COMPUTATIONAL SCREENING OF PHYTOCONSTITUENTS FROM MEDICINAL PLANTS FOR FERTILITY POTENTIALS

Thenmozhi. M[1], Suganya. J[2*], Amudha. P[3], Prabha. S.B[4], Vidya. R[3], Shanmugavani.S[2]

1- Department of Biotechnology, 2- Department of Bioinformatics,
3 - Department of Biochemistry, 4 - Department of Microbiology,
School of Life sciences, Vels Institute of Science, Technology and Advanced Studies,
Chennai, Tamil nadu, India
Corresponding author: suganyaj11@gmail.com
Mobile No: 9042112221

ABSTRACT:

Infertility is a global problem affecting 8-12% married people during their reproductive lives. According to estimates from 2010, roughly 5% of heterosexual couples worldwide have unresolved infertility issues. Ovulatory issues, which are frequently characterized by irregular menstrual cycles, are the most frequent cause of female infertility. There are most important traditional plants like Achyranthes aspera, Justicia insularis, Punica grantum whose previous pharmacological studies were reported to possess anticancer, antidiabetic, antimicrobial and fertility inducer. The ten phytochemicals were identified from the above 3 medicinal plants through literature survey. The estrogenic protein 1A52 was responsible for infertility caused to human. To investigate the binding relationship between the compounds of Achyranthes aspera, Justica adhatoda, and Punica grantum and the protein, 10 compounds were tested for their estrogenic activity against the 1A52 protein using docking experiments. The docking results showed that two compounds had the best binding interactions with the estrogenic protein's binding site.

Keywords: *Medicinal Plants, Phytochemical compounds, Estrogen Protein, Docking.*

INTRODUCTION:

Numerous ethnobotanical surveys on medicinal plants used by the local population have been carried out in several places of the world, such as Morocco, Saudi Arabia, Taiwan, and Trinidad and Tobago[1,2]. Many different plant species have been identified as having fertility-inducing substances[3]. Countries like Ethiopia and India have long practiced traditional medicine that makes married people more fertile[4]. Without a good understanding of how they work, many medicinal plants have been utilized as nutritional supplements, in the treatment of many ailments, and even to stimulate reproduction[5].

Plants have been used as a source of medicine since antiquity, yet scientific therapies frequently overlook the value of herbal therapy[6]. The World Health Organization proposed using potent, locally accessible plants as alternatives to medication[7]. India has always been worried about population growth; therefore, medicinal herbs have been examined for their potential as contraceptives and their impact on fertility[8,9]. It is currently necessary to explore medications with potential fertility-enhancing properties, and plant extracts have frequently been studied for their potential to affect animal fertility[10]. The current study was conducted because herbal medications are widely available and have minimal negative effects. Three plants, *Achyranthes aspera*[11], *Justicia insularis*[12]and *Punica grantum*[13] were identified in a recent study of medicinal plants as having the highest potential for inducing fertility activity.

One of the plant species in the Amaranthaceae family, *Achyranthens aspera*, is a potent ayurvedic herb[14]. In India, Asia, and other parts of the world, it is a widespread weed that is raised, branching, and quadrangular in shape. Since the beginning of time, the plant and all of its parts, including the roots, seeds, leaves, flowers, and fruits, have been used mostly for therapeutic purposes[15]. In traditional Ayurvedic medicine, *Achyranthens aspera* is primarily used to treat a variety of health conditions, including bug and snake bites, leprosy, asthma, haemorrhoids, arthritis, and fertility inducers[16,17].

In Nigeria and India, traditional healers frequently employ the plant *Justicia insularis* to treat a variety of health issues[18]. The herb *Justicia insularis*, which belongs to the Acanthaceae family, is grown in private gardens in Asia, West Africa, and Central Africa[19]. They are edible leaves that have been collected in the wild for use in the area. The leaves are traditionally used to cure and prevent infertility in the aforementioned countries[20].

Punica granatum is a perennial that can withstand drought. Pomegranate trees are frequently grown in semiarid and arid regions[21]. They are commonly grown in Iran, India, and the Mediterranean nations of Morocco, Spain, Egypt, Turkey, and Tunisia. The pomegranate, on the other hand, is classified as a berry and is a member of the Punicaceae plant family[22,23]. Punica is the only genus that only has one dominant species, *P. granatum*. Increasing evidence makes it evident that *Punica granatum* provides a number of health advantages. High blood pressure, high cholesterol, oxidative stress, hyperglycemia, infertility, and inflammatory activities are just a few of the disease risk factors that pomegranates can help treat or prevent.[24,25]

Nowadays, it is common practice to use molecular docking to find novel ligands for targets with established structures[26]. The target protein interactions are indicated by the free energy binding score; the lower the free energy binding, the higher the binding affinity. Additionally, computational techniques can be used to determine the inhibition factor. The most active chemical has the lowest inhibition constant[27]. It denotes the molecular connection

between the target protein's ligands. The potential for chemicals to bind with the target protein is suggested by the high value of surface interaction[28,29]. The aim of this *in silico* study was to explore the fertility inducer property of phytocompounds via molecular docking using Docking software.

MATERIALS AND METHODS:

Selection of small molecules:

From the three medicinal plants *Achyranthes aspera*, *Justicia insularis* and *Punica grantum* ten phytochemicals were identified through literature research. The chemical must have at least a two-dimensional structure in order to be computationally assessed. The two-dimensional structures of the ten compounds were verified and retrieved using the pubchem database[30].

Selection of Protein:

Infertility in humans is spurred on by the complex of the protein estrogen receptor alpha ligand-binding domain and estradiol. The Protein Data Bank (PDB) (http://www.rcsb.org/pdb/) was used to retrieve the three-dimensional structure of the protein which was discovered through experimental research along with its resolution[31].

Analyzing active sites of protein:

Using CASTp (http://sts.bioe.uic.edu/castp), the active sites of small molecules that must be the ligand-receptor binding sites were predicted to be present in the protein. By measuring concave surface regions on three-dimensional protein structures, the Computed Atlas of Surface Topography of Proteins Server seeks to offer an online resource for locating, defining, and thoroughly describing topographic features[32].

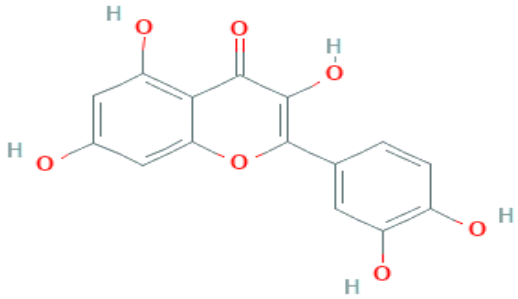
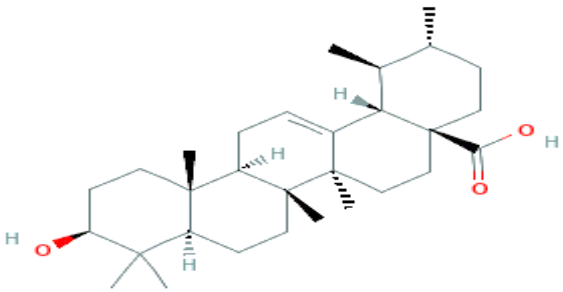
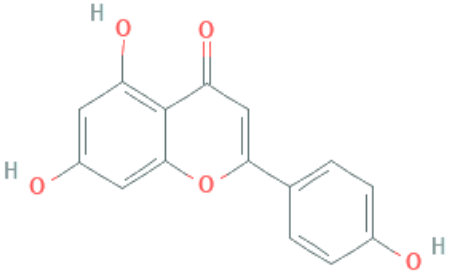
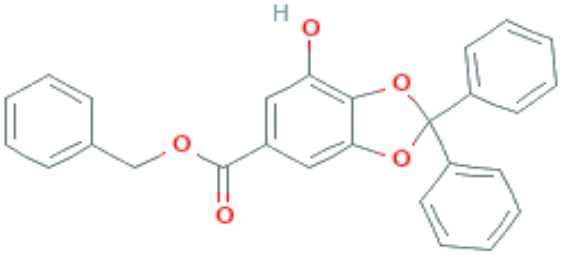
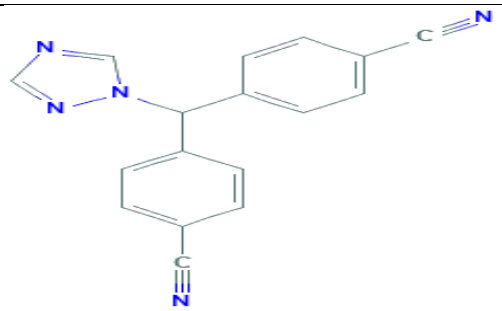
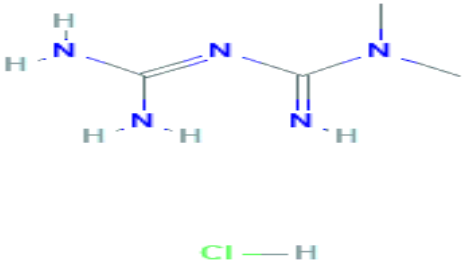
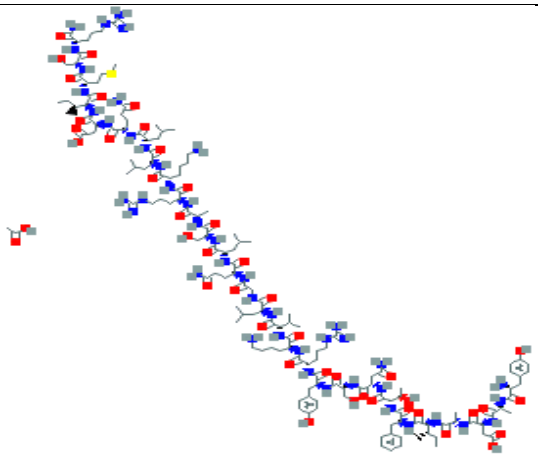
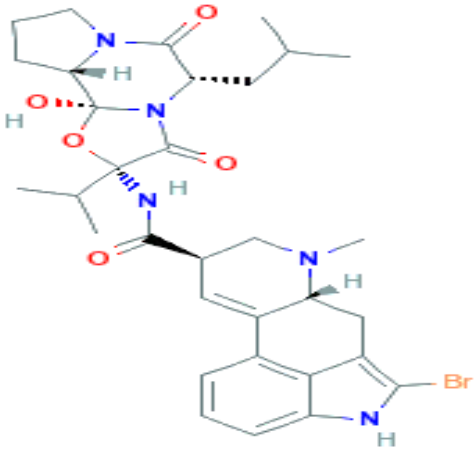
Molecular Docking Interactions:

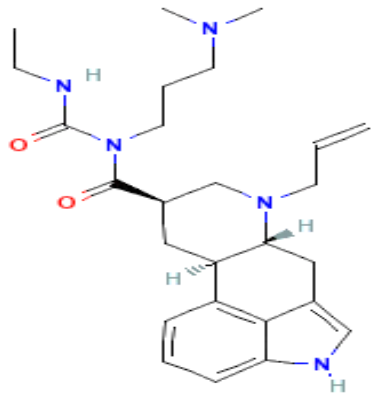
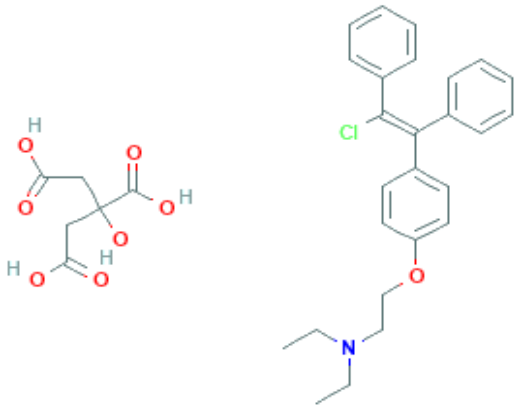
Using ArgusLab 4.0.1, molecular docking studies were carried out after the protein and ligand were prepared to assess the interactions. Free molecular docking software for Windows is called ArgusLab. The protein was loaded, and the chosen amino acid served as the active site. Finally, databases containing the smaller compounds were loaded. Using the shape-based search technique and the AScore scoring function, docking calculations were permitted to proceed. The energy between the ligand and the protein target is determined by the scoring function. The lowest AScore determined by ArgusLab served as the basis for choosing the optimum docking model. Based on hydrogen bond interactions between small molecules and proteins close to the substrate binding site, the best binding interaction was chosen[33].

RESULTS AND DISCUSSION:

The 10 identified bioactive compounds from 3 medicinal plants were selected and subjected to molecular docking with the estrogen receptor. The 2D structures of bioactive compounds were first retrieved from the PubChem database (Table 1).

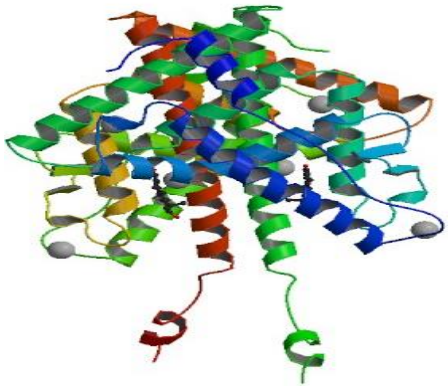
Table1: 2D structure of ten phytochemicals

Phytochemical compounds with its 3D structure and Pubchem ID	
 QUERCETIN - 5280343	 URSOLIC ACID - 64945
 APIGENIN - 5280443	 GALIC ACID 3,4-DIPHENYLMETHYLENE KETAL BENZYL ESTER - 46780424
 LETROZOLE 3902	 METFORMIN HYDROCHLORIDE - 14219
 SERMORELIN ACETATE - 16132412	 BROMOCRIPTINE - 31101

	
CABERGOLINE 54746	CLOMIPHENE CITRATE - 3033832

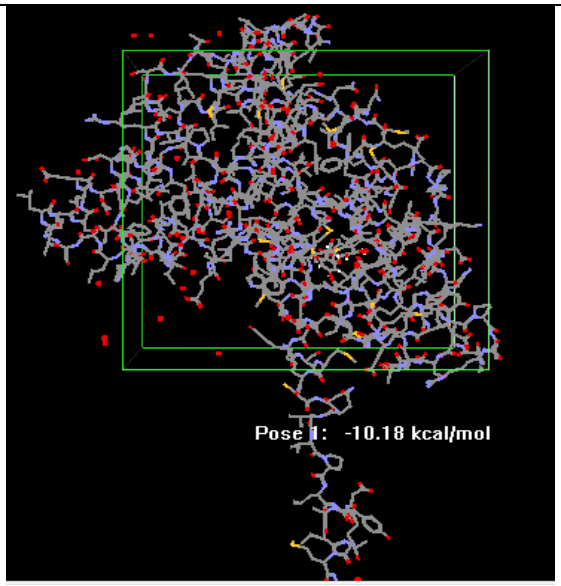
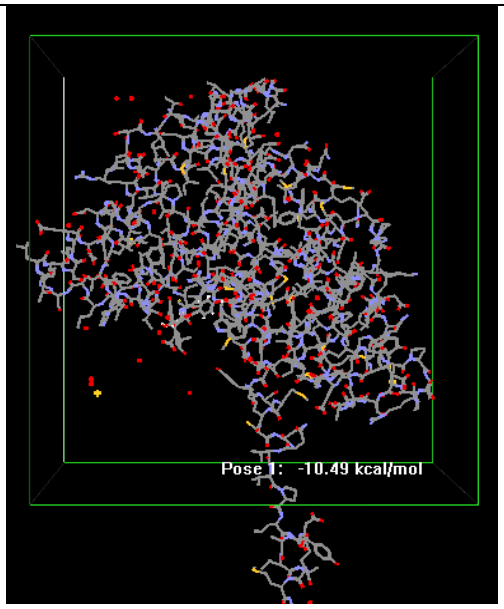
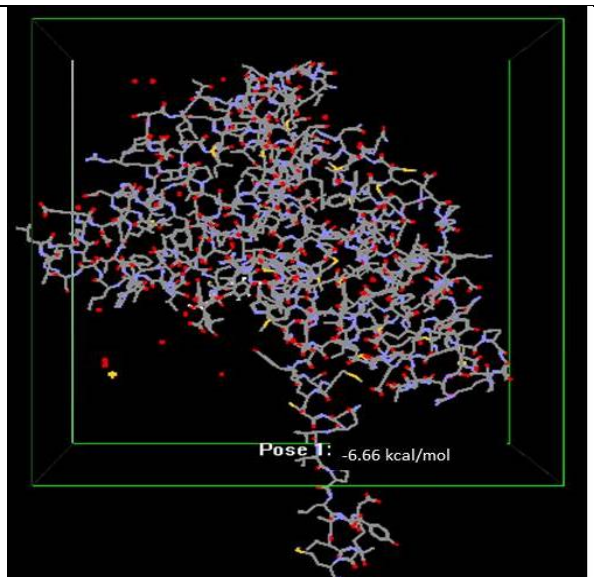
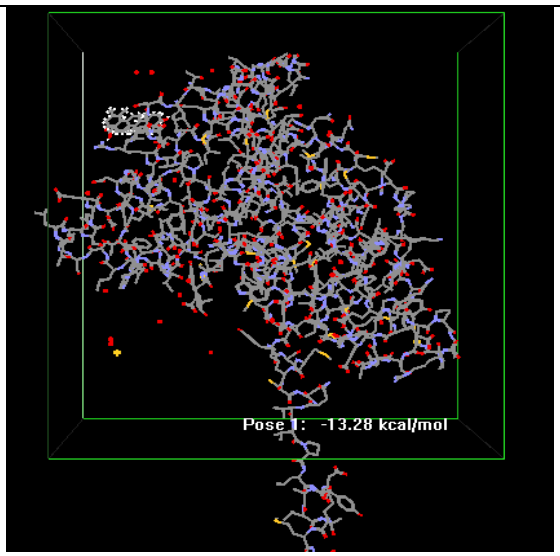
The three-dimensional crystal structure of the estrogen receptor with PDB ID: 1A52 was determined by X-ray crystallography at a resolution of 2.80 Å with Chain A – 258 amino acids and Chain B – 258 amino acids retrieved from the Protein Data Bank. A total of 16 binding sites were assessed in the structure (1A52) through CASTp software with ideal parameters. The 3D structure and binding site were shown in table 2.

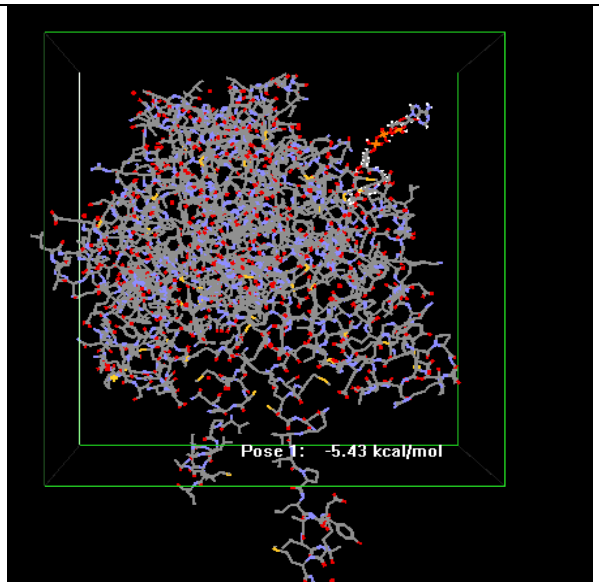
Table 2: 3D structure and binding site of Protein

	S.No	Active Sites	Position	S.No	Active Sites	Position
	1.	GLU	380	2	SER	518
	3	CYS	381	4	SER	518
	5	ALA	382	6	ASN	519
	7	TRP	383	8	LYS	520
	9	LEU	384	10	MET	522
	11	GLU	385	121	TYR	526
	13	ARG	515	14	CYS	530
	15	HIS	516	16	SER	527

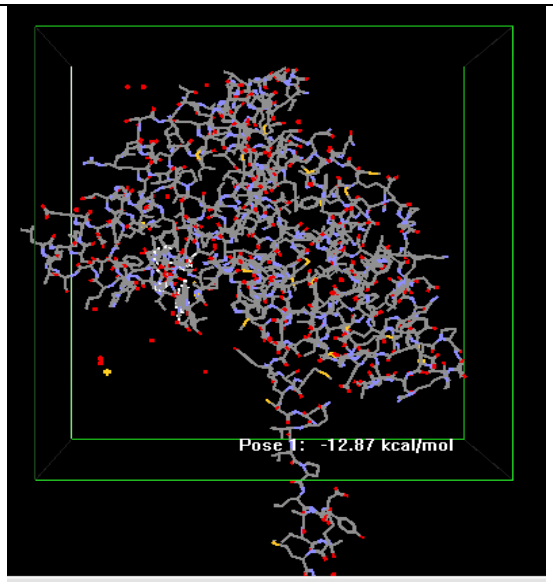
Since the aforementioned three plants have historically been used to cure a variety of ailments, we looked into the ability of bioactive chemicals from *Achyranthes aspera*, *Justica adhatoda,* and *Punica grantum* to bind with important oestrogen targets. The binding analysis between the estrogen receptor and 10 ligands revealed that the binding pattern varied with the nature of the ligands. The docking results of bioactive compounds are shown in table 3.

Table 3- Docking results between bioactive compounds and receptor

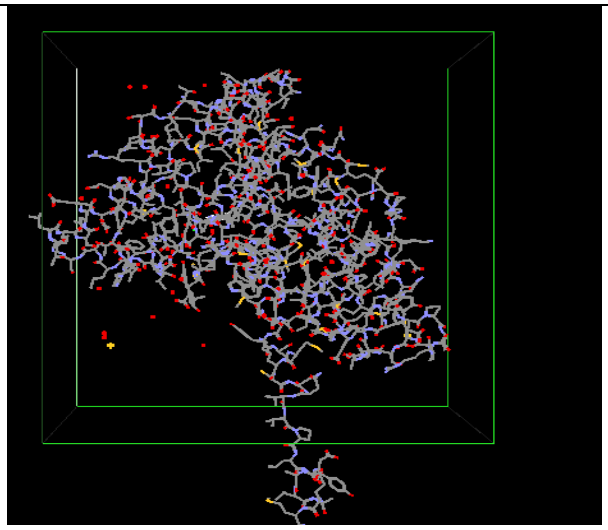
Docking outcome with Protein (1A52) and phytochemicals	
 **QUERCETIN**	 **URSOLIC ACID**
 **APIGENIN**	 **GALIC ACID 3,4-DIPHENYLMETHYLENE KETAL BENZYL ESTER**



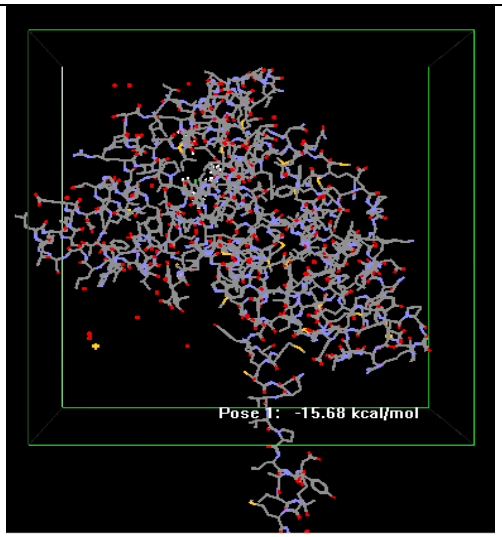
SERMORELIN ACETATE



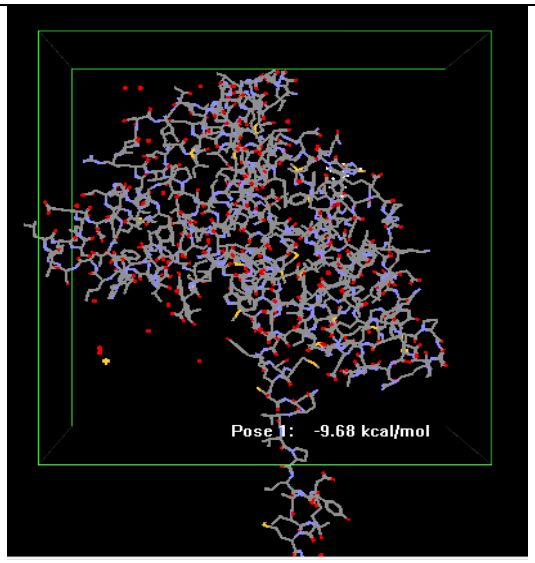
BROMOCRIPTINE



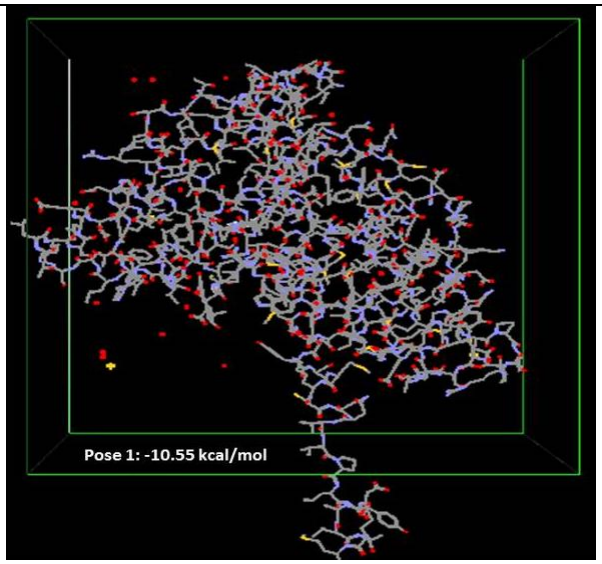
CABERGOLINE



CLOMIPHENE CITRATE



LETROZOLE



METFORMIN HYDROCHLORIDE

The docking results are represented in the form of minimum binding energy values (Tables 4).A high binding affinity between the receptor and ligand molecules was represented by a higher negative docking score, demonstrating the greater effectiveness of bioactive substances. The range of docked ligand scores for receptors was between -15 and -5 Kcal/mol. Among the tested bioactive compounds, Clomiphene Citrate and Galic acid 3,4-diphenylmethylene ketal benzyl ester are the lead compounds in the current study with the highest docking scores. The results of the docking research also showed that various energy sources were reliable and supported the overall potency of the above 2 compounds' interactions with the target protein. Sermorelin Acetate received the lowest rating, while cabergoline failed to engage with the receptor by binding. However, above 2, the chemicals were effective as therapeutic inducers of conception.

Table 4 - Docking Interaction with Protein and phytochemicals

S.No	Docking Interaction	Binding Energy
1.	1A52 - Clomiphene Citrate	-15.68kcal/mol
2.	1A52 - Galic acid 3,4-diphenylmethylene ketal benzyl ester	-13.28kcal/mol
3.	1A52 - Bromocriptine	-12.87kcal/mol
4.	1A52 - Metformin Hydrochloride	-10.55kcal/mol
5.	1A52 - Ursolic Acid	-10.49kcal/mol
6.	1A52 - Quercetin	-10.18 kcal/mol
7.	1A52 - Letrozole	-9.68kcal/mol
8.	1A52 - Apigenin	-6.66kcal/mol
9.	1A52 - Sermorelin Acetate	-5.43kcal/mol
10.	1A52 - Cabergoline	No Binding interaction

CONCLUSION:

The process of developing new drugs is significantly influenced by developments in computation. To reduce the price and duration of drug development, virtual screening methods are often and widely used. A key component of structure-based drug design is the discovery of new ligands for proteins using the molecular docking technique. Drug formulations heavily depend on the interaction between the chemicals and the receptor. A wide range of human diseases can be treated with natural products, and many medicines are made from them. Given the development of drug resistance in numerous diseases and the minimal side effects of conventional therapies, plant-based medications are the most prospective solutions. Our findings indicate that the chosen bioactive chemicals can successfully attach to receptors and that molecular docking can be used to identify fertility inducers from drugs. In molecular docking investigations, the bioactive substances Clomiphene citrate and Galic acid 3,4-diphenylmethylene ketal benzyl ester showed encouraging binding affinity towards the two target proteins. As a result of our research, we may be able to create trustworthy and potent medications for a number of ailments using just 2 chemicals. Additional research to analyse its bioactivity and clinical trials are required for the development of new medication formulations.

CONFLICT OF INTEREST:

The authors declare they have no competing interests.

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