Computational Investigation of Isoquinoline Alkaloid from the Stems of Tinospora Cordifolia against Polycystic Ovarian Syndrome

Murali Krishna Moka
SRMIST

M. Sumithra (sumithrm@smist.edu.in)
SRMIST

Research Article

Keywords: ADMET studies, DFT analysis, Docking studies, Isoquinoline alkaloids, Tinospora cordifolia

Posted Date: June 24th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1753057/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Polycystic Ovarian Syndrome (PCOS) is a global, substantial, metabolic, and endocrine health concern affecting one in every five women of progenerative aged (reproductive) women. Despite the fact that systematic screening and early diagnosis have improved the conception rate in recent decades, infertility still claims ~4%-20% of reproductive-aged women across the globe. In contemporary drug development, in-silico techniques are predominantly employed to investigate interactions between drugs and receptors, as well as quantum chemical properties. A computational approach was used in the current study to conform the quantum chemical properties, Density-functional theory (DFT) analysis, ADMET drug-likeness of the Isoquinoline, alkaloid [Berberine (BER), Palmatine (PAL), Jatrorrhizine (JAT), &Magnoorine (MAG)], isolated from Tinospora cordifolia stem, and the four receptors were selected from PDB database.

Methods Docking studies were carried out with Human Androgen Receptor(1E3G), Insulin receptor(3EKK), Estrogen Receptor beta(1U3S), and Human steroidogenic cytochromeP450 17A1(6WR0) by using AutoDockVina with a pliable ligand docking approach. ADMET prediction and toxicological predictions were performed using SwissADME. DFT analysis was used to predict the optimized structures and molecular electrostatic potential surfaces of the isolated compounds, GROMACS for Molecular Dynamic (MD) Simulation Studies.

Results and Discussion BER and PAL shown better docking scores against the 1E3G human androgen receptor. The absorption of molecules through the skin was measured in cm/s by the skin permeability value (Kp). Higher negative values of MAG (-6.44), JAT (-5.9), PAL (-5.79), and BER (-5.78) resulted in lower skin permeation (log Kp, cm/s). CYP17A1 has a remarkable binding affinity for all four Isoquinoline alkaloids [BER (-5.46), JAT (-5.26), MAG (-6.13), and PAL (-6.01)]. Isoquinoline alkaloids (BER & PAL) have potential roles against various diseases, and in specific PCOS, scientific evaluation has been put forth based on virtual screening.

1. Introduction

Hyperandrogenism, Insulin resistance, Estrogen dominance are the prime defining traits of women with PCOS. These traits lead to disruption of normal adrenal or ovarian functions resulting in impaired folliculogenesis [1] and eventually resulting in excess androgen production. The androgen receptor (AR) [2], Insulin receptor, Estrogen receptor [3] and CYP17A steroidogenesis receptors are linked to the PCOS phenotype and may also implicate in folliculogenesis, disruption of these receptors is responsible for the development of PCOS. Isoquinoline alkaloids are being employed to control androgen synthesis. Isoquinoline alkaloids [4] have a wide variety of medicinal value, therefore, of great interest. Berberine, magnoorine, palmatine, jatrorrhizine, papaverine, morphine, codeine, corydaline, emetine and chelerythrine are among the most often used in traditional medicine. Tinospora cordifolia is one such a plant reported to be having rich in isoquinoline alkaloids like berberine, magnoorine, palmatine, jatrorrhizine.

To understand the drug-receptor relationship and quantum chemical effects, in-silico studies are frequently employed in drug design with using AutoDock Vina and a versatile ligand docking method to dock Human Androgen Receptor(1E3G), Insulin receptor(3EKK), Estrogen receptor beta(1U3S), and Human steroidogenic cytochromeP450 17A1(6WR0). ADMET was performed by using the SwissADME prediction. DFT has become a strong tool in in-silico approach of chemistry because of its great potential. This approach was developed for spectroscopic investigation of chemical entities as a commanding utility. Frequency calculations and spectral intensity forecasts are now necessary for interpreting the experimental spectra of complex molecules [5]. Recent improvements in the in-silico approaches based on time dependent functional density theory (TDDFT) have resulted in increased interest in electronic structure calculations in exciting states [6]. The work sought to conduct a computational analysis, virtual docking simulation of isoquinoline (Proto-Berberine) compounds of Tinospora cordifolia, and the creation of novel suggested compounds against (PCOS) Androgen Receptor.

2. Materials And Methods

2.1 Virtual screening and docking

For all the compounds' 2D structures (.mol), ChemDraw 16.0 were used to examine the drawings (Fig. 1). Chem3D 16.0 which is used to convert all of the compounds (Berberine (BER) [7], Jatrorrhizine (JAT) [8], Magnoorine (MAG) [9], and Palmatine (PAL) [10]) into a 3D structure (.pdb). Chem3D was utilised to load the 3D coordinates of each compound (.pdb) for energy simplification. For macromolecule preparation, the docking receptor (PDB codes 1E3G, 6WR0, 1U3S, and 3EKK) is a protein target from the RCSB Protein Data Bank. All bound ligands and water molecules were removed from the receptor's active site. The compounds (BER, JAT, MAG, and PAL) were docked against the active site of the protein using molecular docking assessment (AutoDockVina). Post-docking assessments were performed using AutoDock Tools [11].
2.2 Computational Quantum Studies

Gaussian 09 was used to perform density functional theory (DFT) assessments on compounds (BER, JAT, MAG, and PAL), which was visualised using Gauss view 5.0. The structural variables of the lead compounds were improved by employing the B3LYP/6–31 G (d,p) basic level set with no symmetric constraints. The enhanced geometry yielded the molecular electrostatic potential map and the compounds' energies. The HOMO-LUMO energy gap, dipole moment, global softness, and electronegativity were all estimated using Koopman’s approximation [12].

2.3 ADMET prediction

As computer mechanics advanced, in-silico methods such as network analysis and screening were widely used to illuminate the pharmacological underpinnings of traditional medicinal plant activities. Network pharmacology, in-silico screening, and pharmacokinetic screening can be used to supplement active molecules in these approaches. The Swiss Institute of Bioinformatics’ (http://www.sib.swiss) ADME software (www.swissadme.ch) was used to estimate individual ADME behaviours of compounds using a web server that displayed the Swiss ADME Submission page. The list is constructed using the simplified molecular input line-entry system describes one source molecule per line with multiple inputs (SMILES) [13].

2.4 Molecular Dynamic (MD) Simulation Studies

For the MD simulation studies, the GROMOS 54A7 force field was employed. To convert pdb to gro format, which is accessed through GROMACS, the pdb2gmx utility was utilised. The PRODRG server was used to determine the ligands architecture and characteristics. After the 100ns simulation was finished, the data was evaluated using the GROMACS simulation programme. The root means square deviation (RMSD)(Backbone), root mean square fluctuation (RMSF)(c-alpha), radius of gyration (RG), and Molecular Mechanics Poisson Boltzmann Surface Area were all calculated using the gromacs package (MM PBSA).

3. Result And Discussion

Women with polycystic ovary syndrome (PCOS) have excessive androgen secretion, leading to ovulatory infertility and follicular maturation arrest, insulin resistance, and obesity. Recent research strongly suggests that insulin stimulates ovarian and adrenal steroidogenesis and pituitary LH release in PCOS patients by acting through its receptor. PCOS is a common endocrine disorder characterized by abnormal estrogen and estrogen receptor function in women [14–16].

Molecular docking was performed against the PCOS inhibitory receptor using AutoDockVina software. BER, JAT, MAG, and PAL were docked individually against with all the specified receptors such as androgen receptor [17], insulin receptor [18], estrogen receptor beta[19], and CYP17A1[20]. And the molecular docking results confirm the efficacy of BER and PAL with the H-bond interactions, and hydrophilic interactions with the proteins were better than JAT and MAG (Fig. 1). The better binding affinity was visualized in BER and PAL and acted as a potential inhibitor of PCOS (Fig. 2, 3, 4, & 5).CYP17A1 shows significant binding affinity with all the four compounds (BER (-5.46), JAT (-5.26), MAG (-6.13), and PAL (-6.01)) in the Tinospora cordifolia [21]. Binding affinity of berberine and magnoflorine with different receptors is mentioned in the Table 1 & 2. Binding affinity of palmatine and jatrorrhizine with different receptors is mentioned in the Tables 3 & 4.

BER showed best docking results with Androgen receptor, and the ligand interactions to the androgen receptor in the amino acids such as Leu 301, Phe 356, Val338, Leu 339, Gly 342, Trp 312, Glu 305, Met 309, and Pro 227. The docking score showed the binding affinity value of BER was − 8.23 Kcal/mol.

Table 1

<table>
<thead>
<tr>
<th>S.No</th>
<th>Receptors</th>
<th>Ligands</th>
<th>Binding Affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Androgen receptor</td>
<td>BER</td>
<td>− 8.23</td>
</tr>
<tr>
<td>2</td>
<td>Insulin receptor</td>
<td>BER</td>
<td>− 3.9</td>
</tr>
<tr>
<td>3</td>
<td>Estrogen receptor beta</td>
<td>BER</td>
<td>24.9</td>
</tr>
<tr>
<td>4</td>
<td>CYP17A1</td>
<td>BER</td>
<td>−5.46</td>
</tr>
</tbody>
</table>

Page 3/15
MAG showed best docking results with insulin receptor among the four receptors, and the ligand interaction to the insulin receptors in the amino acid consists of Asp 1083, Ala 1028, Val 1010, Met 1076, Met 1139, Val 1060, Gly 1005, Lys 1030, and Ser 1006. The docking score showed the affinity value of MAG was −6.31.

Table 2
Comparative binding affinity of MAG with different receptor

<table>
<thead>
<tr>
<th>S.No</th>
<th>Receptors</th>
<th>Ligands</th>
<th>Binding Affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Androgen receptor</td>
<td>MAG</td>
<td>1280</td>
</tr>
<tr>
<td>2.</td>
<td>Insulin receptor</td>
<td>MAG</td>
<td>-6.31</td>
</tr>
<tr>
<td>3.</td>
<td>Estrogen receptor beta</td>
<td>MAG</td>
<td>6.82</td>
</tr>
<tr>
<td>4.</td>
<td>CYP17A1</td>
<td>MAG</td>
<td>-5.26</td>
</tr>
</tbody>
</table>

PAL showed best docking results with Androgen receptor, and the ligand interaction to the androgen receptors in the amino acid consists of Leu 701, Leu 704, Leu 880, Gln 711, Met 745, Met 749, Met 780, Met 787, Phe 876, Phe 891, and Asn 705. The docking score showed the affinity value of PAL was −6.71.

Table 3
Comparative binding affinity of PAL with different receptor

<table>
<thead>
<tr>
<th>S.No</th>
<th>Receptors</th>
<th>Ligands</th>
<th>Binding Affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Androgen receptor</td>
<td>PAL</td>
<td>-6.71</td>
</tr>
<tr>
<td>2.</td>
<td>Insulin receptor</td>
<td>PAL</td>
<td>-5.16</td>
</tr>
<tr>
<td>3.</td>
<td>Estrogen receptor beta</td>
<td>PAL</td>
<td>23.52</td>
</tr>
<tr>
<td>4.</td>
<td>CYP17A1</td>
<td>PAL</td>
<td>-6.13</td>
</tr>
</tbody>
</table>

JAT showed best docking results with CYP17A1 receptor, and the ligand interaction to the CYP17A1 receptors in the amino acid consists of Leu 452, Leu 370, Arg 440, Ser 441, Phe 435, Pro 434, Ile 371, Gys 442, Val 366, Val 310, and Ala 448. The docking score showed the affinity value of JAT was −6.01.

Table 4
Comparative binding affinity of JAT with different receptor

<table>
<thead>
<tr>
<th>S.No</th>
<th>Receptors</th>
<th>Ligands</th>
<th>Binding Affinity (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Androgen receptor</td>
<td>JAT</td>
<td>230</td>
</tr>
<tr>
<td>2.</td>
<td>Insulin receptor</td>
<td>JAT</td>
<td>150</td>
</tr>
<tr>
<td>3.</td>
<td>Estrogen receptor beta</td>
<td>JAT</td>
<td>33.8</td>
</tr>
<tr>
<td>4.</td>
<td>CYP17A1</td>
<td>JAT</td>
<td>-6.01</td>
</tr>
</tbody>
</table>

The results of 2-D and 3-D molecular docking visualization provide information that an interaction was formed between the BER, MAG, PAL and JAT, ligand against the various receptors of androgen receptor, insulin receptor, estrogen receptor, and CYP17A1.

BER and PAL showed better binding affinity to the PCOS androgen receptors than the other three receptors, whereas MAG & JAT showed better binding affinity with insulin receptor and CYP17A1 receptor respectively. CYP17A1 had showed significant binding affinity with all the four compounds.
3.1 DFT Analysis of Isoquinoline alkaloids from stems of *Tinospora cordifolia*

BER, MAG, PAL, and JAT were first optimized using the B3LYP algorithm with a 6-31G (d) basis in Gaussian 16 utilizing Fukuis molecular orbital theory. The HOMO energy (EHOMO) and LUMO energy (ELUMO) of molecular orbitals were estimated. EHOMO and ELUMO are key characteristics that describe a molecule’s ability to give and take electrons, respectively. In HOMO and LUMO, maps showing the density of electrons in different locations of the molecules were created and evaluated. The EHOMO and ELUMO values of BER, MAG, PAL, and JAT are shown in Table 5. The molecular orbitals; density maps were created and examined. The energy gap is proportional to the reactivity of a molecule, which can be linked to the change of molecules from HOMO to LUMO. As a result, the band energy gaps of BER, MAG, PAL and JAT have been estimated and displayed (Fig. 17).

The ability of the maps to donate or accept the electrons was calculated using the density functional theory. BER and PAL showed the lowest energy gap between HOMO and LUMO with the value of 0.158 eV and 0.161 eV. The chemical reactivity of a molecule can be linked to its molecular dipole moment since the two are proportionate. The molecular dipole moments of BER, JAT, and PAL were all greater than 2.0 debye, except MAG having the lowest at 0.95 debye. We calculated the values of various descriptors such as Electronegativity (\(\mu\)), Electrophilicity index (\(\chi\)), Global softness (\(\sigma\)), and Absolute hardness (\(\eta\)) of the compounds using the EHOMO and ELUMO values. The electronegativity of a substance influences the ability of a molecule to receive electrons. A molecule's electronegativity determines how effective it is at inhibiting other molecules. BER has the lowest electronegativity among the other compounds (-0.0789), and then PAL has the lowest electronegativity value (-0.0805). The docking analysis is associated with the values of BER, JAT, MAG and PAL using conceptual DFT. In terms of all the compounds, we found that BER was the best scoring molecule among the compounds with a high docking score. Koopman’s approximation was used to predict the HOMO-LUMO energy gap and related reactive elements. (chemical potential, hardness, softness, electronegativity, electrophilicity).

### Table 5

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dipole Moment</th>
<th>(E_{\text{HUMO}}) (eV)</th>
<th>(E_{\text{LUMO}}) (eV)</th>
<th>Energy gap (\Delta E) (eV)</th>
<th>Absolute hardness (\eta)</th>
<th>(\Sigma)</th>
<th>(\mu)</th>
<th>(\chi)</th>
<th>(\sigma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BER</td>
<td>3.44</td>
<td>-0.199</td>
<td>-0.0411</td>
<td>0.158</td>
<td>0.079</td>
<td>6.32</td>
<td>-0.0789</td>
<td>0.0394</td>
<td></td>
</tr>
<tr>
<td>JAT</td>
<td>5.54</td>
<td>-0.195</td>
<td>-0.032</td>
<td>0.163</td>
<td>0.081</td>
<td>6.13</td>
<td>-0.0815</td>
<td>0.0410</td>
<td></td>
</tr>
<tr>
<td>MAG</td>
<td>0.95</td>
<td>-0.188</td>
<td>-0.005</td>
<td>0.183</td>
<td>0.091</td>
<td>5.55</td>
<td>-0.0915</td>
<td>0.0460</td>
<td></td>
</tr>
<tr>
<td>PAL</td>
<td>5.91</td>
<td>-0.196</td>
<td>-0.035</td>
<td>0.161</td>
<td>0.080</td>
<td>6.21</td>
<td>-0.0805</td>
<td>0.5031</td>
<td></td>
</tr>
</tbody>
</table>

In the above Table 5, \(\sigma\): Global softness, \(\mu\): Electronegativity, \(\chi\): Electrophilicity.

The results show that the BER had the smallest energy gap compared to the other compounds, indicating a high level of chemical reactivity and intramolecular charge transfer from HOMO to LUMO groups.

### 3.2 ADME Predictions of Isoquinoline alkaloids from stems of *Tinospora cordifolia*

**ADME Predictions of Compounds**
Table 6
ADME Predictions of Ligand Calculated by SwissADME

<table>
<thead>
<tr>
<th>S.No</th>
<th>Ligands</th>
<th>Mol.Wt. (g/mol)</th>
<th>Number of Rotatable bonds</th>
<th>Number of Hydrogen acceptors</th>
<th>Number of Hydrogen donors</th>
<th>TPSA (Å²)</th>
<th>LogP</th>
<th>Lipinskis rule of five violation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>BER</td>
<td>336.36 g/mol</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>40.80</td>
<td>-1.27</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>JAT</td>
<td>338.38 g/mol</td>
<td>3</td>
<td>4</td>
<td>1</td>
<td>51.80</td>
<td>-1.49</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>MAG</td>
<td>342.41 g/mol</td>
<td>2</td>
<td>4</td>
<td>2</td>
<td>58.92</td>
<td>-2.00</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>PAL</td>
<td>352.40 g/mol</td>
<td>4</td>
<td>4</td>
<td>0</td>
<td>40.80</td>
<td>-1.46</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 7
ADME Predictions of Ligands Calculated by SwissADME

<table>
<thead>
<tr>
<th>S.No</th>
<th>Ligands</th>
<th>Skin Permeation Value (log Kp) cm/s</th>
<th>GI Absorption</th>
<th>BBB Permeability</th>
<th>Inhibitor Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P-gp Substrate CYP1A2 Inhibitor CYP2C19 Inhibitor CYP2C9 Inhibitor CYP2D6 Inhibitor CYP3A4 Inhibitor</td>
</tr>
<tr>
<td>1.</td>
<td>BER</td>
<td>-5.78</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2.</td>
<td>JAT</td>
<td>-5.9</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>3.</td>
<td>MAG</td>
<td>-6.44</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>4.</td>
<td>PAL</td>
<td>-5.79</td>
<td>High</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Computer-aided drug design has been used in the prediction of ADMET characteristics of medicines, resulting in early-stage drug development. Lipinskis rule [22] of five states that the drugs i.e. compounds, molecules and/or candidates must stick to the four parameter rule, according to this rule HBDs (hydrogen bond donors) must be less than five, HBAs (hydrogen-bond acceptors) must be less than ten, the value of log P cannot be less than 5,molecular mass must be less than 500 Da but according to Veber's rule Topological polar surface area (TPSA) must not exceed 140. "Drug-likeness" refers to a prediction that an organic compound exhibits features that suggest it could be an orally active drug. The SwissADME predictions suggested that all of the compounds fulfilled Lipinski's rule of five, indicating that they are likely to be orally active in the current investigation. SwissADME was used to predict the Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) studies of BER, MAG, PAL, and JAT. The absorption of molecules through the skin is measured in cm/s by the skin permeability value (Kp). Lower skin permeation (log Kp, cm/s) was obtained with a higher negative value as MAG (-6.44), JAT (-5.9), PAL (-5.79), and BER (-5.78). According to the SwissADME prediction variables, all of the compounds have enhanced gastrointestinal absorption, blood-brain barrier penetration, and are permeability glycoprotein substrates (P-gp). None of the substances are inhibitors of CYP2C19 or CYP2C9, according to the CYP interaction data. (Fig. 18). The PAL is also not an inhibitor of CYP1A2, while the other compounds are inhibitors of CYP1A2, CYP2D6, and CYP3A4. The method estimates bioavailability radar based on six physicochemical properties: polarity, lipophilicity, size, insolubility, flexibility, and insaturation, in order to discover drug-likeness. BER, MAG, PAL, and JAT are potential drug candidates from Tinospora cordifolia, according to bioavailability radar. These prognostic results should be verified in vitro and in vivo functional and pharmacological studies to treat PCOS.

The isolated compounds[23] optimized structures and molecular electrostatic potential surfaces were studied. The docking results in this study revealed that BER and PAL had higher docking scores against PCOS as an inhibitor. All four compounds (BER, JAT, MAG, and PAL) from stems of Tinospora cordifolia, according to SwissADME predictions, satisfies Lipinski's rule of five with zero violations. BER has a reduced electronegativity and global softness, implying that intramolecular charge transfer between electron-donor and electron-acceptor
groups is significant, better bioactivity and chemical reactivity are predicted. This study reveals that BER and PAL from stems of *Tinospora cordifolia* may act as a lead molecule in inhibiting the formation of PCOS condition.

### 3.3 Molecular Dynamics (MD) Stimulatory studies

The complexes formed by docking two ligands were subjected to molecular dynamics (MD) simulation to better understand the conformational changes and stability of the ligand bound complexes, since it provides information on complex behaviour at the atomic level. GROMOS 54A7 force field was used for the simulation. The pdb2gmx tool was used to convert pdb to gro format, which is accessible via gromacs. The architecture and properties of the ligand were obtained using the prodrug server. Following that, a protein-ligand complex was created. The complex was solvated using the SPC water model and placed in the centre of a cubic periodic box. The system net charge was then neutralised by introducing counterions as needed. With the addition of 0.150 M of NaCl, the ionic strength was adjusted. The protein structure is then subjected to energy reduction using the steepest descent approach to eliminate unnecessary bonds, conflicts, and obtain a globally reduced state. After energy reduction, the system was equilibrated using two ensembles: Number of atoms, Volume of the system, and Temperature of the system (NVT) and Number of atoms, Pressure of the system, and Temperature of the system (NPT). The system was put through a production run once it reached equilibrium. The produced data was analyzed using the GROMACS simulation software once the 100ns simulation was completed. Using the gromacs package, we estimated root mean square deviation (RMSD)(Backbone), root mean square fluctuation (RMSF)(c-alpha), radius of gyration(RG), and Molecular Mechanics Poisson Boltzmann Surface Area(MM PBSA).

#### 3.3.1 Molecular Dynamics (MD)

MD simulation was run on the Crystal Structure of the Human Androgen Receptor Complex with chosen ligands from docking PAL (Palmatine) and BER (Berberine). To understand the stability of the above-mentioned protein-ligand complexes, RMSD (Root Mean Square Deviation), RMSF (Root Mean Square Fluctuations), RG (Radius of Gyration), H-Bonds (Hydrogen bonds), and MMPSA calculations were carried out.

#### 3.3.2 Root mean square deviation

It is a key criterion for determining the differences between the two confirmations. The greater the variance, the higher the RMSD value. For PAL, the RMSD values were determined to be steady from 30ns to 100ns, with a little fluctuation from 85ns to 90ns, and for BER, it was stable from 40ns to 100ns. In the RMSF plots, the amino acids implicated in bringing about the overall structural deviation are investigated. The RMSD results for PAL and BER are depicted in Figs. 7 & 8.

#### 3.3.2 Root mean square fluctuation (RMSF)

RMSF study determines which amino acids in a protein cause more vibrations in the presence and absence of ligands, resulting in protein instability. A 0 to 100ns simulation timeframe is used to calculate the RMSF values. During the simulation, it was discovered that residues in the loop area fluctuate more. This means that during the 50ns simulation periods, the compound did not fluctuate.

#### 3.3.3 Radius of Gyration (RG)

The radius of gyration may be used to evaluate the protein's compactness. The RG values versus a simulated timeline of 0 to 100000ps for PAL and BER were used to examine protein folding and unfolding.

#### 3.3.4 Hydrogen Bond (H-bond)

The formation of hydrogen bonding stabilizes protein-ligand complexes. The hydrogen bonds produced in the molecular docking study are validated by simulation analysis in our research.

#### 3.3.5 MMPBSA

MMPBSA determines how much energy is needed for ligands to bind to protein.
Table 8
MMPBSA of complicated PAL and BER of 100ns simulation

<table>
<thead>
<tr>
<th>Target</th>
<th>Ligand code</th>
<th>Binding energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAL</td>
<td>-211.219 +/- 9.358 kJ/mol</td>
<td></td>
</tr>
<tr>
<td>BER</td>
<td>-93.681 +/- 11.349 kJ/mol</td>
<td></td>
</tr>
</tbody>
</table>

Conclusion

In this research to assess the potential Isoquinoline alkaloids against PCOS for drug interactions, drug-likeness, quantum chemical properties, and binding energy calculations, we used bioinformatics tools, including AutodockVina for molecular docking, Gauussian 09 for DFT analysis and SwissADME for admet analysis and GROMACS for molecular dynamic studies. BER and PAL have good binding affinity and better docking scores against the target receptor. CYP17A1 also shows significant binding affinity with all the four compounds (BER (-5.46), JAT (-5.26), MAG (-6.13), and PAL (-6.01)) in the Tinospora cordifolia. BER & PAL has superior bioactivity and chemical reactivity, according to DFT findings, since it has considerable intramolecular charge transfer between electron-donor and electron-acceptor groups. BER and PAL may serve as a lead molecule, and more research into functional group inclusion and modification is needed in order to generate novel compounds with therapeutic potential against PCOS disorders, according to the findings of this study. In addition to docking and DFT, the prediction of pharmacokinetics with special reference to ADME, bioavailability, and drug-like features of four compounds, BER and PAL, can be effective molecules for the novel drug candidate. However, further in vitro and in vivo assays for PCOS conditions are advised to confirm the prevailing predictions.

Declarations

Acknowledgement

Sincerely thanks to Dean Dr V Chitra for our support and motivation towards research

Authors contribution

Murali Krishna Moka (MKM): Methodology & experimentation; Writing and original draft preparation; Visualization; Sumithra M (SM): conceptualization; Investigation supervision; Writing-Reviewing editing.

Disclosure statement

The Authors declare No conflict of Interest

References

7. Mohan MC, Abhimannue AP. Identification and characterization of BER in tinosporacordifolia by liquid chromatography Quadrupole time of flight mass spectrometry (LC MS/MS q-tof) and evaluation of its anti-inflammatory potential. Pharmacognosy Journal.

Figures
Figure 1
Autodock of isoquinoline alkaloid BER with androgen receptor, insulin receptor, estrogen receptor, and CYP17A1:

Figure 2
Autodock of isoquinoline alkaloid MAG with androgen receptor, insulin receptor, estrogen receptor, and CYP17A1.

Figure 3
Autodock of isoquinoline alkaloid PAL with androgen receptor, insulin receptor, estrogen receptor, and CYP17A1.
Figure 4

Autodock of isoquinoline alkaloid JAT with androgen receptor, insulin receptor, estrogen receptor, and CYP17A1

Figure 5

Transfer of charge from electron donor (HOMO) to electron acceptor (LUMO) groups shows within a single molecule.
Figure 6

BER, JAT, MAG, and PAL, bioavailability radar (pink region depicts ideal range of particular property) (LIPO = lipophilicity as XLOGP3; SIZE = size as molecular weight; INSOLU = insolubility in water by log S scale; FLEX = flexibility as per rotatable bonds; POLAR = polarity as TPSA (Topological polar surface area) and INSATU = instauration as per fraction of carbons in the sp3 hybridization

Figure 7
RMSD plots of respective compounds (BER & PAL) from GROMACS

Figure 8
Root means square deviation of backbone atoms of PAL and BER complex

Figure 9
RMSF plots of respective compounds (BER & PAL) from GROMACS
Figure 10

Root means square fluctuation of c-alpha atoms of PAL and BER

Figure 11

RG plots of respective compounds (BER & PAL) from GROMACS
Figure 12
Legend not included with this version

Figure 13
H-Bond of complex PAL and BER