequent molecular docking studies.

Molecular Docking Procedure

Molecular docking was performed using AutoDock Tools (ADT) 4.2 following the standard protein and ligand preparation protocol.

1. **Preparation of the Receptor**

The prepared protein structure (PDB format) was imported into AutoDock Tools. Polar hydrogen atoms were added to the receptor, followed by the assignment of Kollman united atom charges. The receptor was then saved in PDBQT format, which is required for AutoDock docking simulations.

2. **Preparation of the Ligand**

The ligand structure was imported into AutoDock Tools and prepared by adding polar hydrogen atoms and assigning Gasteiger charges. Rotatable bonds were identified by defining the torsion tree, detecting the root atom, and assigning the appropriate number of torsions to allow ligand flexibility during docking. The prepared ligand was subsequently saved in PDBQT format.

3. **Grid Box Generation**

The receptor was selected as the macromolecule, and AutoGrid parameters were generated. Grid maps were prepared by selecting the ligand atom types and defining a grid box that completely enclosed the active site of the target protein. The grid box dimensions and center coordinates were adjusted to ensure complete coverage of the ligand-binding pocket. The grid parameter file (GPF) and grid dimension file were generated and saved for AutoGrid calculations.

4. Docking Simulation

Docking calculations were carried out using the Lamarckian Genetic Algorithm (LGA) implemented in AutoDock 4. The prepared receptor (PDBQT) and ligand (PDBQT) files were loaded, and docking parameter files (DPF) were generated. AutoGrid was first executed to calculate the interaction energy maps, followed by AutoDock to perform the docking simulations and predict the optimal ligand-binding conformations within the receptor active site.

5. Analysis of Docking Results

Upon completion of the docking simulation, the docking log (DLG) file was generated and analyzed.

The binding affinity (binding energy) of each docking pose was examined, and the conformation exhibiting the lowest binding energy (most negative docking score) with an acceptable clustering result was selected as the best binding pose. The selected docked complex was exported in PDBQT format for further analysis.

6. Visualization of Protein–Ligand Interaction

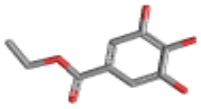
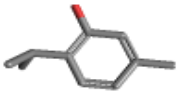
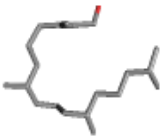
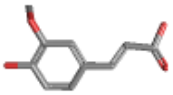
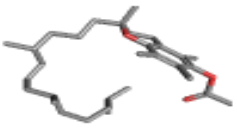
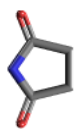
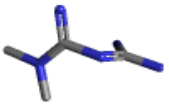
The docked protein–ligand complex was imported into BIOVIA Discovery Studio Visualizer for interaction analysis. Two-dimensional (2D) and three-dimensional (3D) interaction diagrams were generated to identify key molecular interactions, including hydrogen bonds, hydrophobic interactions, π–π interactions, π–alkyl interactions, electrostatic interactions, and van der Waals contacts between the ligand and the amino acid residues present in the binding site of the target protein.

Table:1 CBEL phytoconstituents selected from GCMS for molecular docking against different types of receptors related to PCOS

S.No	Phytoconstituent	Molecular formula	Molecular Weight (g/mol)
1.	Ethyl gallate	$C_9H_{10}O_5$	198.17
2.	Thymol	$C_{10}H_{14}O$	150.22
3.	Phytol	$C_{20}H_{40}O$	296.53
4.	Ferulic acid	$C_{10}H_{10}O_4$	194.18
5.	Avenasterol	$C_{29}H_{48}O$	412.69
6.	Ergostane derivative	$C_{30}H_{50}O_6$	506.72
7.	α-Tocopherol acetate	$C_{31}H_{52}O_3$	472.75
8.	Pyrrolidine-2,4-dione	$C_4H_5NO_2$	99.09

9.	Metformin (standard)	$C_4H_{11}N_5$	129.16

Table 2: 3D Structures of CBEL phytoconstituents

S. No	Phytoconstituent	Structure
1.	Ethyl gallate	
2.	Thymol	
3.	Phytol	
4.	Ferulic acid	
5.	Avenosterol	
6.	Ergostane derivative	
7.	Alpha- tocopherol acetate	
8.	Pyrrolidine-2,4-dione	
9.	Metformin (standard)	

Protein selected for docking studies:

- 1XQ3 (androgen receptor)
- 1IRK (insulin receptor)
- 1XWD (follicle stimulating hormone receptor)
- 7FIJ (luteinizing hormone receptor
- 3S79 (aromatase enzyme receptor)

Protein Information 1XQ3

- DOI: https://doi.org/10.2210/pdb1XQ3/pdb
- Classification: Transcription
- Organism: *Homo sapiens*
- Expression System: *Escherichia coli* BL21(DE3)
- Mutation: No
- Deposited: 2004-10-11
- Released: 2004-11-16
- Depositing authors: He, B., Gampe Jr., R.T., Kole, A.J., Hnat, A.T., Stanley, T.B., An, G., Stewart, E.L., Kalman, R.I., Minges, J.T., Wilson, E.M.

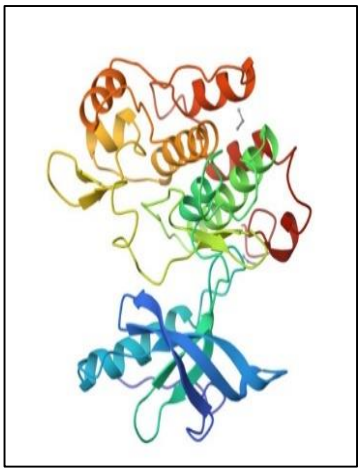


Fig 1: Structure of 1XQ3 protein

Protein information 1IRK

- DOI: https://doi.org/10.2210/pdb1IRK/pdb
- Classification: Transferase (Phosphotransferase)
- Receptor Type: Insulin Receptor (IR) – Receptor Tyrosine Kinase (RTK)
- Organism: *Homo sapiens*
- Expression System: *Escherichia coli*
- Mutation: No
- Deposited: 1995-01-02
- Released: 1995-02-27
- Depositing authors: Hubbard, S.R., Wei, L., Ellis, L., Hendrickson, W.A

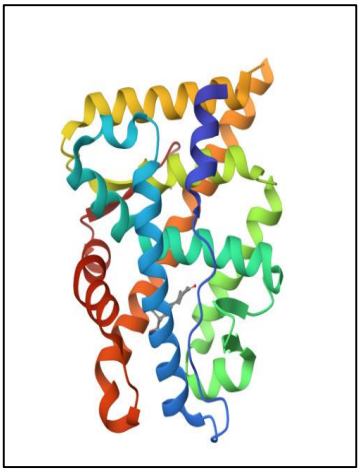


Fig 2: Structure of 1IRK protein

Protein Information 1XWD

- DOI: https://doi.org/10.2210/pdb1XWD/pdb
- Classification: Hormone/Growth Factor
- Receptor Type: Follicle-Stimulating Hormone Receptor (FSHR) – G protein-coupled receptor (GPCR), Glycoprotein hormone receptor
- Organism: *Homo sapiens*
- Expression System: *Trichoplusia ni* (High Five insect cells; Baculovirus expression system)
- Mutation: No
- Deposited: 2004-10-30
- Released: 2005-01-25
- Depositing authors: Fan, Q.R., Hendrickson, W.A.

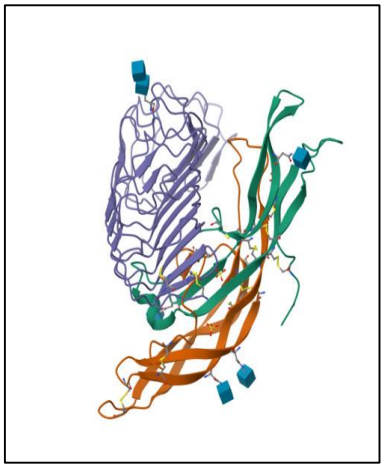


Fig 3: Structure of 1XWD protein

Protein Information 7FIJ

- DOI: https://doi.org/10.2210/pdb7FIJ/pdb
- Classification: Signaling Protein
- Receptor Type: Luteinizing Hormone/Choriogonadotropin Receptor (LHCGR) – G protein-coupled receptor (GPCR), Glycoprotein hormone receptor
- Organism: *Homo sapiens*
- Expression System: *Spodoptera frugiperda* (Sf9 insect cells; Baculovirus expression system)
- Mutation: No (wild-type receptor)
- Deposited: 2021-03-11
- Released: 2021-10-13

- Depositing authors: Duan, J., Xu, P., Cheng, X., Mao, C., Croll, T., He, X., Shi, J., Luan, X., Yin, W., You, E., Liu, Q., Zhang, S., Jiang, H., Zhang, Y., Jiang, Y., Xu, H.E

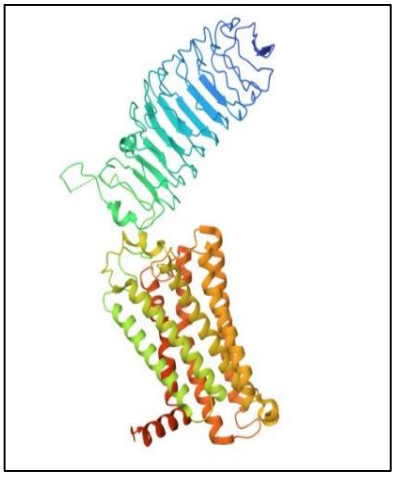


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Fig 4: Structure of 7FIJ protein

Protein Information 3S79

- DOI: https://doi.org/10.2210/pdb3S79/pdb
- Classification: Oxidoreductase
- Protein Type: Cytochrome P450 Aromatase (CYP19A1) – Heme-containing monooxygenase enzyme (Cytochrome P450 family)
- Organism: *Homo sapiens*
- Expression System: *Escherichia coli*
- Mutation: No
- Deposited: 2011-05-26
- Released: 2012-06-06
- Depositing authors: Ghosh, D.

Fig 5: Structure of 3S79 protein.

Results:

Phytochemical analysis:

Table 3: Phytochemical screening of *Croton bonplandianus* ethanolic leaf extract (CBEL)

S.NO	CHEMICAL TEST	Presence (+)/ Absence (-) of Phytoconstituents in CBEL
1.	**Detection of Alkaloids**	
	a. Dragendroff's test	(+)
	b. Hager's test	(+)
	c. Mayer 's test	(+)
	d. Wagner test	(+)
2.	**Detection of carbohydrates**	
	a. Barfoed's test	(-)
	b. Molisch's test	(+)
	c. Selliwanoff 's test	(+)
3.	**Detection of reducing sugars**	
	a. Bendict's test	(-)
	b. Fehling's test	(+)
4.	**Detection of glycosides**	
	a. Borntrager's test	(+)
	b. Modified Borntrager's test	(+)
5.	**Detection of proteins and Amino acids**	
	a. Biuret test	(-)
	b. Ninhydrin test	(+)
	c. Xanthoprotein test	(+)
6.	**Detection of flavonoids**	
	a. Alkaline reagent test	(+)
	b. Ferric chloride test	(+)
	c. Lead acetate test	(+)
7.	**Detection of Phenolic compounds**	

	a. Iodine test	(+)
	b. Ferric chloride test	(+)
	Detection of tannins	
8.	a. Gelatin test	(+)
	b. NaOH test	(+)
9.	**Detection of saponins**	
	a. Foam test	(+)
10.	**Detection of Phytosterols**	
	a. Salkowski's test	(+)
	b. Libermann Burchard test	(+)
11.	**Detection of quinones**	
	a. Alcoholic KOH test	(-)
	b. Con.Hcl test	(-)
12.	**Detection of fixed oils and Fats**	
	a. Stain test	(+)

Table 4: Mass spectral analysis (GCMS) of CBEL

s.no	*Name*	*Molecular formula*	*Area*	*Retention time*
1.	**Pyrrolidine-2,4-dione**	C4H5NO2	288848	4.176
2.	Ethane, 1,1-dimethoxy	C4H10O2	2459	4.502
3.	2-Methyl-5-hexen-3-ol	C7H14O	9693	4.789
4.	Glycerin	C3H8O3	5191	4.945
5.	Propane, 2-methyl-2-nitro-	C4H9NO2	4250	5.485
6.	Methane, diethoxy-	C5H12O2	18874	6.505
7.	3-Hexanol, 2-methyl-	C7H16O	2555	5.822
8.	2-Buten-1-ol, propanoate	C7H12O2	8844	7.281
9.	3-Fluoro-5-trifluoromethylbenzoic acid, neopentyl ester	C13H14F4O2	4893	14.306
10.	Benzenemethanol, .alpha.,.alpha.-dimethyl-	C9H12O	7202	14.556
11.	Diethyl Phthalate	C12H14O4	552035	15.552
12.	Hydroperoxide, 1,4-dioxan-2-yl	C4H8O4	114628	16.095
13.	N-Ethylformamide	C3H7NO	11852	16.458
14.	Cyclopentane, 1,1,3-trimethyl-	C8H16	52719	16.654
15.	Octane, 1-iodo-	C8H17I	7222	17.05
16.	3-Buten-2-ol	C4H8O	2912	17.638
17.	3-Methyl-5-nonylpyrrolizidine	C17H33N	8471	18.303
18.	R(-)3,7-Dimethyl-1,6-octadiene	C10H18	14987	18.768
19.	Octane, 1-iodo-	C8H17I	6396	19.816
20.	tert-Butyl cyclopropylmethyl sulfoxide	C8H16OS	8123	19.886
21.	Allyl hyponitrite	C6H10N2O2	5559	20.697
22.	**3,7,11,15-Tetramethyl-2-hexadecen-1-ol (phytol)**	C20H40O	195421	22.095
23.	cis-2-Nitro-4-t-butylcyclohexanone	C10H17NO3	5653	22.317
24.	Oxalic acid, cyclobutyl heptyl ester	C13H22O4	9045	22.683
25.	Butane, 2,2-dimethyl-	C6H14	9214	22.78
26.	2,3-Epoxyjuanislamin	C26H36O9	9153	24.909
27.	1,2,2-Trimethylpropyl trifluoroacetate	C8H13F3O2	6320	25.01
28.	1,3-Dioxan-4-one, 2-(1-methylethyl)-5-methyl	C8H14O3	4563	25.089
29.	**Ethyl gallate**	C22H24O5	4280	25.258
30.	2-Butanone, 1-(2-furanyl)-	C8H10O2	9000	26.002
31.	Ethyl 3-(6-methoxy-3-methyl-2-benzofuranyl)-3-(p-methoxyphenyl)propionate	C22H24O5	3849	25.667
32.	Sulfurous acid, butyl octyl ester	C12H26O3S	24868	26.293
33.	1,5-Hexadien-3-ol	C6H10O	12658	26.795
34.	Pentanenitrile, 4,4-dimethyl-5-oxo-	C7H11NO	14465	27.026

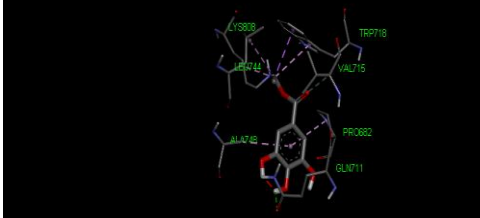
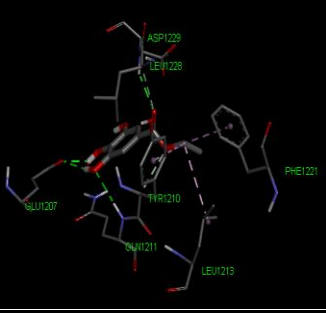
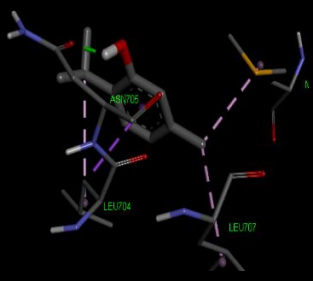
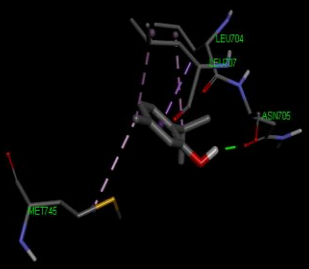
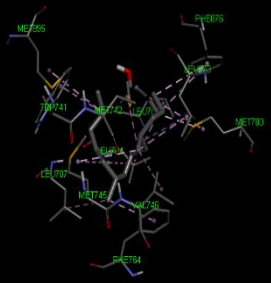
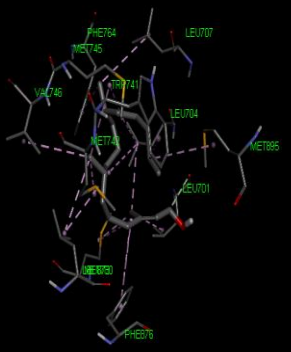
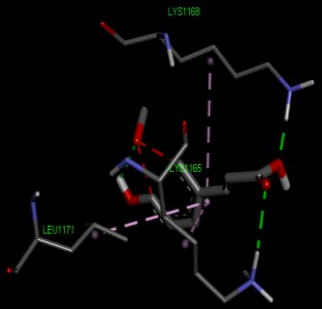
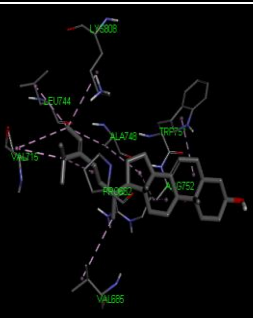
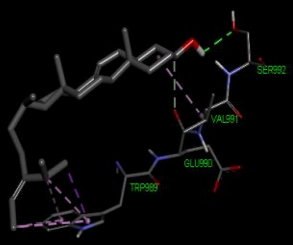
35.	Pyrrol-2(5H)-one, 4-acetyl-5-(2,4-dichlorophenyl)-1-(2-furfuryl)-3-hydroxy-	C17H13Cl2NO4	20930	27.192
36.	2-Cyclopenten-1-one, 2-methyl-	C6H8O	20151	27.299
37.	Caprolactone oxime, (NB)-O-[(diethylboryloxy)(ethyl)boryl]-	C12H25B2NO2	49342	27.448
38.	5-(Furan-2-ylmethylsulfanyl)-8-nitro-quinoline	C14H10N2O3S	57407	27.617
39.	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	C18H26O	380301	28.169
40.	Cyclopenta[e]tetrahydropyran-2-one, 5-(5-hydroxyindan-1-yl)-4a-methyl-	C18H22O3	21048	28.437
41.	1,1,3,3,5,5,7,7-Octamethyl-7-(2-methylpropoxy)tetrasiloxan-1-ol	C12H34O5Si4	18837	28.533
42.	2-Propyn-1-ol, propionate	C6H8O2	25461	28.659
43.	1,3-Bis-(2-cyclopropyl,2-methylcyclopropyl)-but-2-en-1-one	C18H26O	280259	28.996
44.	Propanoic acid, 4-hexen-1-yl ester	C9H16O2	18697	29.291
45.	2-Hexyne, 6-iodo-	C6H9I	1641	29.5
46.	Cyclopropane, 1-(5-hexenyl)-2-iodo-	C9H15I	11080	29.601
47.	2-Fluoro-3-trifluoromethylbenzoic acid, heptadecyl ester	C25H38F4O2	8345	30.05
48.	Glycine, N-(4-ethylbenzoyl)-, hexadecyl ester	C27H45NO3	6051	30.147
49.	2-Fluoro-3-trifluoromethylbenzoic acid, 6-chlorohexyl ester	C14H15ClF4O2	3739	30.375
50.	1-Benzyl-4-chloro-5-methyl-2-phenylimidazole	C17H15ClN2	13426	30.5
51.	4-Methoxymandelic acid,	C15H26O4Si2	11799	30.665
52.	Furan-2-carboxamide, N-benzyl-N-nonyl-	C21H29NO2	7572	30.783
53.	Silane, [(10-iododecyl)oxy]trimethyl-	C13H29IOSi	41530	31.046
54.	Propanedinitrile, cyclohexyl(2-methylcyclohexyl)-	C16H24N2	6607	31.158
55.	N-(3-Methyl-6-nitrobenzothiazolin-2-ylidene)-m-toluamide	C16H13N3O3S	28534	31.341
56.	Tricyclo[5.2.1.0(2,6)]dec-8-ene-3,5-dione, 4-(1,5-dimethyl-3-oxo-2-phenyl-2,3-dihydro-1H-pyrazol-4-yl)-10-oxa-4-aza-	C19H17N3O4	32012	31.512
57.	Succinic acid, 2,2,3,3,4,4,4-heptafluorobutyl 2-methylhex-3-yl ester	C15H21F7O4	41298	31.783
58.	**18-O-Feruloyloxyoctadec-9-enoic acid, methyl ester, trimethylsilyl ether**	C32H52O6Si	73777	32.025
59.	1,3,5,7,9-Pentaethyl-1,9-dibutoxypentasiloxane	C18H48O6Si5	44630	32.145
60.	cholest-20(22)-en-3-one, 4,5-epoxy-11-hydroxy-	C27H42O3	62019	32.249
61.	Pentadecafluorooctanoic acid, dodec-2-en-1-yl ester	C20H23F15O2	94702	32.4
62.	Cyclopentanecarboxamide, N-(2-phenylethyl)-N-hexadecyl-	C30H51NO	108486	32.526
63.	**Ergostane-3,5,6,12,25-pentol, 25-acetate, (3.beta.,5.alpha.,6.beta.,12.beta.)-**	C30H52O6	194339	32.664
64.	(E,E,E)-(5-Phenylsulfonylgeranyl)geraniol	C26H38O3S	194305	32.8

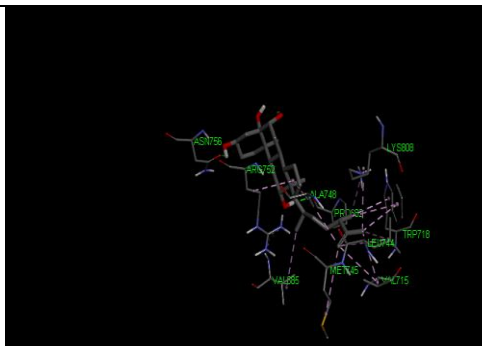
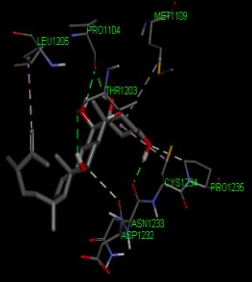
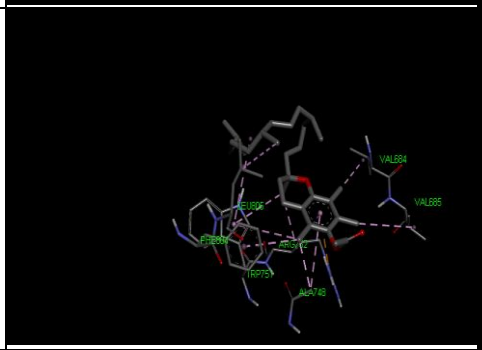
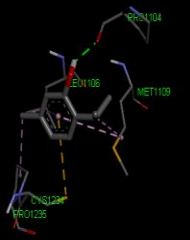
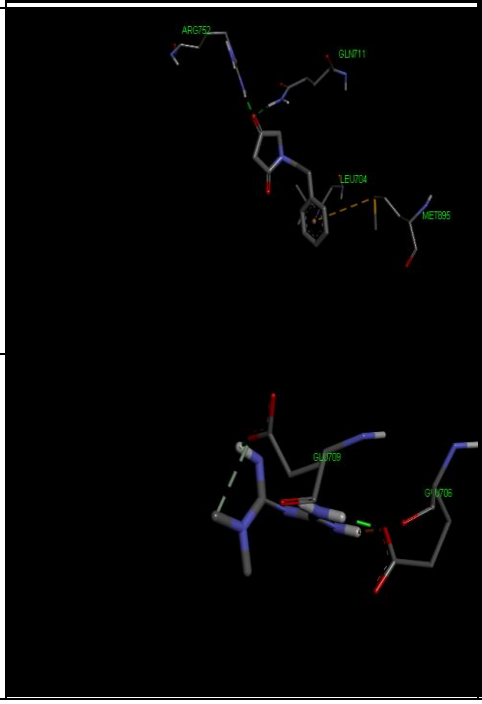
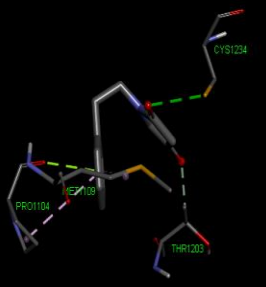
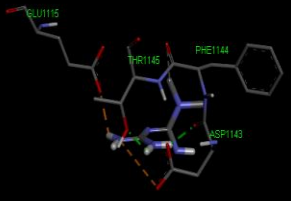
65.	2-(2,6-Dimethyl-4-morpholinyl)-6-(3-methyl-1-piperidinyl)-5-nitro-4-pyrimidinamine	C19H34N6O3	91801	33.008
66.	18-Methyl-nonadecanol	C23H50OSi	136281	33.199
67.	Glutaric acid, dodec-2-en-1-yl dodec-9-yn-1-yl ester	C29H50O4	119977	33.283
68.	**Tocopherol acetate**	C31H52O3	211834	33.406
69.	Furazano[3,4-E][1,2,4]-triazolo[4,3-a]pyrazin-5-amine	C5H3N7O	252531	33.629
70.	.beta.-Methyl xyloside	C6H12O5	76263	33.717
71.	**Thymol**	C13H22OSi	65884	33.792
72.	1H-Indole-2,3-dione, 1-(tert-butyldimethylsilyl)-5-chloro-, 3-(O-ethyloxime)	C16H23ClN2O2Si	80441	34.016
73.	5-Decyne	C10H18	102024	34.107
74.	**Silane, trimethyl[[(3.beta.)-stigmasta-7,24(28)-dien-3-yl]oxy]-**	C32H56OSi	60650	34.236
75.	(E,E,E)-(5-Phenylsulfonylgeranyl)geraniol	C26H38O3S	78533	34.333
76.	Glutaric acid, 2,7-dimethyloct-5-yn-7-en-4-yl tridecyl ester	C28H48O4	90366	34.5

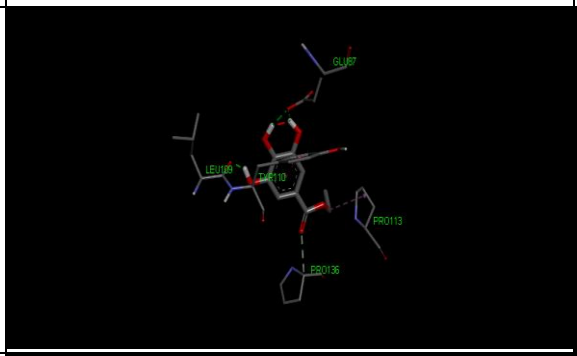
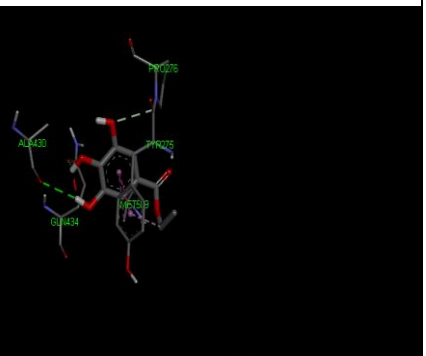
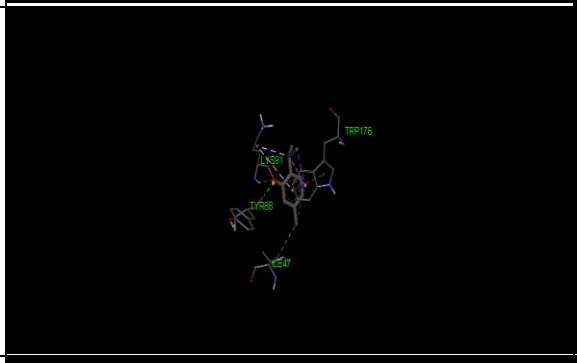
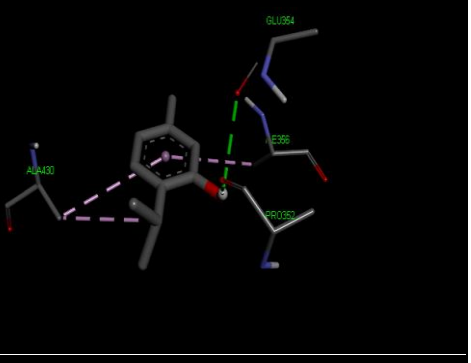
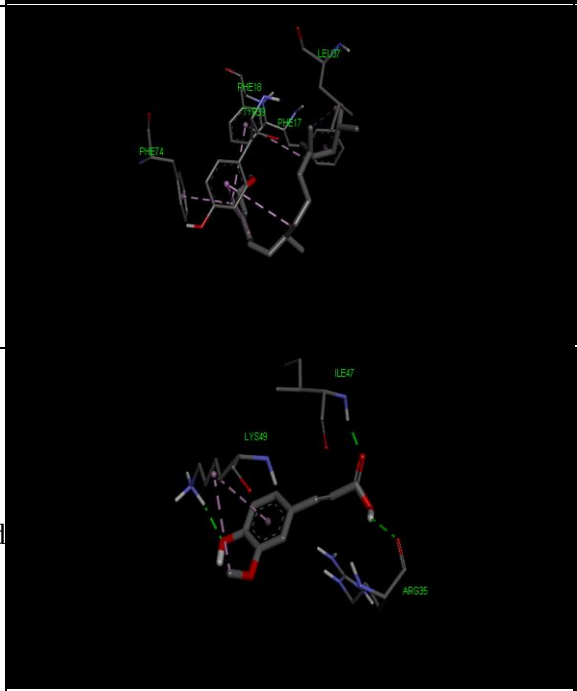
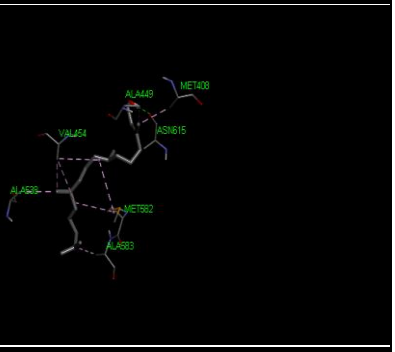
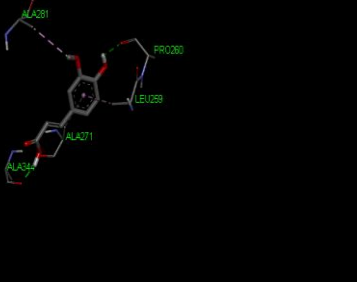
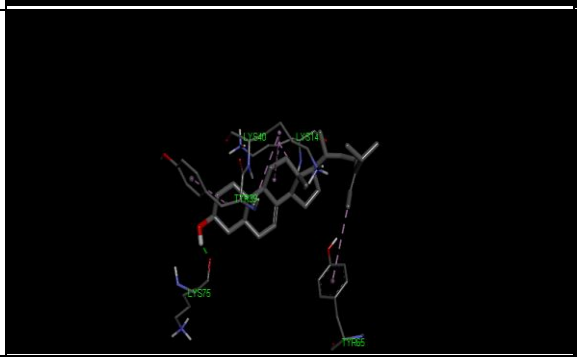
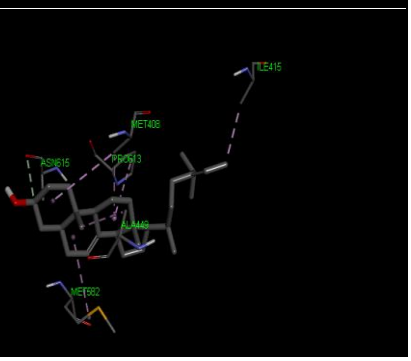
Table 5: Binding affinity of CBEL phytoconstituents to various receptor

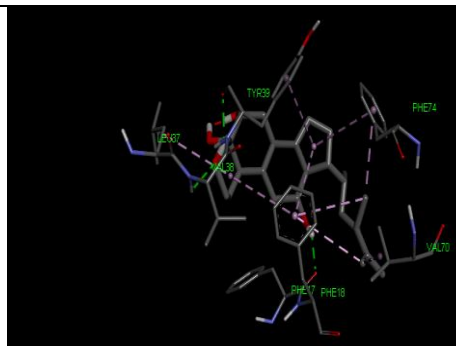
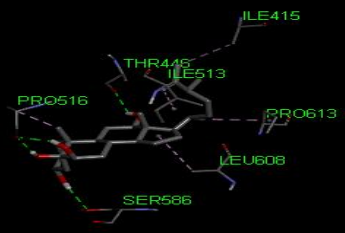
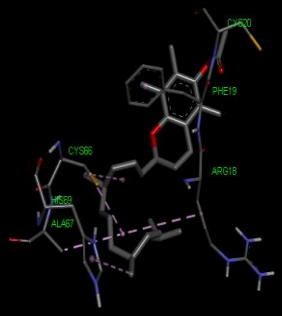
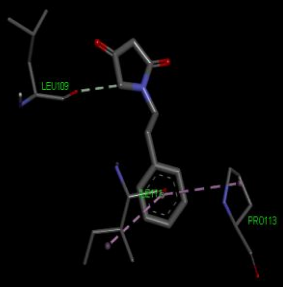
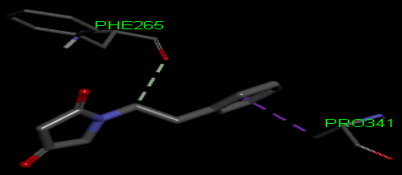
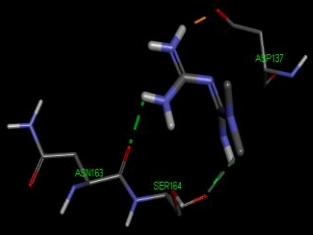
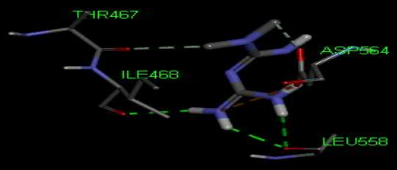
S.no	CBEL Phytoconstituents	Molecular docking (Kcal/mol)				
		1XQ3	1IRK	1XWD	7FIJ	3S79
1.	Ethyl gallate	-8.599	-7.062	-9.104	-7.017	-8.354
2.	Thymol	-6.633	-5.514	-6.139	-5.320	-4.728
3.	Phytol	-10.355	-11.638	-9.654	-9.164	-10.862
4.	Ferulic acid derivative	-8.533	-6.411	-7.247	-6.179	-8.218
5.	Avanosterol	-11.758	-9.519	-10.275	-10.941	-10.607
6.	Ergostane derivative	-12.577	-10.353	-11.253	-10.774	-11.821
7.	Tochopherol acetate	-12.125	-11.903	-10.828	-10.679	-11.397
8.	Pyrrolidine-2,4-dione	-8.153	-6.150	-6.604	-5.670	-6.914
9.	metformin	-4.701	-4.882	-5.434	-3.925	-4.526

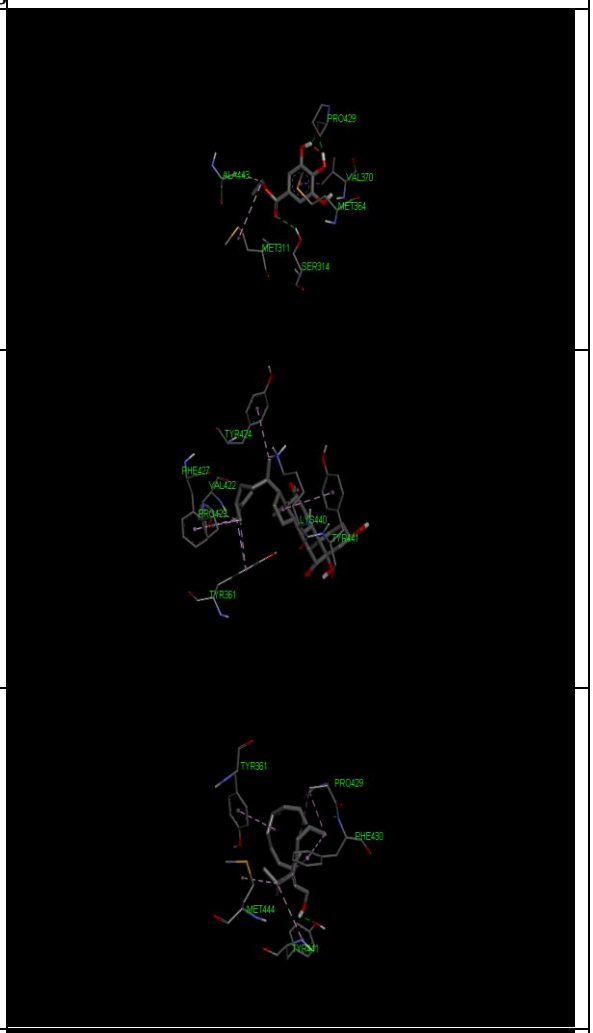
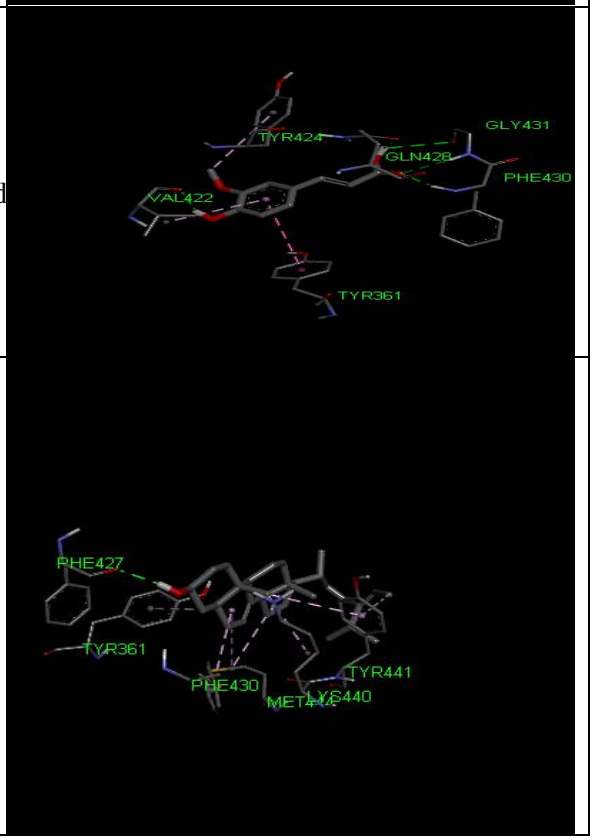
Table 6 : Amino acid binding sites of the ligands

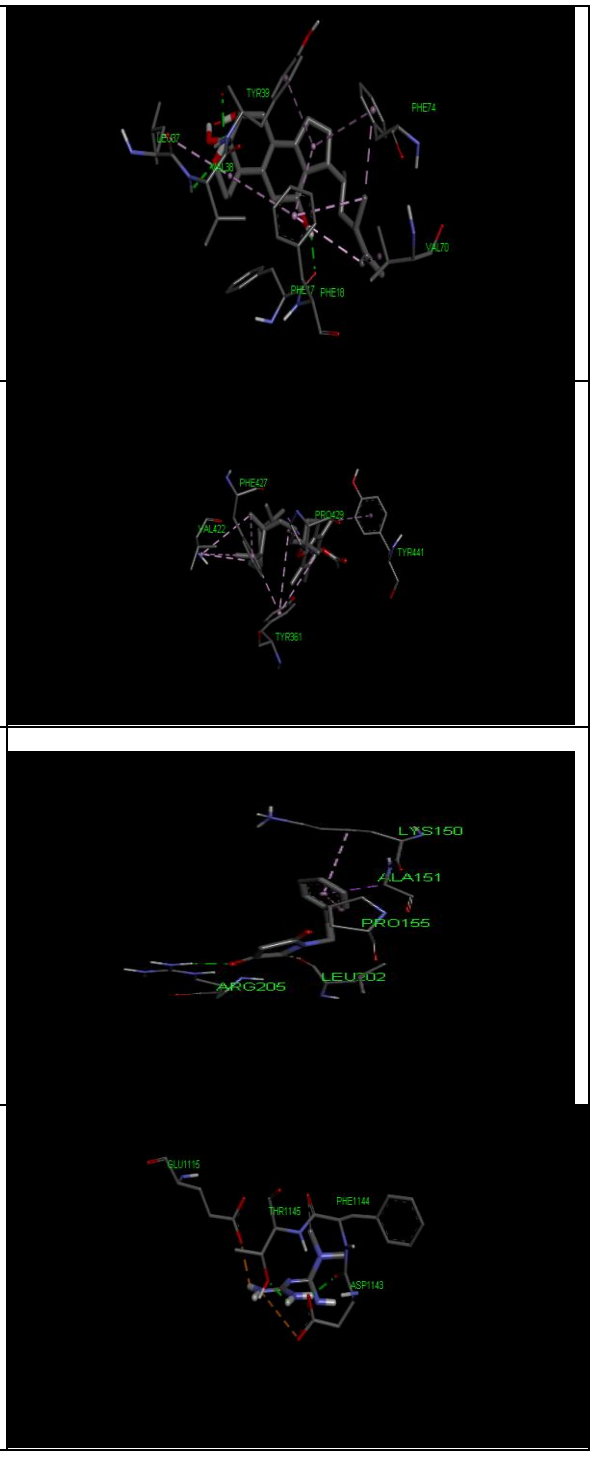
CBEL phytoconstituents	1XQ3	1IRK
Ethyl gallate		
thymol		
Phytol		
Ferulic acid derivative		
avenosterol		

Ergostanol derivative		
Tochopherol acetate		
Pyrrolidine-2,4-dione		
Metformin		

CBEL phytoconstituents	1XWD	7FIJ
Ethyl gallate		
thymol		
Phytol		
Ferulic acid derivative		
avenosterol		

Ergostane derivative		
Tochopherol acetate		
Pyrrolidine-2,4-dione		
Metformin		

CBEL phytoconstituents	**3S79**
Ethyl gallate	
thymol	
Phytol	
Ferulic acid derivative	
avenosterol	

Ergostane derivative	
Tochopherol acetate	
Pyrrolidine-2,4-dione	
Metformin	

Discussion:

The preliminary phytochemical screening of *Croton bonplandianus* ethanolic leaf extract (CBEL) confirmed the presence of several important bioactive compounds, including alkaloids, flavonoids, phenolic compounds, glycosides, tannins, saponins, phytosterols, amino acids, carbohydrates, and fixed oils. These phytochemicals are known for their antioxidant, anti-inflammatory, and hormone-regulating properties, which may be beneficial in the management of polycystic ovary syndrome (PCOS). Quinones and proteins were not detected, indicating variations in the chemical composition of the extract.

GC-MS analysis identified several major phytoconstituents such as phytol, ethyl gallate, thymol, tocopherol acetate (vitamin E), sterol derivatives (avenosterol and ergostane derivatives), and pyrrolidine-2,4-dione. These compounds have been reported to possess antioxidant, anti-inflammatory, insulin-sensitizing, and lipid-lowering activities. Since oxidative stress, chronic inflammation, insulin resistance, and hormonal imbalance are key factors involved in PCOS, these compounds may contribute to the therapeutic potential of CBEL.

The molecular docking studies further supported these findings, as the selected phytoconstituents showed favorable binding interactions with PCOS-related target proteins (1XQ3, 1IRK, 1XWD, 7FIJ, and 3S79). Among them, ethyl gallate, phytol, thymol, tocopherol acetate, sterol derivatives, and pyrrolidine-2,4-dione exhibited good binding affinity, indicating their possible role in regulating pathways associated with insulin signaling, inflammation, oxidative stress, and hormonal imbalance. Metformin, used as the standard drug, served as a reference for comparing the binding efficiency of the phytocompounds.

Overall, the phytochemical screening, GC-MS analysis, and molecular docking results suggest that *Croton bonplandianus* ethanolic leaf extract is a promising source of bioactive compounds with potential therapeutic benefits against PCOS. However, further in vivo studies are required to validate these findings and establish their clinical significance.

Conclusion:

The present study showed that the ethanolic leaf extract of *Croton bonplandianus* contains various bioactive phytochemicals with potential medicinal properties. GC-MS analysis identified important compounds such as phytol, ethyl gallate, thymol, tocopherol acetate, and sterol derivatives. Molecular docking revealed that these compounds showed good binding affinity with PCOS-related target proteins, indicating their possible role in managing PCOS. Overall, the results suggest that ethanolic extract of *Croton bonplandianus* leaves may serve as a promising natural source for the treatment of PCOS. However, further in vivo studies are required to confirm its efficacy and safety.

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