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ORIGINAL ARTICLE

Predicting Anti-Diabetic Property of a Compound Extracted from *Hygrophilla auriculata* Seed using Molecular Docking for GCMS, Physical Characteristics, Drug Disposition, Drug Toxicity and Protein Ligand Interaction

Gokilavani Myilraj[1], Arunkumar Radhakrishnan[1]*
Department of Pharmacology, Chettinadu Academy of Research and Education, Chennai, Tamil Nadu, India.
Corresponding Author Email: arunrmbbs1978@gmail.com

ABSTRACT
The seeds from Hygrophilla auriculata is traditionally used by rural populations to treat diabetes and infertility. Plant-based secondary metabolites are crucial for developing new drugs. In vitro and in vivo studies are substantially supported by early screening of bioactive chemicals for a therapeutic activity. The objective of this study is to screen antidiabetic bioactive compounds in H. auriculata seed using in silico molecular docking approach. In the H. auriculata seed, GCMS analysis identified 15 compounds with medicinal properties. Out of these, twelve compounds were evaluated for drug efficacy, drug-like properties, and drug disposition and toxicity using the SWISS ADME online tool. The compounds, including beta sitosterol, estradiol, squalene, octadecanoic acid, naphthalene, N-hexa decanoic acid, decanoic acid, stigmasterol and vaccenic acid were found to have drug-like properties suggesting that they could be cell-permeable and orally active. The ADMET analysis predicted that above mentioned compounds are safe and readily absorbed. Molecular docking studies revealed a strong binding affinity like -11.67 kcal/mol (beta sitosterol), -11.2 kcal/mol(stigmasterol), -9.87 kcal/mol(estradiol), with docking scores ranging from -2.90 kcal/mol (vaccenic acid) to -11.67 kcal/mol (beta sitosterol). This strong binding provides evidence that beta sitosterol, stigmasterol and esterdiol compounds may have antidiabetic activity and warrants further research to understand their mode of action
KEYWORDS: *Hygrophilla auriculata*, anti-diabetic, molecular docking, and ADMET.

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INTRODUCTION

In the last several years traditional medicine is popularly used across the countries because of their availability and acceptability due to less side effects [1]. Phytotherapies have been historically practiced to treat a wide range of acute or chronic health conditions from Musculo skeletal disease to memory loss [2]. World health Organisation has also been advocating the use of herbal medicine for primary health care needs in most of the countries. According to the International Diabetes Federation (IDF), the global burden of diabetes continues to increase at an alarming rate. In 2024, it was estimated that there were approximately 589 million adults between the ages of 20 and 79 living with diabetes worldwide [3]. Furthermore, projections indicate that this number is expected to rise significantly, reaching an estimated 853 million by the year 2050. This upward trend highlights the urgent need for effective strategies to prevent, manage, and treat diabetes on an international scale. Considerable number of these people go untreated, especially in low- and middle-income nations and may increases the risk of complications such as kidney failure (nephropathy), cardiovascular illnesses (heart attack, stroke), and nerve damage [4].
Beyond its impact on personal health, diabetes places a tremendous financial burden on healthcare systems around the world. Due to drug resistance, drug interactions, complications and painful in administering, and side effects, the traditional medicine is preferred over conventional therapy (5).

However, the effectiveness and safety of traditional medicinal herbs and practices for diabetes are yet to be scientifically validated. Hence, the research is conducted to identify active components and comprehend their mechanisms of action. This evidence-based approach is crucial to integrate traditional medicine effectively and safely into modern diabetes care, offering a more comprehensive and personalized treatment strategy for patients worldwide (5). One of the important proteins play a major role for treating diabetes is insulin. Apart from insulin other proteins-based drugs also plays a remarkable role to treat diabetes are GLP-1 Receptor Agonists (e.g., Semaglutide, Liraglutide, Dulaglutide), Amylin Analogues (e.g., Pramlintide). The same way some enzymes are targeted to treat diabetes, Alpha-Glucosidase and Alpha-Amylase, Dipeptidyl Peptidase-4 (DPP-4), Insulin-Degrading Enzyme (IDE), SNO-CoA-assisted Nitrosylase (SCAN), Protein Tyrosine Phosphatase 1B (PTP1B), Sphingosine Kinase 2 (SphK2), Pyruvate Kinase M2 (PKM2) (6).

While insulin remains the most direct protein-based therapeutic, the landscape of diabetes treatment heavily relies on modulating the activity of various enzymes through inhibitors to improve glucose control and address underlying pathologies like insulin resistance. Emerging research continues to identify new enzymatic and protein targets for novel drug development (7). Naturopathic remedies with medicinal plants have sparked interest in treating numerous medical conditions. *H. auriculata* (K. Schum) Heine (H. auriculata) is a semi-woody plant belonging to Acanthaceae, grows in damp areas of India close to the banks of freshwater or stagnant ditches and swampy areas. It is frequently combined with sedges and marshy grasses. Phytosterols, fatty acids, minerals, polyphenols, proanthocyanins, mucilage, alkaloids, enzymes, amino acids, terpenoids, vitamins, glycosides, carbohydrates, hydrocarbons, and flavonoids are among the several categories of phytoconstituents found in *H.auriculata* seeds, and it is useful in the treatment of diabetic, infertility, diseases of urinogenital tract, dropsy of chronic Bright's disease, hyperdipsia, skin diseases, flatulence, diarrhoea, dysentery, leucorrhoea, gonorrhoea, asthma, blood diseases, gastric diseases, painful micturition, menorrhagia etc (8-10). However, the most potent compound that has anti-diabetic properties has not been reported. The objective of the present study is to identify and evaluate bioactive compounds with anti-diabetic properties with binding affinity and its molecular interactions with potential blood sugar regulating substance proteins such as, 3DH4, 3IOL, 2ZOX and enzymes are Sodium glucose co transporter-1Galactose, Glucagon like peptide-1 and cytosolic beta-glucosidase.

MATERIAL AND METHODS

Gas Chromatography and Mass Spectrometry (GCMS)

The chemical constituents of cold macerated ethanolic seed extract of hygrophilla auriculata, analysed using Agilent GC 7890 A gas chromatography connected with an MS- 5975C mass spectrometer instrument detector. Autosampler system-7693 (ALS 7693) was used in the sample injection process. The carrier gas- Helium was used at 1ml/minute constant flow rate, and the 15 spitless flow rate was 1ml/minute. The DB-5MS non-polar capillary column (5% diphenyl, 95% dimethyl polysiloxane) with the dimensions of 30m length, a 0.25mm inner diameter and a 0.25µm of film thickness was used in this study. The initial oven temperature was kept at 50ºC for 1 minute and programmed to reach 300 ºC held for 2 minutes. The total run time 28 minutes was programmed for the analysis, and the injection volume was 1µl. The detector operated in 50-550 mass range with 0.5s scan interval. The obtained chromatogram of seed extract was analysed in mass spectrometry to identify the mass of detected fractions. Eluted chemical constituents were further identified based on the retention time and mass spectra. The comparison of eluted compounds made with standard mass spectra data library National Institute Standard and Technology-14.0 (NIST 14.0) version to determine the name, molecular weight and structure of the eluted chemical constituents [11].

Physical characteristics and Druglike properties

The SWISS ADME online server was utilized to calculate the physical characteristics and drug like properties parameter based on Lipinski, Ghose, Veber, Egan and Muegge rules. The PubChem database (or another designated source like Chem Spider, ZINC, etc.) was used to obtain the Simplified Molecular Input Line Entry System (SMILES) notation for every test compound. The prediction software's input format was SMILES strings. Several pre-validated drug-likeness rules were used to evaluate the compounds' potential for oral bioavailability and good pharmacokinetics.

The Rule of Five (Ro5) by Lipinski was used to forecast poor absorption/permeation using molecular weight lipophilicity, hydrogen bond donors, and hydrogen bond acceptors. Ghose Filter: focuses on characteristics such as number of atoms (20 to 70), range of -0.4 to 5.6, and range of 160 to 480. Veber rules were used to evaluate oral bioavailability by emphasizing the quantity of rotatable bonds and polar surface area. Egan Rules was used for predicting passive oral absorption using TPSA and thresholds.

Muegge Filter was used for counting particular functional groups, lipophilicity, and molecular size [12, 13].

Drug disposition and toxicity properties

ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) prediction provides valuable facts about the compound that could be evidenced for drug design. The computational pkCSM tool (http:// biosig.unimelb.edu.au/pkcsm/prediction) was used to conduct ADMET studies. To compute the ADMET characteristics, the molecules were supplied in the standard SMILE format. A positive ADMET profile is crucial for compounds in the development of new drugs. The absorption characteristics indicate whether a substance is effectively absorbed through oral or intestinal administration, as well as its solubility in water. Caco-2 cells are commonly employed as an in vitro model to assess absorption efficiency across epithelial barriers associated with cellular transport within the body, owing to their similarity to epithelial cells in the small intestine. A drug is deemed permeable if the permeability of Caco-2 cells exceeds 8×10-6 [14].

Protein ligand interaction prediction

Molecular docking simulation were performed using Autodock4 (version 4.2.8). A scoring interaction prediction based on energy is used to rank the receptor-ligand poses got during docking calculations. The best-docked confirmation has been visualized using the Discovery studio visualizer (BIOVIA), and the docking site, binding interaction, and bond length were identified. The best binding mechanism, affinity, and intermolecular interactions between the test substances (ligands) and the target protein (receptor) were predicted using molecular docking simulations. The docking computations were performed using Auto Dock 4.2.8, which used its empirical binding free energy function to score the poses and its robust Lamarckian Genetic Algorithm (LGA) for conformational searches. This method is commonly employed in modern structure-based drug design research and is widely recognized for assessing ligand-receptor binding [15].

RESULTS AND DISCUSSION

The present study is the first to report in silico findings of *H. auriculata* seed extract compounds with the target proteins.

GC-MS analysis:

The cold macerated procedure using to extract Ethanolic seed extract of *H. auriculata* was analysed in GC-MS to identify the bioactive compounds responsible for anti-diabetic pharmacological actions. Overall, 40 peaks were obtained after a full run of 28 minutes (Fig. 1). The chromatogram displays the retention time (RT), peak area, and peak area percentage of the bioactive chemicals.

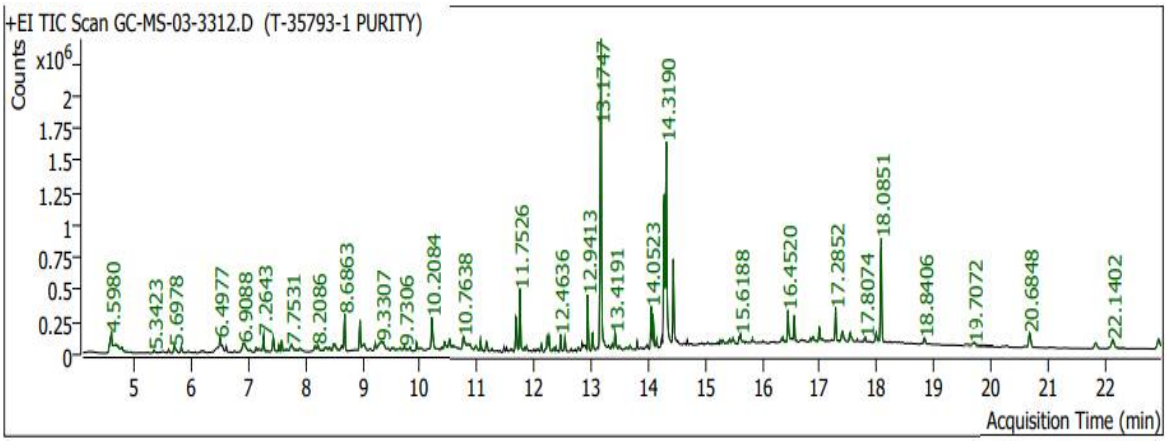


Fig: 1 Chromatogram of Ethanolic seed extract of *Hygrophilla auriculata* analysed in GC-MS in order to identify the anti-diabetic bioactive compounds.

Among the several chemical constituents, twelve peaks with moderate to higher concentrations were selected for the data analysis. The first compound obtained was hexanoic acid- at 4.598 min of retention time. Consequently, beta sitosterol, stigmasterol, squalene, estradiol, cis-vaccenic acid, n-hexa decanoic acid, decanoic acid, etc compounds were identified (Table 1). The drug-like characteristics of the 12 compounds were evaluated using Swiss ADME. All these 12 compounds were found to meet the drug-likeness criteria according to Lipinski's rule of five. According to Lipinski's rule of five, a molecule is considered drug-like only if its molar weight (MW) is below 500 Daltons (Da); the logarithm of the

octanol/water partition coefficient (Q Plog Po/w) is under 5, the number of hydrogen bond acceptors (HBA) is fewer than 10, and the number of hydrogen bond donors (HBD) is less than 5.

Drug disposition and Toxicity properties:

By using PKCSM, these chosen compounds were further assessed for their drug-like behaviour (Table 2). The phytocompounds investigated in this work are easily absorbed in the gut, as evidenced by their high permeability to Caco-2 cells and high human intestinal absorption (HIA) rates ranging from 89.0 to 96.5 percent indicating that these molecules are readily absorbed in the gut. Additionally, the high skin permeability activity of all the screened phytocompounds was demonstrated by Log Kp values being less than -3.67. From the cells, the P-glycoprotein extrudes poisons. According to this research, none of the substances were P-glycoprotein substrates except C6- C8. These substances are overexpressed on cell surfaces and stop medications from leaking out too much. Only water- and lipid-soluble, selective transport molecules, such as glucose transporters and plasma glycoprotein, can cross the blood-brain barrier and enter the central nervous system, according to the distribution analysis [16]. CYP2D6 and CYP3A4 are two CYP450 isoforms used in this investigation to assess metabolism. CYP450 is an enzyme that assists in the metabolism of xenobiotics by oxidizing them and making detoxification easier [17]. In the metabolic analysis, all compounds except C1, C2, C3, C4, C8, C10, C11 and C12 were CYP3A4 substrates. It was therefore anticipated that these substances would be efficiently digested in the liver. Since total clearance controls a drug's half-life, bioavailability, dose concentrations, and frequency, it is a crucial pharmacokinetic feature that influences its metabolism and excretion [18]. In the excretion, the organic cation transporter substrate was investigated. The renal clearance by renal OCT2 substrate and total clearance values of all the identified phytocompounds were substantial.

The harmful nature of each ligand was also examined utilizing a variety of assays in the toxicity prediction. The AMES toxicity and hepatotoxicity tests indicated that all the medication candidates identified in this investigation were non-toxic and anti-diabetic. Oral rat acute toxicity (LD50), oral rat chronic toxicity (LOAEL), and maximum tolerated dose (human) were provided in the ADMET analysis (Table 2). The biomarker that causes the cardiotoxicity of a wide variety of medicinal drugs is typically the hERG potassium channel (human ether-a-go-go-related gene). It has a direct role in cardiac activity, which controls heartbeat. Human QT interval lengthening and serious cardiovascular problems are caused by blocking the hERG potassium channel, which is a major problem in pharmaceutical trials. The risk of cardiovascular problems is decreased because of all drugs evaluated for hERG activity do not block hERG except C1. All of the tested phytocompounds were found to be non-hepatotoxic, less deadly, and non-carcinogenic to rats, according to an overall toxicity study. The compound sitosterol demonstrated a strong binding affinity towards the target proteins, resulting in anti-diabetic, as supported by the molecular docking studies and ADMET analysis. Further to examine the drug development from the aforementioned substances, in vitro research, clinical trials, and other cutting-edge methodologies could be used [19].

TABLE: 1 List of compounds identified in cold macerated ethanolic seed extract of *Hygrophilla auriculata* subjected to GC-MS. Physical characteristics and Druglike properties of the ligands were detailed

S.no	Compound Name	Formula	Retention time (sec)	Mol. Weight (g/mol)	Area (%)	HBA	HBD	LogP	Lipinski violation
1	Gamma sitosterol	$C_{29}H_{50}O$	22.9	414.7	0.94	1	1	5.07	1
2	Stigmasterol	$C_{29}H_{48}O$	22.1	412.7	1.42	1	1	7.80	1
3	1-h cyclopenta phenanthren-3-ol	$C_{27}H_{46}O$	20.7	190.2	1.13	0	0	3.80	0
4	Squalene	$C_{30}H_{50}$	18.0	410.7	5.05	0	0	10.6	0
5	Estradiol	$C_{15}H_{16}N_2OS$	17.2	204.2	1.16	4	2	0.56	0
6	Naphthalene	$C_{17}H_{18}O_5$	16.9	128.1	1.17	0	0	2.83	1
7	Phthalic acid di ester	$C_{24}H_{38}O_4$	16.6	222.2	1.21	4	0	2.04	0
8	Glycerol- 1 palmitate	$C_{19}H_{38}O_4$	16.4	330.5	1.49	4	2	4.36	0
9	n-decanoic acid	$C_{18}H_{36}O_2$	14.4	172.2	5.24	1	1	3.21	0
10	Cis-vaccenic acid	$C_{18}H_{34}O_2$	14.3	282.4	12.3	1	1	6.10	1
11	Octa decanoic acid	$C_{18}H_{36}O_2$	14.4	284.4	3.12	1	1	6.33	1
12	n-hexa decanoic acid	$C_{16}H_{32}O_2$	13.2	256.4	15.5	1	1	5.55	1

TABLE 2: ADMET Properties of GCMS analysed and selected compound for docking analysis.

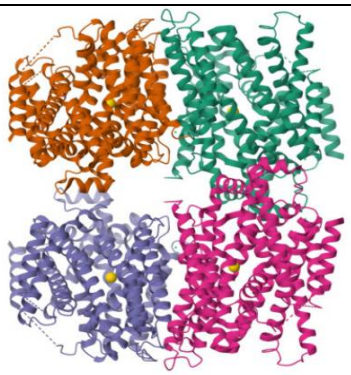
Pharmacokinetic properties		C 1	C 2	C 3	C 4	C 5	C 6	C7	C8	C9	C10	C11	C12	Unit
Absorption	Water solubility	-6.77	-6.68	-4.81	-8.40	-0.49	-3.49	-2.42	-5.38	-3.30	-5.92	-5.97	-5.56	log mol/L
	Human intestinal Absorption (HIA)	94.4	94.9	96.5	89.0	76.2	95.1	96.5	90.9	94.0	91.8	91.3	92.0	% Absorbed
	Skin permeability	-2.7	-2.78	-1.92	-2.76	-3.67	-1.43	-2.53	-2.81	-2.68	-2.72	-2.72	-2.71	Log Kp
	P-glycoprotein Substrate	N	N	N	N	N	Y	N	Y	N	N	N	N	Yes/No
	BBB permeability	0.78	0.77	0.49	0.96	-0.24	0.43	0.13	-0.87	0.14	-0.16	-0.19	-0.11	log BB
	CNS permeability	-1.70	-1.65	-1.63	-0.93	-3.0	-1.25	-2.83	-3.39	-2.14	-1.65	-1.70	-1.81	log PS
Metabolism	CYP2D6 substrate	No	No	No	No	No	No	No	No	No	No	No	No	Yes/No
	CYP3A4 substrate	Yes	Yes	Yes	Yes	No	No	No	Yes	No	Yes	Yes	Yes	Yes/No
	CYP2D6 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	Yes/No
	CYP3A4 inhibitor	No	No	No	No	No	No	No	No	No	No	No	No	Yes/No
Excretion	Total clearance	0.62	0.618	0.06	1.79	0.46	0.19	0.86	1.97	1.55	1.88	1.83	1.76	log ml/min/kg
	Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	No	Yes/No
Toxicity	AMES toxicity	No	No	No	No	No	No	No	No	No	No	No	No	Yes/No
	Maximum tolerated dose (Human)	-0.62	-0.66	0.10	-0.53	1.20	0.70	1.37	0.34	-0.05	-0.81	-0.79	-0.70	log mg/kg/day
	hERG inhibitor	Yes	No	No	No	No	No	No	No	No	No	No	No	Yes/No
	Oral rat acute toxicity (LD50)	2.55	2.54	2.16	1.89	1.83	1.94	2.09	1.70	1.53	1.41	1.40	1.44	mol/kg
	Hepatotoxicity	No	No	No	No	No	No	No	No	No	No	No	No	Yes/No
	Skin sensitivity	No	No	Yes	No	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes/No

C_1: beta sitosterol; C_2- stigmasterol; C_3- 1-h cyclopenta phenanthren-3-ol; C_4- squalene; C_5- estradiol; C_6- naphthalene; C_7- phthalic acid di ester; C_8- glycerol- 1 palmitate; C_9- n-decanoic acid; C_{10}- cis vaccenic acid; C_{11}- octa decanoic acid; C_{12}- n-hexa decanoic acid

Target proteins:

Three target proteins were selected from the RCSB PDB database and earlier studies, and their structures and functions (Table 3)

Table. 3. Target diabetic pathogenic proteins with their characteristics and Structure

Protein Name and enzyme	PDB code	Function of protein	Protein 3D Structure
3DH4 Sodium glucose co transporter-1 Galactose	3DH4	specifically, a sodium/sugar symporter (vSGLT) from Vibrio parahaemolyticus, is a transmembrane protein responsible for transporting sodium and sugars across cell membranes	
3IOL Glucagon like peptide-1	3IOL	The protein structure 3IOL, as detailed in the RCSB Protein Data Bank, represents the crystal structure of Glucagon-Like Peptide-1 (GLP-1) in complex with the extracellular domain of the GLP-1 receptor. This complex is crucial for understanding how GLP-1, a peptide hormone, binds to and activates its receptor, which is involved in regulating insulin release and other metabolic processes.	

2ZOX cytosolic beta-glucosidase	2ZOX	It is the PDB (Protein Data Bank) code for the crystal structure of the covalent intermediate of human cytosolic beta-glucosidase. This enzyme is also known as klotho-related protein (KLrP) or GBA3. It hydrolyses various beta-D-glucosides, including glucosylceramide, and is involved in a non-lysosomal catabolic pathway of glucosylceramide	

Molecular docking:

Phytocompounds had been derived from diverse remedial plants, for instance, flavonoids, phenols, alkaloids, saponins, tannins, terpenoids, glycolipids, glycosides, anthocyanins and carotenoids are known to possess anti-diabetic activity. In numerous findings, flavonoids and polyphenols exhibit blood glucose inhibition by enhancing the GLUT-2 expression within the pancreatic beta cells and GLUT-4 translocation expression. Oxidative stress and inflammation are associated with the pancreas, linked to the progression and the pathogenesis of diabetes [20].The free radicals produced from protein glycosylation and hyperglycaemia-induced glucose autoxidation has an imperative role in DM pathogenesis.

All the identified ligands were put through docking with the target proteins following the initial drug-like property studies. There were six *in silico* docking analyses conducted in all. The current computer investigation looks for an effective medication candidate from the seed of *H. auriculata* that inhibits the target proteins that cause diabetic development. The binding energy analysis indicates that the chosen compounds including stigmasterol (-11.2 kcal/mol), sitosterol (-11.6 kcal/mol), vaccenic acid (-2.90 kcal/mol), N- decanoic acid (-4.82 kcal/mol), decanoic acid (-2.87 kcal/mol), and esterdiol (-9.87 kcal/mol) demonstrated strong binding affinity towards the target proteins among the docked complexes. [Table 4]. Depending on the type of ligand, different binding patterns are analysed between the protein and the selected ligands.

Table 4. Binding energy scores of docked complexes

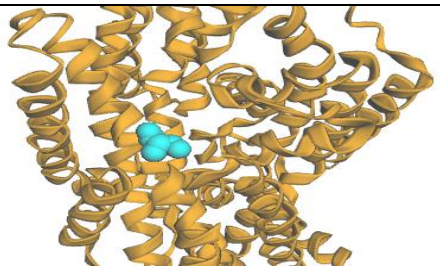
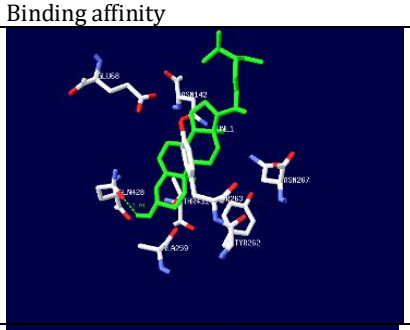
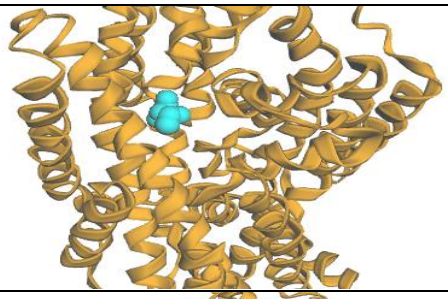
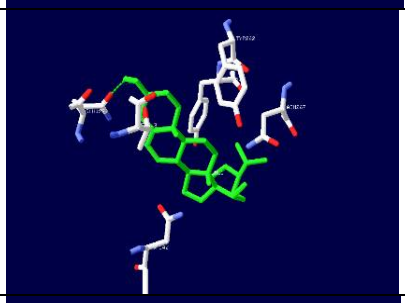
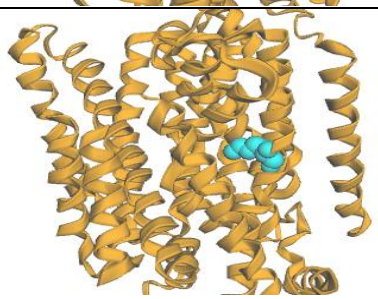
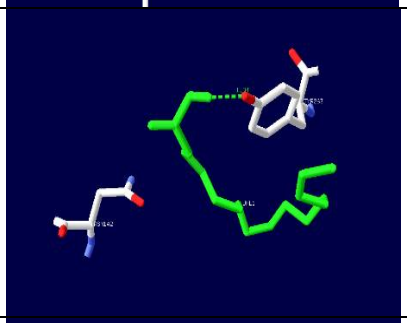
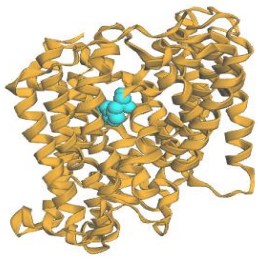
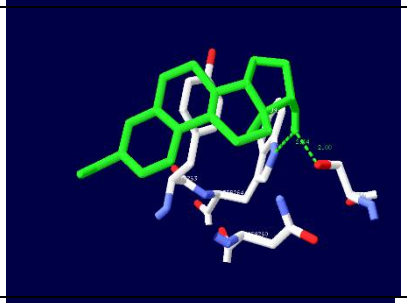
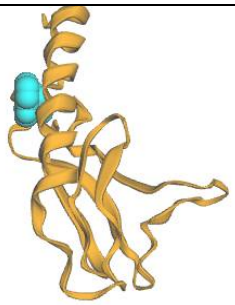
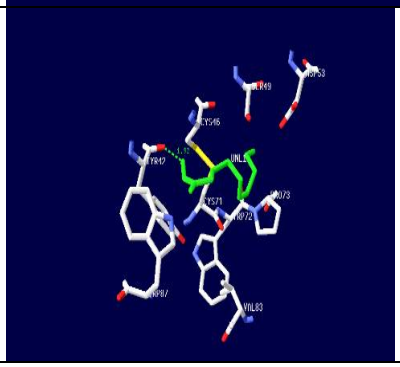
S. No	Compound Name	Hydrogen Bond Distance	Ligand		
			2ZOX	3DH4	3IOL
1	Stigmasterol	2.01	-	-11.2	2.01
2	Sitosterol	2.09	-	-11.6	-
3	vaccenic acid	2.90	-2.90	-	-
4	n- hexa decanoic acid	1.84	-	-4.82	-
5	decanoic acid	1.92	-	-	-2.87
6	Estradiol	2.00, 2.14	-	-9.87	-

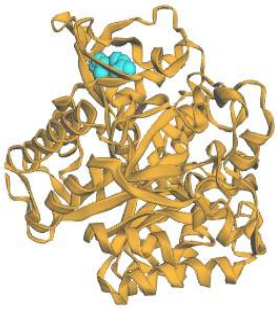
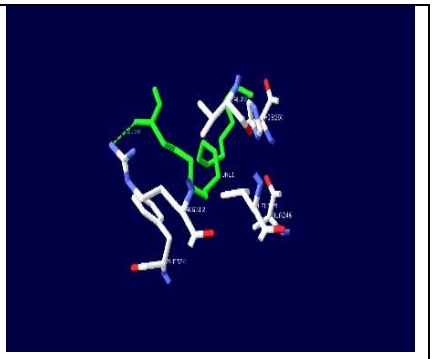
The more negative docking scores is the more favourable binding affinity. The strongest binding affinity is indicated by the largest value in molecular docking investigations. The effectiveness of a protein-ligand interaction depends on the ligand's proactive fit into the receptor pockets [21]. With sitosterol and stigmasterol, sodium glucose co transporter- 1 exhibited the best docking interaction (-11.6 kcal/mol; 1 H-bond and -11.2 kcal/mol; 2 H-bond, respectively). Similarly, decanoic acid, N- decanoic acid, vaccenic acid and esterdiol were found to dock well with galactose when compared to other substances. There are two H-bond contacts with sitosterol and three H-bond interactions with esterdiol in the galactose complex docking profile. The maximum binding energies were -11.6 kcal/mol and -11.2 kcal/mol, respectively. Glucagon like peptide 1 protein docking profile revealed that sitosterol and stigmasterol had superior binding affinities (-11.6 kcal/mol; 3 H-bond and -11.2 kcal/mol; 2 H-bond, respectively).

Additionally, the protein Sodium glucose co transporter-1 had the best docking contacts with esterdiol and N-hexa decanoic acid, which were followed by this. The binding energies of the esterdiol and N- hexa decanoic acid complexes were -9.87 and -4.82 kcal/mol, respectively, indicating that the esterdiol complex interacted with two H-bonds, while the N- hexa decanoic acid complex interacted with alkyl and nonbonded interactions. Discovery Studio was used to illustrate the 2D interactions, which are shown in

Figure 2. Thus, In the current docking experiment, the bioactive components of *H. auriculata* seed extract interact with GLP-I, which breakdown of sugar molecule process and possess anti diabetic activity. The ligands proteins stigmasterol, sitosterol, esterdiol, N- hexa decanoic acid, decanoic acid and vaccenic acid demonstrated greater than -11 to -4 kcal/mol binding energies with Galactose, cytosolic beta-glucosidase, Glucagon like peptide-1 in the current investigation.

Fig 2: Docking compound with their acting site and binding affinity with proteins

S.no	Compound name	Active site	Binding affinity
1	Stigmasterol		
2	Sitosterol		
3	n- hexa decanoic acid		
4	Estradiol		
5	Decanoic acid		

6	Vaccenic acid		

For anti-diabetic activity, galactose, cytosolic beta-glucosidase, glucagon like peptide-1 enzyme inhibition is therefore essential. The molecular docking further shows that the ligands stigmasterol and sitosterol exhibited a higher binding affinity galactose. Because these ligands inhibit the galactose and glucagon like peptide 1 protein, they are likely to trigger the beta-cells-dependent insulin pathway, which in turn promotes anti diabetic activity [22]. Among other chosen chemicals, the vaccenic acid, decanoic acid and esterdiol had a high affinity with all of the target proteins, which may support anti diabetic activity. Thus, based on the current computational analysis, it can be concluded that stigmasterol and sitosterol strong metabolic activity of insulin warrant more in vitro and in vivo studies to examine its anti -diabetic properties [23].The results demonstrated a legitimate in silico study that evaluated phytochemicals from *H auriculata* seed as viable therapeutic candidates for anti-diabetic and open the door for future medication development. Additional in vivo studies are needed to reveal the mode of action for possible usage as anti-diabetic medications [24, 25].

CONCLUSION

In this investigation, the bioactive components from *H. auriculata* were used to analyse the anti- diabetic medication development process. The compound stigmasterol, esterdiol and sitosterol exhibits a high binding affinity towards Galactose, cytosolic beta-glucosidase, Glucagon like peptide-1. Therefore, the current study shows that *H. auriculata* seed has an important anti-diabetic quality against a variety of proteins that contribute to beta cell mediated insulin aetiology.

Declaration of interest

The authors have no competing interests to declare relevant to this article's content.

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Author's contributions

M. Gokilavani - conceptualization, methodology, analysis and writing the original draft preparation.
Dr. Arunkumar - Providing research guidance, constructing the study, revising the manuscript.

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