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Original Article

Scrutinizing Potential Phytoconstituents from *Bauhinia variegata* in Mitigating the Symptoms of Polycystic Ovarian Syndrome: A Computational Approach

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ABSTRACT

Background: Polycystic ovarian syndrome is a metabolic disorder majorly caused by the hormonal fluctuations in female and the current scenario explains that adolescents remain predominantly affected with this disorder. *Bauhinia variegata* assist in the treatment of various ailments and are used as an ingredient in targeting uterine disorders, yet the exact constituent that contributes on activity remains unknown. **Aim:** The main aim of our study is to determine the potent phytoconstituent of *Bauhinia variegata* to fight against the symptoms of PCOS through computational techniques. **Materials and Methods:** Five *in silico* techniques like Molecular docking analysis, Pharmacokinetics, toxicity prediction of the compounds, Biological activity and Molecular dynamics simulation studies were performed to identify the potent phytoconstituent. **Results and Discussion:** Molecular docking studies show that the major constituent lupeol had a good binding interaction and high docking score of -10.31 Kcal/mol and -11.52 Kcal/mol with both the proteins 3RUK and 1E3K. Pharmacokinetics, toxicity and biological activity studies reveal that it had ideal drug likeliness properties with proper biological activity values and were found to non-toxic in the analysed parameters. Lupeol complex was found to be potentially stable throughout the molecular dynamic's simulation for 100 ns. **Conclusion:** Thus, through *in silico* analysis it is evident that from the list of phytoconstituents of *Bauhinia variegata*, lupeol possess potent activities in mitigating the symptoms of PCOS. Further *in vitro* and *in vivo* analysis on PCOS model is expected to yield favourable results.

Keywords: *Bauhinia variegata*, Molecular docking, Molecular dynamics, Pharmacokinetic analysis, Toxicity prediction.

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INTRODUCTION

Women of reproductive age majorly possess metabolic abnormalities which results in the prevalence of polycystic ovarian syndrome.[1] Worldwide diagnostic report from National Institutes of Health (NIH) denotes 4-10% of reproductive women are affected with PCOS. One among ten women of the world population are diagnosed with the disorder and many are left out without proper diagnosis.[2,3] Though the women of reproductive age are predominantly affected, prevalence of the disorder is seen in late adults even after the child bearing age.[4] The factors that play a wide role in the occurrence includes, hyperandrogenism, hereditary, excessive stress, anxiety and atypical sleep patterns.[5]

The precise pathophysiology of PCOS is yet to be determined, however few factors like chronic low grade inflammation that leads to excessive oxidative stress,[6] increase of prolactin and decline of melatonin levels, imbalance in the Hypothalamic-Pituitary-Ovarian (HPO) axis which leads to the excessive secretion of GnRH and Insulin resistance remain as contributing elements (Figure 1).[7]

PCOS likewise termed as Stein-Leventhal syndrome is mainly diagnosed with the help of a criteria called Rotterdam criteria which was introduced in the year 2003[8] and underwent few amendments at recent times. In accordance to the criteria, women with positive results for two among the three conditions are referred to be categorized under PCOS. The conditions include, presence of ≥20 antral follicles in either of the ovaries with accumulation of ovarian volume greater than 10 cm^3, Oligo anovulation characterized by less than 8 menstrual cycle per year and hyperandrogenism determined through ideal clinical and biochemical methods.[9,10] PCOS is often diagnosed with the

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help of phenotypes classification that is currently under main role.[11] Various hormonal fluctuations are noticed in the affected women as they possess hyperandrogenism characterised mainly due to excessive unbound free testosterone in the blood stream, abnormal surge in Luteinizing hormone and reduction in follicle stimulating hormone. Hyperinsulinemia leads to decrease in the Sex Hormone Binding Globulin (SHBG) which leads to the clinical symptoms of PCOS like hirsutism, acne, alopecia and acanthosis nigricans.[12,13] Women with PCOS often tend to have a hike in the body weight and are accompanied by anovulation for a longer duration. The major complications of untreated PCOS includes, development of type-II diabetes mellitus, cardiovascular abnormalities,[14] pulmonary hypertension,[15] Non-alcoholic fatty liver disease[16] and endometrial cancer.[17]

The therapy that is currently under practice for PCOS can be categorized like Pharmacological and Non-Pharmacological treatment. The Pharmacological treatments mainly include administration of synthetic drugs like Oral contraceptives including estrogen and progesterone derivatives, anti-androgens like levonorgestrel, Desogestrel, Flutamide, spironolactone and cyproterone acetate. Insulin sensitizers like pioglitazone and rosiglitazone are prescribed to reduce insulin resistance, free androgens and ovarian dysfunction.[18,19] Letrozole, clomiphene and anastrozole are administered to induce ovulation[20] and few supplements like Vitamin-D, probiotics, prebiotics and calcium are given to increase the ovulatory phase and for promoting follicular growth. Few surgical treatments like ovarian drilling are also recommended for patients with PCOS.[21] Non-pharmacological treatment includes practice of regular exercise, proper healthy food intake, maintenance of standard sleep cycle and dietary lifestyle.[22,23] Yoga and acupuncture techniques have also proven to have beneficiary effects on woman with PCOS.[24]

While there are several synthetic medications under practice, a major disadvantage of the synthetic therapy is occurrence of various adverse effects. Hence usages of herbal medicines efficiently bypass the adverse effects produced. Herbal therapy play a pivotal role in the management of PCOS symptoms.[25] Several home remedies are under practice but a proper research is essential to prove the potency of herbal medications. Herbal extract and polyherbal formulations are currently under major usage in reducing the symptoms of the disorder. *Bauhinia variegata* (BV) is one of the major herbs that are included in the herbal formulations for PCOS.[26,27] BV has been reported to possess several biological activities like Anti-inflammatory,[28] anti-carcinogenic, anti-mutagenic,[29] anti-proliferative,[30] anti-oxidant,[31] anti-bacterial,[32] anti-diabtetic,[33] hepatoprotective,[34] neuroprotective[35] and apoptotic activities.[36] Though *Bauhinia variegata* is used in combined formulations for treating PCOS, the exact phytoconstituent that is responsible for activity is unknown. Thus, the aim of our current work is to determine the potential phytoconstituent of *Bauhinia variegata* that play a predominant role in fighting against the symptoms of PCOS through several computational techniques. *In silico* technique paves way in reducing the depletion of time and resources and assist in scrutinizing compounds to further proceed for *in vitro* and *in vivo* analysis.[37] A series of major and minor phytoconstituents reported in the literatures and traditional books were considered in the study.[38,39]

MATERIALS AND METHODS

Preparation of chemical structures

The phytochemical constituents of *Bauhinia variegata* were selected and sketched with the help of ChemDraw ultra 12.0 (Table 1). They were converted to the three dimensional form using Chem3D pro 12.0 software.[40] The lowest energy conformation of the structures were effectively attained with the help of minimisation of the structures using Avogadro 1.2.0 software.[41]

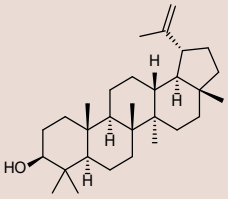
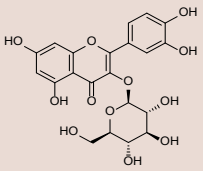
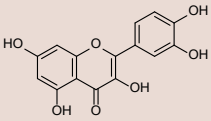
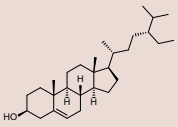
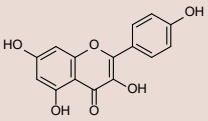
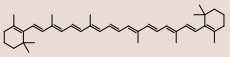
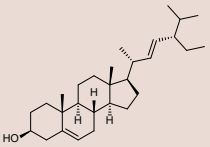
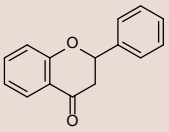
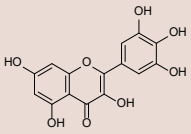
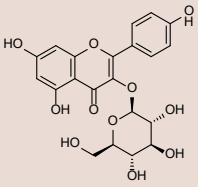
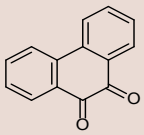
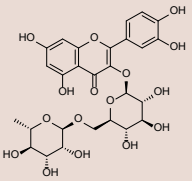
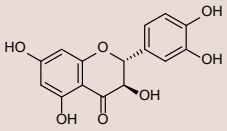
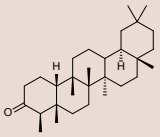
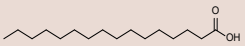
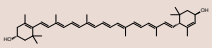
Selection of Target protein

The proteins act as a predominant target in the human body. Selection of two different proteins namely, Human cytochrome P450 CYP17A1 (Anti-androgen protein) with PDB ID 3RUK and Human progesterone receptor with PDB ID 1E3K from RCSB protein data bank was performed in the study.[42] The hormones that play a vital role in the pathophysiology of PCOS were analysed and these proteins were selected in accordance. Co-crystallized ligands and non-interactive water molecules of the selected proteins were removed with the help of Molegro molecular viewer 2.5 software and taken up into use.[43]

Molecular Docking studies

Molecular docking studies helps in determining the binding interaction of the ligands with the protein and effectively help in scrutinizing the affinity of the compounds with the binding score.[44,45] AutoDock tools 1.5.7 software was used in performing the Molecular docking studies.[46] The prepared proteins were subjected to mgl tools, where hydrogen bonds and Kollman chargers were added, gasteiger charges were computed and the protein was saved. The energy minimised ligand molecules were added into the directory, torsions were analysed and the ligands were saved in pdbqt format. The grid selection was performed by picking the protein and ligand molecule in the directory followed by setting dimensions of 120*120*120 Å with a grid space 0.40. The docking run was performed by fixing the receptor rigid, while 16 possible conformers of each ligand molecule were obtained and incorporated in the run. Lamarckian genetic algorithm was used, then the binding energy and interactions obtained were analysed and reported.

Table 1: Chemical structures of the constituents of *Bauhinia variegata*.

 Lupeol	 Isoquercetin
 Quercetin	 β-sitosterol
 Kaempferol	 β-carotene
 Stigmasterol	 Hentriacontane
 Flavanone	 Myricetol
 Kaempferol-3-glucoside	 Phenanthriquinone
 Rutin	 Taxifolin
Octacosanol	 Friedelin
 Palmitic acid	 Xanthophyll

Visualization of Ligand-protein interactions

MOE 2022 software was used in visualizing the binding interactions of the ligands with the protein. The final docking complexes were subjected into the software, various hydrogen and hydrophobic interactions were observed and recorded.[47]

Pharmacokinetic analysis of the chemical constituents

Pharmacokinetic parameter helps in identifying the nature of the selected compound and their drug likeliness properties. SwissADME online tool was used in determining the pharmacokinetic and solubility parameters of the considered chemical constituents.[48] The SMILES code of the constituents were added into the online tool from which various Physicochemical and Pharmacokinetic parameters like Molecular weight, GI absorption, BBB permeation, skin permeation, solubility, PAINS score, bioavailability score and many other factors were calculated. The synthetic accessibility of the constituents was assessed by analysing the medicinal chemistry score of the compound. Various rule violations like Lipinski, Egan, ghose and veber possessed by the constituents were also determined.[49]

Toxicity prediction

Presence of toxicity paves way in producing untoward effects in the body. Analysation of toxicity levels in the constituents plays a critical role in dose fixation and other parameters of treatment. OSIRIS toxicity predictor was used in analysing the toxicity of selected chemical constituents of *Bauhinia variegata.* The online tool helps in determination of four different toxicity parameters like Mutagenicity, tumorigenicity, eye and skin irritation and reproductive toxicity which helps in screening out the toxic compounds and eliminating them before pursuing the pre-clinical studies thus reducing the sacrifice of animal models.[50]

Biological activity prediction

PASS online tool was used in determining the biological activity of selected chemical constituents. The tool follows a Bayesian algorithm and they possess a data greater than 3500 biological parameters which are often updated in regular intervals.[51] The tool mainly works on a principle which uses the structural and functional group similarity of the added compounds with the drugs in the database to predict biological activity of the compounds.[52] The sketched and energy minimised chemical constituents were subjected into the tool and parameters relevant to pathophysiology and symptoms of PCOS were selected and recorded.

Molecular dynamics simulation

Molecular dynamics studies help in determining the complex stability of the compounds in the applied environment. They help in scrutinizing the best possible complex throughout the system. Desmond V 5.9 package Schrodinger LLC suite software

under Ubuntu environment was used to perform the molecular dynamics studies.[53,54] The best derived complex from docking analysis was chosen and added into dynamics determination. Then an orthorhombic water box with a size of 10Å was fixed and TIP3P (Three site transferable water molecule) model environment was selected. The force field of OPLS 2005 was fixed and counter ions like Na^+ and Cl^- were added to neutralize the environment. A 2000 step energy reduction was successively performed before selection of 100ns production cycle. The NPT and NVT parameters were selected as per the protocols, maintenance of 1 atm pressure and 300K temperature was done in the system. A grid spacing of 0.8 was fixed in the panel of PME approach. The system was allowed to run for a 100ns simulation with relaxation time of 20ps and complex stability was analysed with the help of simulation interaction diagram. RMSD, RMSF, H-bond and SASA plot analysis were carried out to determine the complex stability.

RESULTS

Molecular docking studies

The phytoconstituents of *Bauhinia variegata* were subjected to molecular docking studies with the help of AutoDock 1.5.7 software. Rigid-flexible docking was performed in which the target protein was placed rigid and ligands were kept flexible during the study. The binding energy score of all individual constituents were compared with each other for both the proteins 3RUK and 1E3K (Table 2). Abiraterone was taken as the standard for Anti-androgen receptor (3RUK) and Nomegestrol acetate was the standard selected for Progesterone receptor (1E3K). Binding energy score of the standards are given in Table 3. The hydrogen and hydrophobic interactions of standards were compared with the constituents to determine the good binding affinity. The constituents had a binding score ranging from -5.10 Kcal/mol to -10.31 Kcal/mol with protein 3RUK and they had a score ranging from -2.32 Kcal/mol to -11.52 Kcal/mol with protein 1E3K respectively. Constituents like Lupeol, β-sitosterol, Stigmasterol and Friedelin had good binding score when compared to other phytoconstituents. Among which Lupeol had the highest binding score of -10.31 Kcal/mol with the protein 3RUK and -11.52 Kcal/mol with the protein 1E3K. The binding score obtained for lupeol was greater than that of the standard Abiraterone with a score -9.78 Kcal/mol. Table 4 clearly shows that constituents with high docking score had closely similar hydrogen and hydrophobic interactions with the standards taken. Lupeol had hydrogen bond interactions with Arg 209 and similar hydrophobic interactions to that of abiraterone with Leu 179, Ala 272 and Val 452 for the protein 3RUK. They possessed hydrogen bond interactions with Trp 765 and Gln 815 while hydrophobic interactions with Met 692, Ile 699, Leu 758 and Phe 818 with the protein 1E3K. The binding interaction images of constituents with good docking score and standards are given in Figure 2.

Table 2: Docking score of chemical constituents of *Bauhinia variegata*.

Sl. No.	Chemical constituent	Code	With 3RUK Kcal/mol	With 1E3K Kcal/mol
1	Lupeol	LUP	-10.31	-11.52
2	Isoquercitrin	IQU	-8.70	-4.01
3	Quercetin	QUE	-7.37	-5.30
4	β-sitosterol	BES	-10.20	-10.44
5	Kaempferol	KAE	-7.23	-7.44
6	β-carotene	BCA	-8.93	-7.92
7	Stigmasterol	STI	-9.71	-10.75
8	Hentriacontane	HEN	-5.25	-2.58
9	Flavanone	FLA	-6.14	-7.90
10	Myricetol	MYR	-7.10	-5.85
11	Kaempferol-3-glucoside	KOG	-8.50	-4.20
12	Phenanthriquinone	PHE	-5.31	-7.68
13	Rutin	RUT	-5.10	-2.32
14	Taxifolin	TAX	-6.53	-5.86
15	Octacosanol	OCT	-6.12	-2.34
16	Friedelin	FRI	-10.10	-10.25
17	Palmitic acid	PAL	-8.02	-5.48
18	Xanthophyll	XAN	-8.83	-8.45

Table 3: Docking score of the selected standards.

Sl. No.	Standard	Code	With 3RUK	With 1E3K
1	Abiraterone	ABI	-9.78	-
2	Nomegestrol acetate	NOM	-	-12.50

Table 4: Binding interactions of the constituents with good docking score.

Code	3RUK		1E3K	
	Hydrogen bond	Hydrophobic interactions	Hydrogen bond	Hydrophobic interactions
LUP	Arg 209	Ile 175 Leu 179 Ala 272 Val 452	Trp 765 Gln 815	Met 692 Ile 699 Leu 758 Phe 818
BES	Arg 209 Cys 412	Phe 84 Ile 269 Ala 337 Val 452	-	Glu 695 Asp 697 Pro 696 Val 698
STI	Arg 409 Cys 412	Ile 175 Glu 275 Leu 331 Ala 418	Trp 765 Gln 815	Pro 696 Val 698 Gly 762 Arg 766
FRI	Phe 84 Arg 209	Leu 179 Ala 272 Val 336 Ile 413	Gly 762 Arg 766	Met 692 Pro 696 Val 729 Leu 763
STANDARDS				
ABI	Asn 172 Cys 412	Leu 179 Ala 272 Ile 413 Val 452	-	-
NOM	-	-	Arg 766 Gln 725	Met 759 Val 760 Phe 778 Leu 763

Pharmacokinetic study

Pharmacokinetic properties of a compound help in understanding the physical and chemical nature of it. SwissADME was the online tool used in determining the Pharmacokinetic properties of the constituents. Various parameters like physico-chemical properties, Drug-likeliness profile, solubility and Pharmacokinetics of the constituents were predicted (Table 5). An ideal compound is known to possess a molecular weight within a range of 150-500 daltons. In this study, it was observed that few constituents like BCA, RUT and XAN had molecular weight greater than the 500 daltons and they also violated the Lipinski rule of 5 with other parameters. TPSA value between 0-140Å is considered to be normal range while few constituents like IQU, MYR, KOG and RUT exceeded the limit which denote that these constituents do not possess good solubility. A lipophilic-hydrophobic balance is essential for ideal solubility of a drug molecule. iLogP within a range of 0-5 is considered to be good however, few constituents like BCA, HEN, OCT and XAN had values above 5 denoting poor hydrophobicity. Constituents like FLA, PHE and PAN were found to be BBB permeant while BES, BCA, STI, RUT and XAN had poor synthetic accessibility. Hence, from the list of the constituents, it was observed that LUP and FRI possess ideal Pharmacokinetic and Drug-likeliness properties over other phytoconstituents in the study.

Toxicity Prediction

OSIRIS property explorer, an online tool was used to predict four different toxicity parameters like Mutagenicity, Tumorigenicity, eye and skin irritation and reproductive toxicity of the subjected constituents. The results obtained is denoted in Figure 3.

The graph clearly explains that constituents like Quercetin and Phenanthriquinone showed high Mutagenicity and tumorigenicity. Palmitic acid showed high tumorigenicity and eye and skin irritation. Kaempferol and myricetol possessed high tumorigenicity. While the constituents with high docking scores like Lupeol, β-sitosterol, Stigmasterol and friedelin were found to be safe and non-toxic in all the four calculated parameters.

Biological activity prediction

In silico biological activity prediction helps in determining the Pa and Pi value of the deployed constituents. PASS online tool was used to determine the activity from which various parameters were displayed in the results. While, parameters having closer relation with the pathophysiology and aetiology of PCOS like Androgen antagonist, Anti-infertility, Oxytocic property, Female Contraceptive activity, Anti-inflammatory, Estrogen agonist, Progesterone agonist activities, Anti-acne and Alopecia treatment were taken up in our study (Table 6). Pa>Pi is considered to be an ideal biological activity. Lupeol and Friedelin was found to possess high Anti-inflammatory property with value greater than 0.70. Lupeol, β-sitosterol and stigmasterol was found to possess good Anti-infertility property with value greater than 0.5. On observation, it was evident that constituents like Lupeol, β-sitosterol, stigmasterol and Friedelin showed biological activity values for all the considered parameters and they were found to have high Pa than that of Pi value.

Molecular Dynamics

Root mean square deviation analysis

From the results obtained by molecular docking studies, Lupeol was the constituent with high binding score and good interaction with both the proteins. Hence it was subjected to the molecular dynamic's simulation. When the ligand comes in contact with the protein they result in the fluctuations of the system. Root mean square deviation analysis is the core analytical part of the molecular dynamics study as they help in the determination of complex stability of ligand and protein in particular environment throughout the system. Complex with fluctuations less than 3Å are represented to be ideal. The RMSD plots of Lupeol (LUP) with both the proteins are given in Figure 4. For 3RUK, the graph evidently shows that the Cα backbone of the protein was found to be prominently stable throughout the simulation time and had an average fluctuation less than 1Å which proves that the complex is stable throughout the simulation time and average ligand fluctuation was less than 1.5Å. For protein 1E3K, the Cα backbone fluctuation and ligand fluctuation was found to be less than 1Å. Hence, the RMSD plot proves that lupeol complex is stable throughout the simulation time from 0-100ns and the fluctuations are under ideal range in the system.

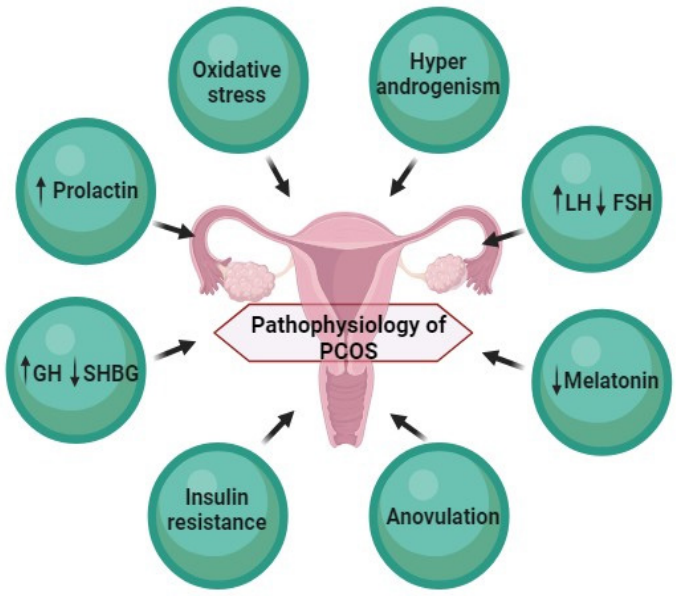


Figure 1: Illustration on Pathophysiology of PCOS.

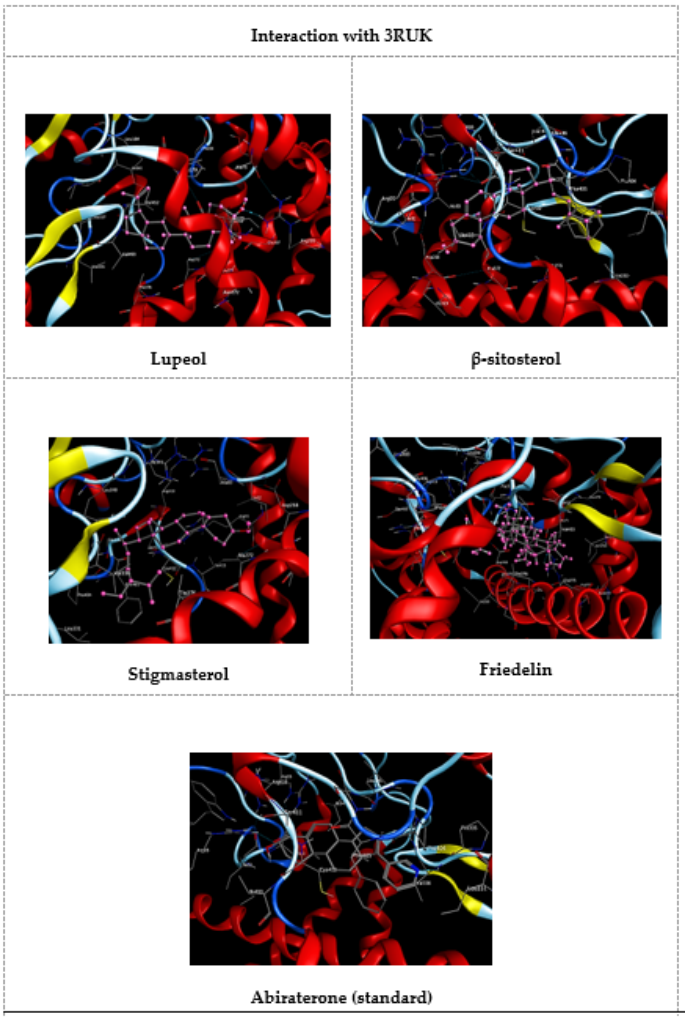


Figure 2: Binding interaction images of constituents with best docking score and standards. A) Lupeol, B) β-sitosterol, C) Stigmasterol, D) Friedelin and E) Abiraterone with the protein 3RUK. F) Lupeol, G) β-sitosterol, H) Stigmasterol, I) Friedelin and J) Nomegestrol acetate with the protein 1E3K.

Table 5: Physicochemical, Pharmacokinetic and Drug likeliness properties of selected Phytoconstituents.

Sl. No.	Code	Physico-chemical Properties					Drug Likeliness And Medicinal Chemistry			Solubility		Pharmacokinetics	
		Mol. Wt	No. of rotatable bonds	No. of H bond acceptor	No. of H bond donor	TPSA Å	Bioavailability score	PAINS Alert	Synthetic accessibility	iLogP	LogS	GI Absorption	BBB Permeant
1	LUP	426.72	1	1	1	20.23	0.55	0	5.49	4.68	-8.64	Low	No
2	IQU	464.38	5	12	8	210.51	0.17	1	5.31	1.55	-2.97	Low	No
3	QUE	302.24	1	7	5	131.36	0.55	1	3.23	1.63	-3.16	High	No
4	BES	414.71	6	1	1	20.23	0.55	0	6.30	4.79	-7.9	Low	No
5	KAE	286.24	1	6	4	111.13	0.55	0	3.14	1.7	-3.31	High	No
6	BCA	536.87	10	0	0	0	0.17	0	6.19	7.79	-11.04	Low	No
7	STI	412.69	5	1	1	20.23	0.55	0	6.21	5.00	-7.46	Low	No
8	HEN	436.84	28	0	0	0	0.55	0	4.06	8.25	-11.04	Low	No
9	FLA	224.25	1	2	0	26.3	0.55	0	2.77	2.41	-3.66	High	Yes
10	MYR	318.24	1	8	6	151.59	0.55	1	3.27	1.08	-3.01	Low	No
11	KOG	448.38	4	11	7	190.28	0.17	0	5.29	0.53	-3.18	Low	No
12	PHE	208.21	0	2	0	34.14	0.55	2	2.33	1.63	-3.27	High	Yes
13	RUT	610.52	6	16	10	269.43	0.17	1	6.52	1.58	-3.3	Low	No
14	TAX	304.25	1	7	5	127.45	0.55	1	3.51	1.3	-2.66	High	No
15	OCT	410.76	26	1	1	20.23	0.55	0	3.72	7.2	-9.25	Low	No
16	FRI	426.72	0	1	0	17.07	0.55	0	5.17	4.52	-8.66	Low	No
17	PAL	256.42	14	2	1	37.3	0.85	0	2.31	3.85	-5.02	High	Yes
18	XAN	568.87	10	2	2	40.46	0.17	0	7.01	7.15	-9.64	Low	No

Table 6: Biological activity of selected Phytoconstituents.

Sl. No.	Code	Androgen antagonist		Anti-infertility, Female		Oxytocic		Contraceptive female		Anti-inflammatory		Estrogen agonist		Progesterone agonist		Antiacne		Alopecia treatment	
		Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
1.	LUP	0,134	0,016	0,524	0,005	0,310	0,021	0,313	0,007	0,708	0,015	0,212	0,017	0,092	0,011	0,514	0,005	0,479	0,060
2.	IQU	-	-	-	-	-	-	-	-	0,772	0,009	0,243	0,014	-	-	-	-	-	-
3.	QUE	0,074	0,033	-	-	0,187	0,137	0,188	0,043	0,689	0,017	0,514	0,005	-	-	-	-	0,552	0,032
4.	BES	0,513	0,005	0,638	0,004	0,505	0,003	0,291	0,008	0,467	0,067	0,224	0,016	0,136	0,006	0,529	0,005	0,378	0,123
5.	KAE	-	-	-	-	0,217	0,093	0,178	0,055	0,676	0,019	0,505	0,005	-	-	-	-	0,566	0,028
6.	BCA	0,163	0,014	0,169	0,147	0,208	0,104	0,145	0,108	0,690	0,017	-	-	-	-	0,934	0,002	0,392	0,112
7.	STI	0,369	0,007	0,507	0,006	0,524	0,003	0,273	0,010	0,542	0,045	0,189	0,020	0,133	0,007	0,553	0,004	0,352	0,146
8.	HEN	0,077	0,031	0,469	0,009	0,407	0,005	0,235	0,017	0,424	0,084	-	-	-	-	0,140	0,037	0,650	0,011
9.	FLA	0,130	0,017	-	-	0,222	0,086	0,233	0,017	0,602	0,031	0,397	0,007	-	-	-	-	0,563	0,029
10.	MYR	0,066	0,038	-	-	0,182	0,147	0,174	0,059	0,720	0,013	0,496	0,005	-	-	-	-	0,518	0,043
11.	KOG	-	-	-	-	-	-	-	-	0,748	0,010	0,305	0,010	-	-	-	-	-	-
12.	PHE	0,073	0,033	-	-	0,328	0,016	0,212	0,026	0,393	0,099	0,093	0,038	-	-	-	-	0,740	0,004
13.	RUT	-	-	-	-	-	-	-	-	0,728	0,013	-	-	-	-	-	-	-	-
14.	TAX	0,076	0,032	-	-	0,200	0,116	0,197	0,036	0,628	0,026	0,290	0,011	-	-	-	-	-	-
15.	OCT	0,068	0,037	0,435	0,013	0,380	0,008	0,245	0,014	0,498	0,058	-	-	-	-	0,184	0,029	0,532	0,038
16.	FRI	0,215	0,011	0,290	0,054	0,318	0,019	0,614	0,003	0,777	0,008	0,235	0,015	0,145	0,006	0,587	0,004	0,531	0,039
17.	PAL	-	-	-	-	0,487	0,003	0,233	0,017	0,515	0,052	-	-	-	-	0,214	0,025	0,655	0,011
18.	XAN	0,112	0,020	-	-	0,244	0,063	-	-	0,463	0,068	-	-	0,033	0,031	0,674	0,003	-	-

Root mean square fluctuation analysis

Proteins undergo fluctuation during the binding of ligands, which enables identical flexibility of the protein for interaction. Minimal helical and loop fluctuations are considered to be normal and the range of fluctuations between 0-3Å is ideal. The RMSF plots of Lupeol (LUP) with both the proteins are given in Figure 5, which clearly explains that the average fluctuations of lupeol with 3RUK was less than 3Å and fluctuations with 1E3K was less than 2.5Å. Thus, these plots explain stability of the complexes.

Protein-ligand contacts

The hydrogen and hydrophobic interactions between ligands and the protein denote stability of the complex. Protein-Ligand complexes of Lupeol with both the targets are given in Figure 6. Interaction fractions explain the intensity of bond formed throughout the simulation time and a value greater than 0.7 is considered to be stable. For protein 3RUK, Lupeol possess hydrogen bond formation with Arg 209 from 0-1 fraction which is 100% that denotes the particular bond formation stays throughout the simulation time and hydrogen bond formation with Ala 75 with 0.8 represents that the specific bonding was found to be stable for 80% of the simulation time. For protein 1E3K lupeol had hydrophobic interactions with Leu 758, Val 698 and Trp 765. Thus, the stronger Protein-ligand contacts denote lupeol with 3RUK possess high complex stability.

Solvent accessible surface area analysis

SASA plot explains the exposure of complex to the external solvent environment. Compounds with less SASA fluctuations denote that the complex is exposed to solvent surface for a maximum duration of time. SASA plots of lupeol with both the proteins are given in Figure S1. The plot explains that lupeol had average fluctuations less than 30Å^2 with protein 3RUK and average fluctuations less than 100Å^2 with protein 1E3K. This explains

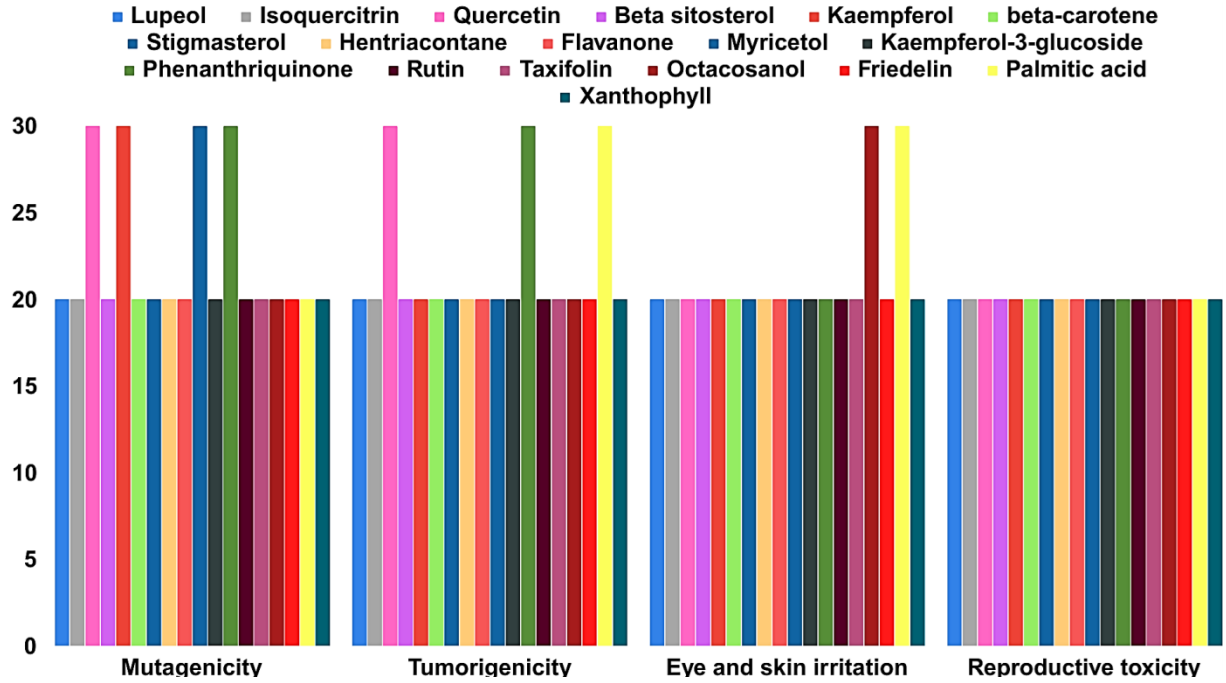


Figure 3: Illustration represents predicted toxicity parameters of *Bauhinia variegata* constituents by OSIRIS online tool.

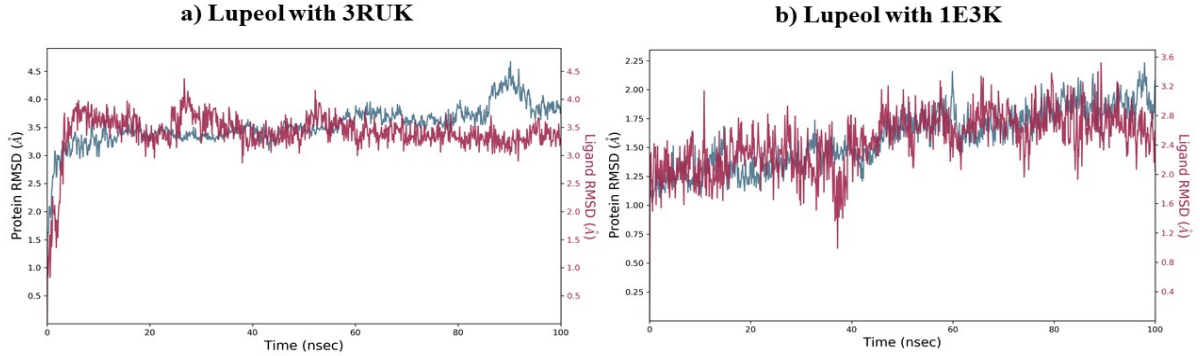


Figure 4: RMSD plots of Lupeol with proteins a) 3RUK and b) 1E3K.

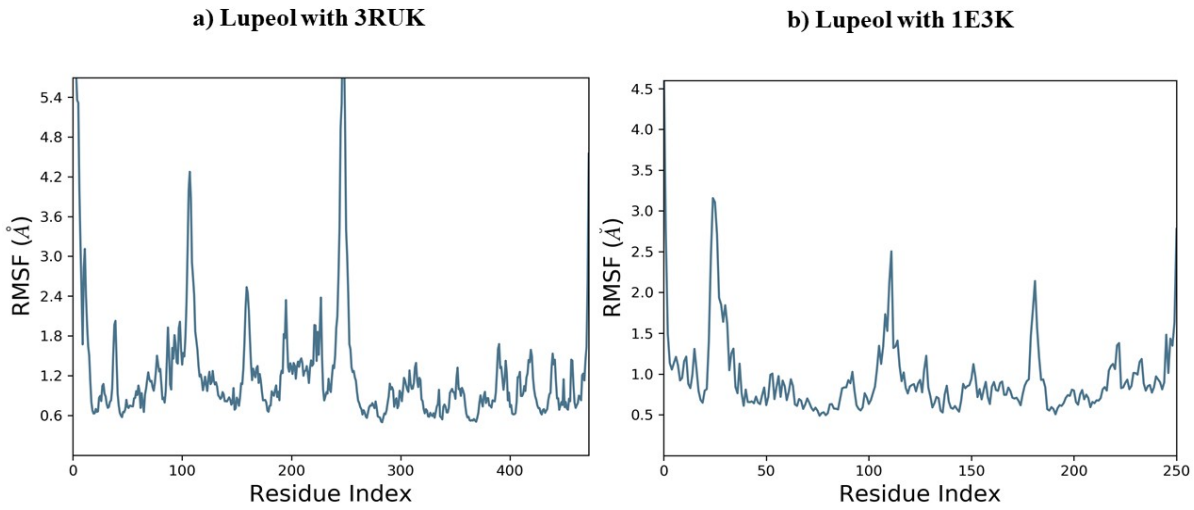


Figure 5: RMSF plots of Lupeol with proteins a) 3RUK and b) 1E3K.

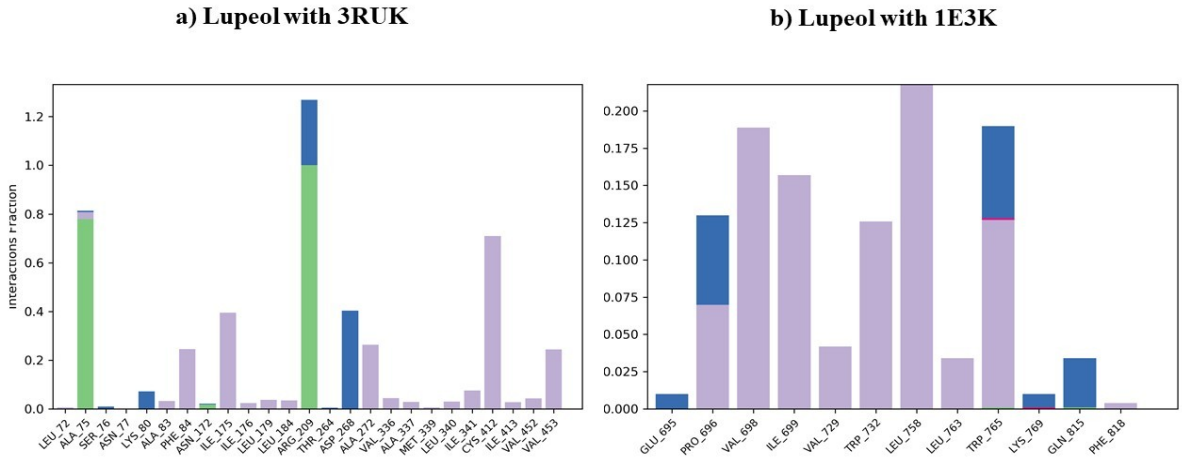


Figure 6: Protein-ligand contact plots of Lupeol with proteins a) 3RUK and b) 1E3K.

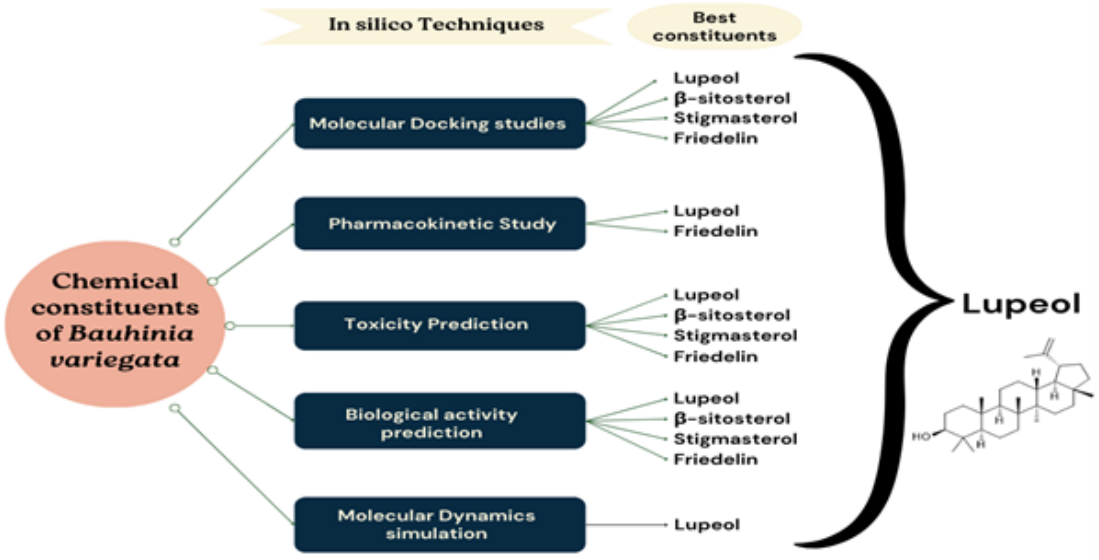


Figure 7: Comparative results for phytoconstituents of *Bauhinia variegata* through various computational techniques.

that lupeol complex was exposed to external environment in a maximal range with the protein 3RUK over 1E3K respectively.

DISCUSSION

Five *in silico* techniques were performed to determine the potent phytoconstituent of *Bauhinia variegata* to fight against the symptoms of PCOS. The results of first computational approach from Molecular docking studies explain that a range of binding energy score and affinity interactions were observed. From which, Lupeol, β-sitosterol, stigmasterol and Friedelin possessed high binding energy and good binding interactions with both the proteins. The result of Pharmacokinetic prediction shows that two constituents namely Lupeol and Friedelin showed ideal drug likeliness and Pharmacokinetic profile when compared to the other phytoconstituents. The toxicity prediction displayed that constituent with good docking score like Lupeol, β-sitosterol, stigmasterol and Friedelin were found to be non-toxic to all the four parameters. The same four constituents were found to possess ideal biological activity scores for all the parameters relative to PCOS. The molecular dynamics simulation proves that Lupeol possess high complex stability with both the proteins, however on comparative analysis lupeol complex with 3RUK protein was found to have prominent RMSD and RMSF fluctuations, they possess ideal SASA values and good Ligand-protein contacts. Comparative results of the whole study is given in Figure 7.

CONCLUSION

On the whole, computational analysis on phytoconstituents of *Bauhinia variegata* enlightens that the major constituent Lupeol (LUP) was found to have high docking score of -10.31 Kcal/mol and -11.52 Kcal/mol with the proteins 3RUK and 1E3K and it had an ideal Pharmacokinetic parameters and Drug-likeliness profile with non-toxic characteristics and also possessed ideal Pa values for all the related parameters to PCOS. Lupeol was found to have greater complex stability with both the selected proteins in Molecular dynamics simulation for 100 ns run. All these *in silico* results clearly explains that lupeol possess anti-androgenic and positive progesterone functioning which enables the compound to fight against the symptoms of PCOS. Hence lupeol deserves further *in vitro* and *in vivo* study on different PCOS models and are expected to yield promising results.

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CONFLICT OF INTEREST

The authors declare no competing interests

ABBREVIATIONS

PCOS: Polycystic Ovarian Syndrome; **BBB:** Blood Brain Barrier; **TPSA:** Topological Polar Surface Area; **ADME:** Absorption Distribution Metabolism Excretion; **RMSD:** Root Mean Square Deviation; **RMSF:** Root Mean Square Fluctuation; **SASA:** Solvent Accessible Surface Area; **BV:** *Bauhinia variegata*; **SHBG:** Sex Hormone Binding Globulin; **HPO axis:** Hypothalamic-Pituitary-Ovarian axis; **LH:** Luteinizing Hormone; **FSH:** Follicle Stimulating Hormone: **GH:** Growth Hormone. **Pa:** Probability of Activity; **Pi:** Probability of Inhibition. **GnRH:** Gonadotropin Releasing Hormone.

SUMMARY

Polycystic ovarian syndrome is a metabolic disorder majorly caused by the hormonal fluctuations in female and the current scenario explains that adolescents remain predominantly affected with this disorder. One among ten women of the world population are diagnosed with the disorder and many are left out without proper diagnosis. *Bauhinia variegata* assist in the treatment of various ailments and are used as an ingredient in targeting uterine disorders, yet the exact constituent that contributes on activity remains unknown. The main aim of our study was to determine the potent phytoconstituent of *Bauhinia variegata* to fight against the symptoms of PCOS through computational techniques. Five *in silico* techniques like Molecular docking analysis, Pharmacokinetics, toxicity prediction of the compounds, Biological activity and Molecular dynamics simulation studies were performed to identify the potent phytoconstituent. Molecular docking studies showed that the major constituent lupeol had good binding interaction and high docking score of -10.31 Kcal/mol and -11.52 Kcal/mol with both the proteins 3RUK and 1E3K. The binding score obtained for lupeol was greater than that of the standard Abiraterone with a score -9.78 Kcal/mol. Pharmacokinetics, toxicity and biological activity studies reveal that it had ideal drug likeliness properties with proper biological activity values and were found to non-toxic in the analysed parameters. Lupeol complex was found to be potentially stable throughout the molecular dynamics simulation for 100 ns. Thus, through *in silico* analysis it is evident that from the list of phytoconstituents of *Bauhinia variegata*, lupeol possess anti-androgenic and positive progesterone functioning which enables the compound to fight against the symptoms of PCOS. Hence lupeol deserves further *in vitro* and *in vivo* study on different PCOS models and are expected to yield promising results.

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Supplementary data

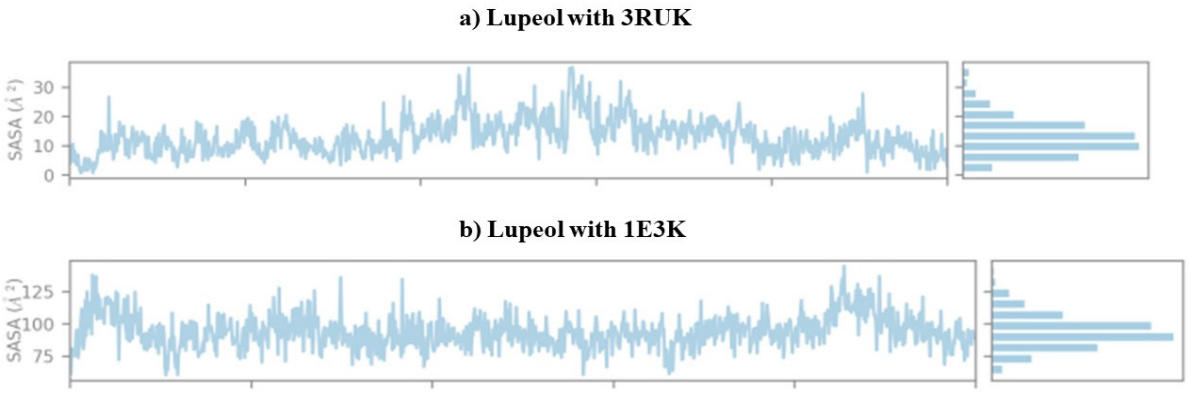

Figure S1: SASA plots of Lupeol with the proteins a) 3RUK and b) 1E3K.