RESEARCH PAPER

Glucose Utilization Capacity of Ethanolic Flower Extract of *Clerodendrum paniculatum* Linn. in Isolated Rat Hemidiaphragm and Molecular Docking Analysis against Therapeutic Targets of Diabetes

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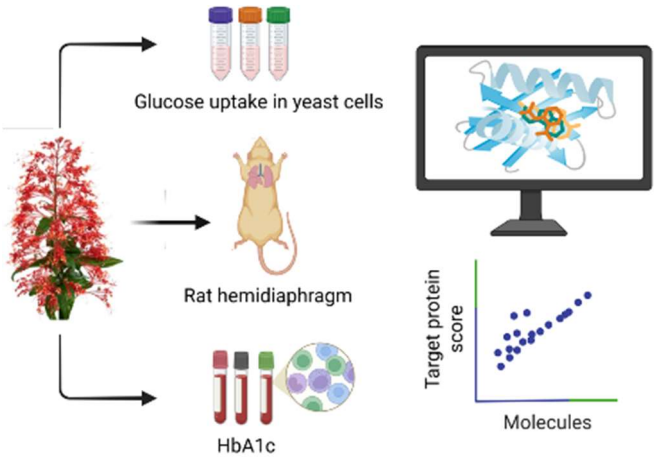
ABSTRACT



The present study focused on glucose utilization capacity of ethanolic flower extract of *C. paniculatum* in isolated rat hemidiaphragm and molecular docking analysis of phytochemicals against four therapeutic targets of DM type-2. The insulin-treated group was slightly higher than that in the flower-extract-treated group. The action of the flower extract was somewhat comparable to that of insulin and the combination of the extract and insulin resulted in an increased glucose uptake. Moreover, the extract demonstrated a concentration-dependent effect on glucose uptake in yeast cells and established a remarkable ability to inhibit glycosylated hemoglobin, with the percentage of glycosylation inhibition being dose-dependent. The physicochemical properties of the Phyto-compounds were investigated based on the Lipinski's rule of five, and all the compounds were meet with the criteria. The compound stigmasta-4,25-dien-3-one (**L3**) showed a good docking score of -11.0 kcal/mol against 1β-HSD1. Similarly, **L3** showed a significant binding energy of -9.2 kcal/mol on PTP1B. This suggests that the **L3** from *C. paniculatum* is a potent candidate for further research and development.

Keywords: *C. paniculatum,* Diabetes mellitus, *In vitro* Studies, Therapeutic Targets, Molecular Docking

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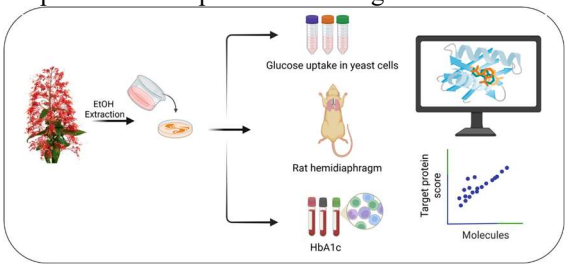
1. Introduction

Medicinal plants are rich source of secondary metabolites, which have unique and complex skeletons that improve human health [1]. The WHO estimated that 80% of people in developed countries still use folk or traditional remedies, mainly derived from plants [2]. However, the potential of higher plants as sources for new phytopharmaceutical drugs is still largely unexplored. Over 7500 plant species have been reported to be used in Indian traditional systems [3]. The concept of one drug–one target–one disease is becoming less popular, and the use of plant active extracts, fractions, and molecules is the new trends in drug discovery [4], which includes bioassay guided isolation of active principles [5]. Numerous plant extracts and fractions have been found to exhibit a wide range of beneficial biological activities, including antioxidant [6], antiviral [7], anticancer [8], and antidiabetic [9] properties. Among these, diabetes mellitus is a serious multifactorial illness that has a significant negative impact on a society's health and finances [10]. The recent estimate from the IDF, over 537 million persons worldwide have diabetes, which results in over 6.7 million fatalities annually [11]. Due to the fact that the medications used to treat diabetes today are synthetic and semi-synthetic and have several side effects [12]. Plants have identified many secondary metabolites with diverse structures and antidiabetic properties to date [13]. Examples of these secondary metabolites include aegeline [14], berberin [15], demethoxycurcumin [16], quercetin [17], gymnemagenin [18], and mangiferin [19]. The scientific community is searching for innovative herbal bio-actives to substitute effective illness management [20].

Clerodendrum paniculatum, a bushy herb also called orange tower flower or pagoda flower, is a member of the Verbenaceae family of plants It originated in Papua New Guinea and tropical Asia. Ethno-botanically, people use different parts of this plant to treat various illnesses and ailments [21]. The plant is one the most spectacular *Clerodendrum* species and have a variety of pharmacological properties, including antibacterial, antioxidant, insecticidal, hypolipidemic, anti-inflammatory, anthelmintic, antimutagenic, cytotoxic, and anti-aging properties [21,22]. Research on *C. paniculatum's* antidiabetic potential is rarely explored. Significantly, we investigated the in vitro inhibition of alpha amylase and alpha glucosidase, as well as streptozotocin-induced in vivo demonstrated the anti-diabetic potential of *C. paniculatum* [23]. A similar study was reported by Venkatesh and colleagues used chloroform leaf extract [24]. From a phytochemical perspective, this plant contains a number of important chemical components that have been identified and reported. These includes various class such as tannins, phenols, phytosterols, flavonoids, and saponins [25–28] etc.

Phytosterols and their derivatives are biologically active and have several medicinal properties. [29] They are known for their ability to lower blood cholesterol levels, which may help prevent obesity and cardiovascular disease.[29] And also have antioxidant properties that can protect against oxidative stress and reduce the risk of cancer. [30] In addition, it has been shown to have anti-inflammatory,[31] anti-cancer, [32] anti-diabetic [33] and immunomodulatory effects [34]. These properties make phytosterols a valuable scaffold for drug research and development. To our knowledge, no research has been conducted on the anti-diabetic properties of the active ingredients found in this plant. A wide variety of phytochemicals, including over 280 different compounds from various Clerodendrum species, have been reported. Recently, Balamurali and colleagues reported that the ability of alcoholic components extracted from *C. paniculatum* to inhibit SARS-CoV-2 was tested using various computational methods. [35] From this perspective, the present study focused on glucose utilization capacity of ethanolic flower extract of *C. paniculatum* in isolated rat hemidiaphragm and molecular docking analysis against therapeutic targets of diabetes (Scheme 1). We hope that this study will help researchers conduct further work on this plant to develop anti-diabetic agents.



Scheme 1. Schematic representation of present study- Glucose utilization capacity of ethanolic flower extract of *C. paniculatum* in isolated rat hemidiaphragm and molecular docking analysis against therapeutic targets of diabetes

2. Materials and methods

2.1. Plant collection and processing

Fresh flower of *C. paniculatum* were collected from the Western Ghats of the northeast region of Kerala (spanning 2,132 sq. km at altitudes between 700 and

2,100 meters). The herbarium was prepared for authentication and identification. The botanical expert in the Department of Botany, University of Calicut, identified the plant and issued the accession number 148233. The flower was shade-dried and powdered. Under controlled temperature the ethanol was used to extract the phytochemical from the flower. The ethanolic crude extract was obtained after filtration and concentration by a rotary evaporator. The extract was packed in an airtight container, and it was stored at 4 °C for further analysis.

2.2. Animals

Adult albino Wistar rats (160–180 g) were selected for this study. Rats were procured from animal house, Karpagam Academy of Higher education, Coimbatore. The present study was approved by the Institutional Animal Ethics Committee (IAEC) constituted for CPCSEA, Government of India (KAHE/IAEC/2019/15-06/002). All rats were housed in polycarbonate cage at 22 °C temperature and 45–65% humidity with free access to water and pellet feed.

2.3. In vitro glucose uptake by isolated rat hemi-diaphragm

The glucose uptake by rat hemidiaphragm was estimated by the modified method described by Walaas and Walaas [36] and Chattopadhyay et. al.,[37] Albino rats of the female sex weighing between 160 and 180 g were selected and maintained as described above. The animals were sacrificed by decapitation, and the diaphragm was dissected out quickly with minimal trauma and divided into two halves. The hemi-diaphragm was then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and was placed in small culture tubes containing 2 ml of Tyrode solution with 2% glucose and was incubated for 30 minutes at 37°C in an atmosphere of 100% O_2 with shaking. Four sets containing five numbers of graduated test tubes were treated as Group (I): served as a control which contained 2 ml of Tyrode solution with 2% glucose and 2.0 ml of distilled water. Group (II): Contains 2 ml of Tyrode solution with 2% glucose, regular insulin (Biocon) 0.62 ml of 0.4 units per ml solution and 1.38 ml of distilled water. Group (III): Contains 2 ml of Tyrode solution with 2% glucose, 0.6 ml of ethanolic extract of *C. paniculatum* (200 µg/ml) and 1.4 ml of distilled water. Group (IV): Contains 2 ml of Tyrode solution with 2% glucose, 0.6 ml of ethanolic extract of *C. paniculatum* (200 µg/ml), 0.62 ml of 0.4 units per ml solution of insulin and ml of distilled water. After incubation, the hemidiaphragms were taken out and weighed. The glucose content of the incubated medium was measured by the GOD-POD method. Glucose uptake per gram of tissue was the difference between the initial and final glucose content in the incubated medium. The uptake of glucose was expressed as mg/g of moist tissue/30 min.

2.4. Glucose uptake in Yeast cells

Yeast cells were prepared following the method outlined by Dinesh et al. (2009).[38] In brief, commercial baker's yeast was subjected to repeated centrifugation (3,000×g for 5 minutes) in distilled water until the supernatant was clear. A 10% (v/v) suspension was then prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1 mL of glucose solution (5, 10, and 25 mM) and incubated together for 10 minutes at 37°C. The reaction was initiated by adding 100 µl of the yeast suspension, which was then vortexed and incubated further at 37°C for 60 minutes. Following incubation, the tubes were centrifuged (2,500 × g for 5 minutes), and glucose concentrations were measured in the supernatant. Metformin was used as the standard drug for comparison. The percentage increase in glucose uptake by the yeast cells was subsequently calculated. All tests were conducted in triplicate.

2.5. Non-enzymatic glycosylation of haemoglobin assay

The antidiabetic activity of the ethanolic leaf extract of *C. paniculatum* was investigated by estimating the degree of non-enzymatic glycosylation of haemoglobin colorimetrically at 520 nm described by Megha et al., (2013) [39]. Solutions of glucose (2%), haemoglobin (0.06%), and sodium azide (0.02%) were prepared in phosphate buffer (0.01 M, pH 7.4). One millilitre of each of these solutions was mixed, and one millilitre of each concentration of the plant sample was added to the mixture. This mixture was then incubated in the dark at room temperature for 72 hours. The degree of glycosylation of haemoglobin was measured colorimetrically at 520 nm. Alpha-tocopherol (Trolax) was utilised as a standard drug, and the percentage inhibition was calculated. All tests were conducted in triplicate.

2.6. Preparation of ligands

The 3D structures of the phytoconstituents such as [**L1**] β-Sitosterol, [**L2**] Oleanolic aldehyde acetate, [**L3**] Stigmasta-4,25-dien-3-one and [**L4**] Lupeol from the plant *C. paniculatum* were sourced from PubChem (https://pubchem.ncbi.nlm). Selected

phytosterol structures were converted from "SDF" to "PDBQT" file format using Open Babel version 2.4.0 (openbabel/2.4.0/). Energy minimisation of the ligands was conducted with Avogadro, providing the necessary inputs for AutoDock Vina to carry out a docking simulation.

2.7. Selection and preparation of receptor

Literature-based background: The proteins selected as binding targets include [**P1**] 11β-HSD1, [**P2**] PTP1B, [**P3**] GFAT, and [**P4**] SIRT6. AutoDock Vina facilitated the docking of crystal structural proteins and selected compounds into the active sites of these target proteins. The Protein Data Bank (PDB) (https://www.rcsb) was utilised to obtain the three-dimensional (3D) crystal structures of the proteins. Molegro software was employed to refine the crystal structures by removing associated water chains and heteroatoms from the downloaded structures, as well as adding polar hydrogen atoms.

2.8. Molecular docking

The algorithm integrated into AutoDock Vina was employed to determine the optimal docked conformation between proteins and ligands. The conformations that displayed the lowest free binding energy (indicating the highest-scoring complex) were selected for analysis of ligand-receptor interactions using PyMOL and the protein-ligand interaction profiler (PLIP).

2.9. In silico ADME properties

The physicochemical, pharmacological, toxicological, and bioactivity properties of the selected phytosterol were analysed using the pkCSM [40] and SwissTargetPrediction[41] web tools. These services were chosen for their capability to provide pertinent pharmacokinetic data for small molecule drug candidates, including aspects such as absorption, distribution, metabolism, excretion, and toxicity. The structures of the phytosterols were converted into simplified molecular-input line entry specification (SMILES) format before being uploaded to the selected web services.

2.10. Statistical analysis

All the experimental results were entered using three parallel measurements of the Mean ± Standard deviation (n=3).

3. Results

Herein, we examined the anti-diabetic properties of the ethanolic flower extract of *C. paniculatum* through in vitro assessments, including glucose uptake by isolated rat hemidiaphragm, glucose uptake in yeast cells, and assays for non-enzymatic glycosylation of haemoglobin. The effect of the ethanolic extract on glucose uptake by the isolated rat hemidiaphragm is tested at 200 µg/ml and depicted in Fig. 1. The flower extract showed 28.72 % of glucose uptake. The results revealed that the glucose uptake in the insulin-treated group was slightly higher than that in the ethanolic-extract-treated group. In other words, the action of the ethanolic flower extract was somewhat comparable to that of insulin. Interestingly, the combination of the ethanolic extract and insulin resulted in an increased glucose uptake. This indicates that insulin may exhibit a synergistic effect with the extract in promoting glucose uptake. In another assay, the ethanolic extract demonstrated a concentration-dependent effect on glucose uptake in yeast cells. The results are comparable to the standard, as shown in Fig 1B. At the highest concentration of 500 µg/mL, the percentage of glucose uptake was measured at 60% for standard metformin (MET) and 59% for the ethanolic flower extract (EFE). The flower extract demonstrated a remarkable ability to inhibit glycosylated haemoglobin, with the percentage of glycosylation inhibition being dose-dependent. At a concentration of 500 µg/mL, a 60% inhibition was observed. Additionally, the standard compound tocopherol (TCP) exhibited a comparable inhibition rate of 61% at the same concentration.

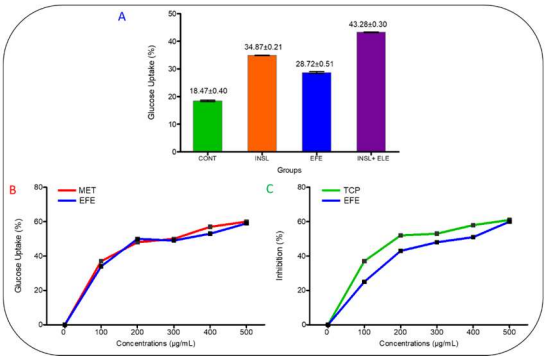


Fig. 1 In vitro anti-diabetic activity of ethanolic flower extracts of *C. paniculatum*. [A]- Glucose uptake by isolated rat hemi-diaphragm; [B]- Glucose uptake in yeast cells; and [C] 4. Non-enzymatic glycosylation of haemoglobin assay. Abbreviations: **CONT**- Control, **INSL**- Insulin, **EFE**- Ethanolic flower extract, **MET**-Metformin, and **TCP**-Tocopherol. All the experimental results were entered using three parallel measurements of the Mean ± Standard deviation (n=3).

The identification of therapeutic target classes is important in drug discovery programme.

We selected the therapeutic targets are related to metabolic diseases, such as type 2 diabetes, obesity, and metabolic syndrome, due to their roles in regulating glucose and lipid metabolism. β-Sitosterol (**L1**) showed 40.0% of the target on the nuclear receptor, followed by 26.7% on the cytochrome P450. In the case of oleanolic aldehyde acetate (**L2**), there was only 44.0% of the target on the enzyme and 16.0% on the nuclear receptor. Stigmasta-4,25-dien-3-one (**L3**) and Lupeol (**L4**) targeted 40.0% and 20.0% of the nuclear receptors, respectively. Moreover, **L4** targeted 33.3% on the enzyme and 20% on the phosphatase. This provided additional information against the various targets, the details of which are illustrated in Figure 2.

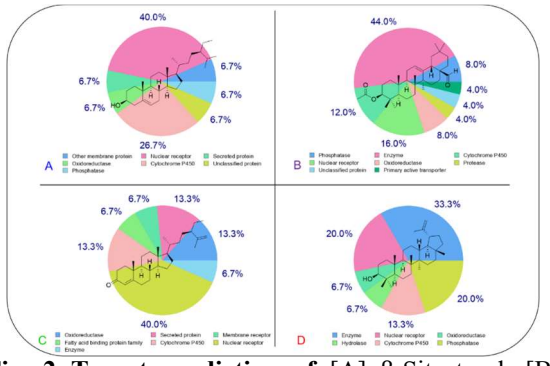


Fig. 2 Target prediction of [A] β-Sitosterol, [B] Oleanolic aldehyde acetate, [C] Stigmasta-4,25-dien-3-one and [D] Lupeol from Swiss-Target-Prediction, an online web tool by using SMILE value. The figure showing most 15 target classes.

The exploration of plant-derived bioactive substances as antidiabetic agents has led to further studies concentrating on pharmaco-informatic analyses of selected phytochemicals from *C. paniculatum*. These analyses target inhibitors such as 11β-HSD1, PTP1B, GFAT, and SIRT6 (Fig. 3). We began our molecular modelling studies by establishing the physicochemical and pharmacokinetic profiles of β-sitosterol, oleanolic aldehyde acetate, stigmasta-4,25-dien-3-one, and lupeol. The physicochemical properties and details are provided in Table 1. According to the Lipinski rule of five, all the compounds satisfy the criteria for solubility, membrane permeability, and efficacy in drug development. The molecular masses of all the compounds are below 500 Da. The lipophilicity (Log P) of β-sitosterol was determined to be 5.05, while the other compounds have a Log P of less than 5, suggesting that these compounds possess the ability to dissolve in fats, oils, and lipids, with a reasonable balance between lipophilicity and water solubility. The compound β-sitosterol found to be highest number of rotatable bonds, and lupeol has only one rotatable bond. Moreover, compounds such as oleanolic aldehyde acetate and stigmasta-4,25-dien-3-one did not find any hydrogen bond donors in the skeleton. The topological polar surface area (TPSA) represents the total surface area of all polar atoms within a compound. The calculated TPSA for the compounds examined was found to be less than 60, suggesting that these compounds exhibit excellent oral bioavailability and membrane permeability. In the pharmacokinetics study, all compounds exhibited low gastrointestinal (GI) absorption and did not penetrate the blood-brain barrier (BBB). Stigmasta-4,25-dien-3-one demonstrated cytochrome inhibition against CYP2C9 enzymes. The other compounds did not show any interaction or inhibition with CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4. Furthermore, the compounds displayed low skin permeability (log □□ > −2.5), details are presented in Table 2.

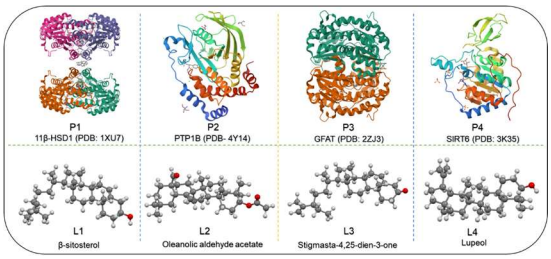


Fig. 3. The 3D structure of proteins and ligand. [**P1**]-11β-HSD1 (PDB: 1XU7): Protein represents the human 11β-hydroxysteroid dehydrogenase type 1 enzyme, [**P2**]-PTP1B (PDB- 4Y14): Protein Tyrosine Phosphatase 1B, [**P3**]- GFAT (PDB: 2ZJ3): Glutamine-fructose-6-phosphate aminotransferase, [**P4**]- SIRT6 (PDB: 3K35): NAD+-dependent deacetylase/ADP-ribosyltransferase, [**L1**]-β-Sitosterol, [**L2**]-Oleanolic aldehyde acetate, [**L3**]-Stigmasta-4,25-dien-3-one and [**L4**]-Lupeol.

The binding capacity and amino acid interactions of phytoconstituents **L1-L4** against the proteins 1β-HSD1 (**P1**), PTP1B (**P2**), GFAT (**P3**), and SIRT6 (**P4**) are illustrated in Figs. 3-6. The compound stigmasta-4,25-dien-3-one exhibited the highest docking score with 1β-HSD (**P1**), with the binding energy calculated at 11.0 kcal/mol. The ligand **L3** is belongs to phyto-steroid class, features a carbonyl group (ketone) at the C3 position and a double bond at the C4 position, thereby forming an unsaturated ketone system within its structure. Ring-B of L3 interacted with three amino acid residues: LYS, VAL, and TYR, located at positions 199, 203, and 280 in chain A, respectively. In comparison, **L1** demonstrated a superior molecular docking score of -9.2 kcal/mol when contrasted with **L2** and **L4** (see Fig. 4). Notably, rings A and B of **L1** interacted with

ILE A:121, with bond lengths measured at 4.69 Å and 4.92 Å. Additionally, the C-24 methyl group was observed to interact with the TYR A:183 residue. The docking scores and corresponding amino acid residues for **L2** and **L4** are presented in Fig. 3. Next, molecular docking was conducted with Tyrosine Phosphatase 1B (PTP1B), a key negative regulator of insulin and leptin signalling. The compound **L4** exhibited the highest docking score of -7.2 kcal/mol, with two interactions, including LEU A: 272 and PRO A: 188. Moreover, the docking scores for compounds **L1**, **L2**, and **L3** were recorded as -5.2, -6.9, and -6.0 kcal/mol respectively (Fig. 5).

Table 1. Computational physico-chemical and molecular docking studies of phytocompounds. [a]

ID	Compounds	Mol. Formula	Mol. Weight (< 500 D)	Log P (< 5)	Rotatable Bonds	HBA (≤ 5)	HBD (≤ 10)	TPSA (< 140 Å²)	Docking Score (kcal/mol)			
									1xu7	4y14	2zj3	3k35
L1	β-Sitosterol	$C_{29}H_{50}O$	414.71	5.05	6	1	1	20.23	-9.2	-5.2	-7.7	-6.2
L2	Oleanolic aldehyde acetate	$C_{32}H_{50}O_3$	482.74	4.64	3	3	0	43.37	-11	-6.9	-7.3	-8.1
L3	Stigmasta-4,25-dien-3-one	$C_{29}H_{46}O$	410.68	4.92	6	1	0	17.07	-7.6	-6.0	-9.2	-6.6
L4	Lupeol	$C_{30}H_{50}O$	426.72	4.72	1	1	1	20.23	-6.73	-7.2	-7.2	-6.6

Table 2. Computational pharmacokinetic studies of phytocompounds. [a]

ID	Compounds	GI	BBB	P-gp	Log Kp	Cytochrome Inhibitors				
						CYP1A2	CYP2C19	CYP2C9	CYP2D6	CYP3A4
L1	β-Sitosterol	Low	No	No	-2.20 cm/s	No	No	No	No	No
L2	Oleanolic aldehyde acetate	Low	No	No	-3.56 cm/s	No	No	No	No	No
L3	Stigmasta-4,25-dien-3-one	Low	No	No	-2.25 cm/s	No	No	Yes	No	No
L4	Lupeol	Low	No	No	-1.90 cm/s	No	No	No	No	No

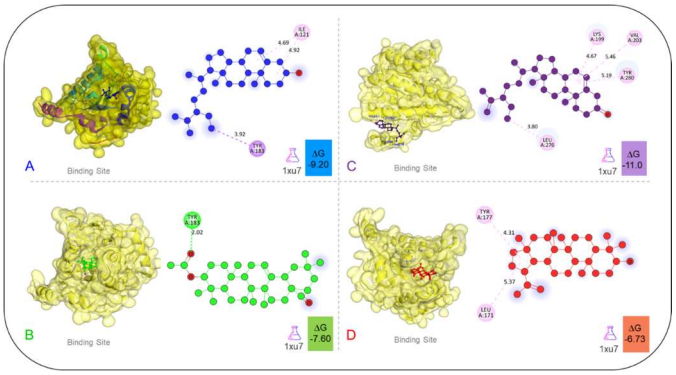


Fig. 4 The molecular docking study of [A] β-Sitosterol, [B] Oleanolic aldehyde acetate, [C] Stigmasta-4,25-dien-3-one and [D] Lupeol against Human 11β-hydroxysteroid dehydrogenase 1 in complex. 11β-HSD1 (PDB: 1XU7): Protein represents the human 11β-hydroxysteroid dehydrogenase type 1 enzyme in complex with an inhibitor, specifically (R)-3-phenyl-2-(pyridin-2-yl)thiazolidin-4-one [1, 2]. 11β-HSD1 is a microsomal enzyme that converts inactive cortisone into active cortisol, making it a target for treating type 2 diabetes and obesity. The figure showing the binding sites and amino acid interactions.

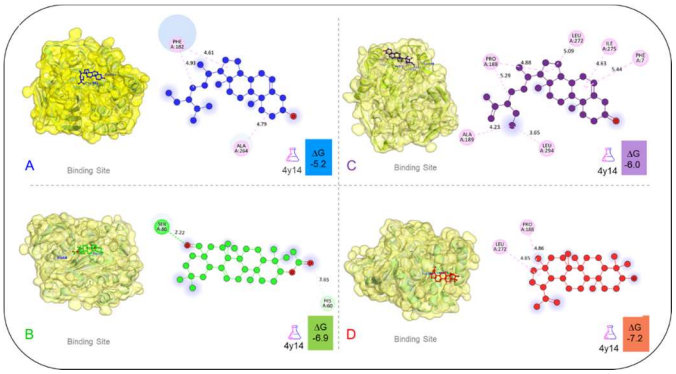


Fig. 5 The molecular docking study of [A] β-Sitosterol, [B] Oleanolic aldehyde acetate, [C] Stigmasta-4,25-dien-3-one and [D] Lupeol against Protein Tyrosine Phosphatase 1B. PTP1B (PDB- 4Y14): Protein Tyrosine Phosphatase 1B. PTP1B is a known negative regulator of insulin signaling, making it a key therapeutic target for type 2 diabetes and obesity The figure showing the binding sites and amino acid interactions.

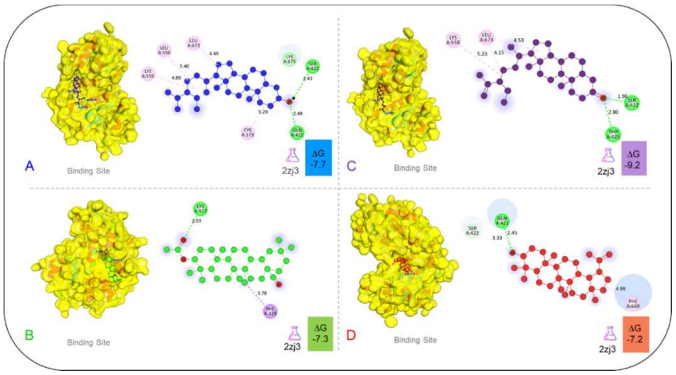


Fig. 6 The molecular docking study of [A] β-Sitosterol, [B] Oleanolic aldehyde acetate, [C] Stigmasta-4,25-dien-3-one and [D] Lupeol against Protein Tyrosine Phosphatase 1B. GFAT (PDB: 2ZJ3): Glutamine-fructose-6-phosphate aminotransferase, specifically the human GFAT1 isomer. This enzyme is the rate-limiting enzyme of the hexosamine biosynthetic pathway. The figure showing the binding sites and amino acid interactions.

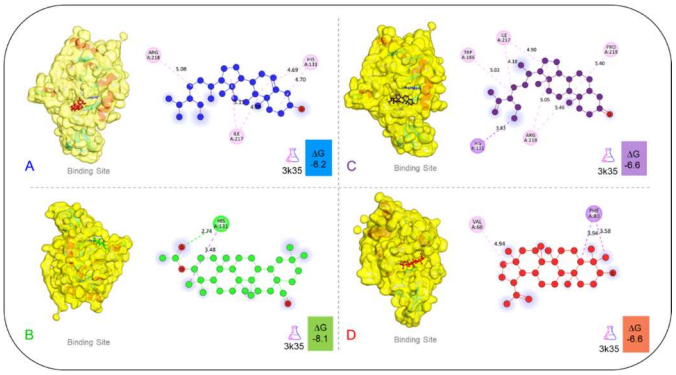


Fig. 6 The molecular docking study of [A] β-Sitosterol, [B] Oleanolic aldehyde acetate, [C] Stigmasta-4,25-dien-3-one and [D] Lupeol against Human Sirtuin 6. SIRT6 (PDB: 3K35): NAD+-dependent deacetylase/ADP-ribosyl transferase involved in regulating glucose homeostasis, inflammation, and DNA repair. The figure showing the binding sites and amino acid interactions.

The docking with protein tyrosine phosphatase 1B (PDB: 2ZJ3), the compound **L3** showed highest binding score of -9.2 kcal/mol within this series. The presence of pentacyclic Ring D, aliphatic chain and keto group at Ring A favors the interaction with various animo acid residues, including LYS A: 559, LEU A:673, SER A:422 and THR A: 425. The other compounds are displayed comparable docking scores; the details are represented in the Fig.6. Interestingly, the compound **L2** demonstrated a favourable binding energy of -8.1 kcal/mol with the SIRT6 protein. The keto (C=O) and C24 methyl groups of **L2** interacted with HIS A:131. Notably, **L3** exhibited the highest number of interactions with the amino acid residues of the SIRT6 protein, which include TRP A:186, ILE A:217, PRO A:219, HIS A:131, and ARG A:218. The docking score for **L3** was recorded at -6.6 kcal/mol, which is comparable to the docking value of **L4**. In this protein, compound **L1** showed the lowest docking score of -6.2 kcal/mol (Fig 7).

4. Discussion

The use of medicinal plants for various diseases, including the treatment of chronic metabolic disorders such as diabetes, has been increasing over the years [42]. There are many excellent drugs derived from plant-based small molecules [43]. Currently, there are several synthetic anti-diabetic drugs available in the market for the treatment of diabetes, which act through different mechanisms, including improving insulin sensitivity, supplementing insulin, increasing insulin secretion, and stimulating glucose uptake.[44] However, such drugs are incompatible with several side effects. Plants are a good source of diverse chemical components and which are relatively safe and much cheaper alternatives to synthetic drugs. Identification of active anti-diabetic agents from plants is a long process in the drug research and development program, which begins with the bioassay-guided fractionation of crude extracts. Several plants and their derived formulations or small molecules have shown promising activity against diabetics.[45] Interestingly, several reports have shown a relationship between antioxidant actives and type 2 DM. *C. paniculatum* is a significant medicinal plant species belonging to the Lamiaceae family and which is used to treat a wide range of illnesses and metabolic disorders [21]. Notably, the leaves and flowers exhibit significant antioxidant properties[46–48]. The primary investigation on the chemical profile revealed the presence of terpenes, flavonoids, tannins, alkaloids, phenolic acids, sterols, and glycosides [49–51]. We investigated the antidiabetic activity of ethanolic flower extract of *C. paniculatum* through in vitro assessments, including glucose uptake by isolated rat hemidiaphragm, glucose uptake in yeast cells, and assays for non-enzymatic glycosylation of haemoglobin. The result indicated that the ethanolic flower extract showed significant antidiabetic potential. The isolated rat hemidiaphragm, due to its high sensitivity to insulin compared to other tissues, serves as a reliable in vitro model for investigating peripheral glucose uptake.[52] The flower extract was tested at a concentration of 200 µg/ml. The difference between the initial and final glucose levels was calculated as the amount of glucose uptake per gram of tissue. Insulin served as a positive control and glucose uptake was found to be 34.87%. Glucose uptake from the extract was shown to be 28.72%. Interestingly, the flower extract showed glucose uptake to an extent equivalent to insulin and the study on combining ethanol extract and insulin increased glucose uptake. This suggests that the extract of the flower may act as a cofactor in promoting glucose uptake. Moreover,

the flower extract has demonstrated a concentration-dependent effect on glucose uptake in yeast cells. The effects are comparable to those of the antidiabetic drug metformin. In addition, the flower extract has demonstrated a remarkable ability to inhibit glycosylated hemoglobin, and the percentage of glycosylation inhibition is dose-dependent. There are several plant extracts were successfully evaluated the glucose uptake capacity through rat hemidiaphragm.[52–58] Notably, Begum et al. (2016) examined the glucose utilization capacity of *Barleria prionitis* and *Hyptis suaveolens*, and found that *H. suaveolens* produced a higher glucose utilization capacity than *B. prionitis*.[58] To our knowledge, there is no report on the glucose uptake capacity of *C. paniculatum* flower extract using isolated rat hemi-diaphrum. Notably, Venkatesh and colleagues reported the antidiabetic activity of chloroform leaf extract of *C. paniculatum* using isolated rat hemi-diaphrum and showed significant glucose uptake of the extract at a dose of 200 mg/kg.[59] In comparison to this study, the flower extract showed a strong glucose uptake capacity. This suggests that further studies are needed to identify the active components of the flower extract responsible for its antidiabetic potential. Chemically, there are several classes of phyto-chemicals were identified from the various parts of *C. paniculatum* including 3-epicaryoptin,[60] *β*-sitosterol, lupeol, oleanolic aldehyde acetate, stigmasta-4,25-dien-3-one, (3β)-stigmasta-4,22,25-trien-3-ol,[61] quercetin,[62] rutin,[63] (24s)ethylcholesta–5,22,25–triene–3β–ol, β-amyrin,[64] etc.

In the context of the COVID-19 pandemic, virtual screening has been frequently used for drug discovery and repositioning. It helps to further *in vitro* and *in vivo* experimental evaluation. Therefore, identifying drug candidates through molecular docking for the treatment and management of DM has many advantages such as reducing costs, increasing accuracy, and accelerating all discoveries. In this scenario, we aimed to evaluate molecular interactions existing between selected bioactive compounds in *C. paniculatum* and targeted proteins related to Type 2 DM. This process involved the molecular docking of 3D structures of those substances into 4 targeted proteins (**P1-P4**) namely 11-β hydroxysteroid dehydrogenase type 1, glutamine: fructose-6-phosphate amido transferase, protein-tyrosine phosphatase 1B and mono-ADP-ribosyl transferase sirtuin-6. In the next step, the two-dimensional chemical structures of selected compounds such as β-sitosterol, oleanolic aldehyde acetate, stigmasta-4,25-dien-3-one, and lupeol (**L1-L4**) with canonical smiles were used to interpret the molecular structure and physicochemical properties based on Lipinski's rule (Pfizer) of five, molecular weight (< 500 D), lipophilicity (log P < 5), number of H-bond acceptors (≤ HBA), number of H-bond donors (≤ HBD), and TPSA (< 140 Å²), respectively. All **L1-L4** compounds met the criteria and suggested that the compounds were suitable for good oral bioavailability. However, Lipinski specifically states that the rule of 5 only applies to compounds that are not substrates for active transporters.[65] Lipinski strictly considers all nitrogen and oxygens as H-bond acceptors, and all nitrogen and oxygen with at least one hydrogen as H-bond donors. Compounds **L1-L4** had no nitrogen atom in the skeleton, and the oxygen atom was attached to the C-3 position in the form of hydroxy (-OH), ester (-COOR), and keto (>C=O) groups, primarily these groups facilitate ligand binding through polar interactions, especially hydrogen bonding and dipole-dipole interactions.

From the literature, molecular docking studies on 1β-HSD1, PTP1B, GFAT, and SIRT6 have been conducted on several phytochemicals. Notably, Narayanaswamy and colleagues reported molecular docking analysis of selected phyto-constituents from *Urtica dioica* on 1β-HSD.[66] Out of a total of 24 molecules examined, the highest molecular docking scores were found for stigmastane-3, 6-diol (-9.5 kcal/mol) and ethyl iso-allocolate (-9.3 kcal/mol). In our study, the compound stigmasta-4,25-dien-3-one (**L3**) exhibited a good docking score of -11.0 kcal/mol. However, β-sitosterol showed a similar docking score of -9.2 kcal/mol. In another study, among 20 bioactive compounds from *Euphorbia thymifolia*,[67] taraxerol showed a remarkable docking score of -12.1 kcal/mol, and three other molecules such as β-amyrin, 24-methylene cycloartenol, and stigmasterol showed binding energies of -11.6, -11.1, and -11.0 kcal/mol. Similarly, compound **L3** showed a significant binding energy of -9.2 kcal/mol compared to PTP1B. This indicates that the selected compound **L3** from *C. paniculatum* is a potent drug for further research and development. Moreover, in our investigation the compounds **L4**, **L3**, and **L2** showed significant molecular docking score on PTP1B, on GFAT and on SIRT6. Computational target perdition is widely used in drug discovery to understand molecular mechanisms, rationalize side effects, and identify off-targets.[68] In this case, we predicted the molecular targets of each compound. β-Sitosterol (**L1**) demonstrated a 40.0% affinity for the nuclear receptor, with a subsequent 26.7% targeting cytochrome P450. In contrast, oleanolic aldehyde acetate (**L2**) exhibited only a 44.0% affinity for the enzyme and 16.0% for the nuclear receptor.

Stigmasta-4,25-dien-3-one (**L3**) and lupeol (**L4**) showed affinities of 40.0% and 20.0% for the nuclear receptors, respectively. Furthermore, **L4** targeted the enzyme with a 33.3% affinity and 20% for the phosphatase. Similarly, several phytoconstituents have been successfully screened for molecular targets[69], which has helped in identifying potent small molecules from plants. This study will help in identifying antidiabetic agents from *C. paniculatum.*

5. Conclusion

C. paniculatum L. is an important medicinal plant species belonging to the Lamiaceae family. Modern pharmacological studies have demonstrated multiple therapeutic properties including antioxidant, hepatoprotective, anti-inflammatory, antibacterial, anti-cancer and anti-diabetic activities. In this study, we mainly focused on the glucose utilization capacity of ethanolic flower extract of *C. paniculatum* in isolated rat hemidiaphragm and molecular docking analysis of selected phytochemicals against therapeutic targets of diabetes. The flower extract was tested at a concentration of 200 µg/ml. The difference between the initial and final glucose levels was calculated as the amount of glucose uptake per gram of tissue. Insulin served as a positive control and glucose uptake was found to be 34.87%. The glucose uptake from the extract was shown to be 28.72%. Interestingly, the flower extract showed glucose uptake to be equivalent to insulin, and a study on combining ethanol extract with insulin increased glucose uptake. This suggests that the flower extract may act as a cofactor in promoting glucose uptake. In addition, the flower extract has demonstrated a concentration-dependent effect on glucose uptake in yeast cells. The results are comparable to those of the antidiabetic drug metformin. In addition, the flower extract has demonstrated a remarkable ability to inhibit glycosylated hemoglobin, and the percentage of glycosylation inhibition is dose-dependent. Molecular target prediction of selected phytochemicals from *C. paniculatum* revealed that β-sitosterol (**L1**) exhibited 40.0% affinity for the nuclear receptor, followed by 26.7% affinity for cytochrome P450. In contrast, oleanolic aldehyde acetate (**L2**) exhibited only 44.0% affinity for the enzyme and 16.0% affinity for the nuclear receptor. Stigmasta-4,25-dien-3-one (**L3**) and lupeol (**L4**) showed 40.0% and 20.0% affinities for the nuclear receptors, respectively. Moreover, all compounds met the criteria of Lipinski's rule, which included five. Furthermore, molecular docking of selected phytochemicals was targeted to 4 proteins (P1-P4) associated with DM type 2. The compound stigmasta-4,25-dien-3-one (**L3**) showed a good docking score of -11.0 kcal/mol compared to 1β-HSD1. Similarly, compound **L3** showed a significant binding energy of -9.2 kcal/mol on PTP1B. This suggests that the selected compound **L3** from *C. paniculatum* is a potent drug for further research and development.

Abbreviations

CONT- Control, **INSL**- Insulin, **EFE**- Ethanolic flower extract, **MET**-Metformin, and **TCP**-Tocopherol. **L1**-β-Sitosterol, **L2**-Oleanolic aldehyde acetate, **L3**-Stigmasta-4,25-dien-3-one, **L4**-Lupeol, **HBA**- hydrogen bond acceptors, **HBD**- hydrogen bond donors, **P1**-11β-HSD1 Protein represents the human 11β-hydroxysteroid dehydrogenase type 1 enzyme, **P2**-PTP1B Protein Tyrosine Phosphatase 1B, **P3**- GFAT Glutamine-fructose-6-phosphate aminotransferase, **P4**- SIRT6 NAD+-dependent deacetylase/ADP-ribosyltransferase,

Ethics approval.

The present study was approved by the Institutional Animal Ethics Committee (IAEC) constituted for CPCSEA, Government of India (KAHE/IAEC/2019/15-06/002).

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Competing interests.

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Availability of data and materials.

The authors declared that data is available within the manuscripts

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