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Withania somnifera modulates glucose metabolism by inhibiting SGLT2, α- glucosidase and α-amylase: An *in silico* and *in vitro* study

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Diabetes is a growing metabolic disease worldwide, and effective approaches to treatment are needed. The study sought to comprehend the mechanistic role of Ashwagandha (*Withania somnifera L.*) root extract in regulating hyperglycaemia. *Withania somnifera* (*W. somnifera*) is known for its medicinal properties. *In vitro* and *in silico* approaches were employed to explore the antidiabetic properties of *W. somnifera* metabolites identified by GC-MS analysis.This work investigated the antidiabetic activities of *W. somnifera* metabolites using GC-MS analysis *in vitro* and *In silico*. We performed a qualitative phytochemical screening as well as a free radical scavenging studies using the $DPPH^+$ and $ABTS^+$ assays. Additionally, α-amylase and α-glucosidase inhibition experiments were performed. Molecular docking studies were done using compounds found from *W. somnifera* root extract against target proteins sodium-glucose transport protein 2 (SGLT2), α-glucosidase, and α-amylase. A qualitative examination revealed that *W. somnifera* contained phenols, steroids, and terpenoids. The $DPPH^+$ and $ABTS^+$ free radical scavenging experiments demonstrated high antioxidant activity, with IC_{50} values of 37.8 and 34.4 µg/mL, respectively. *W. Somnifera* inhibited α-amylase and α-glucosidase enzymes at doses of 3.25–50 µg/mL. Docking experiments demonstrated high binding affinities of -9.6 to -3.5 kcal/mol, -11.8 to -5.5 kcal/mol, and -9.3 to -4.5 kcal/mol, respectively. In conclusion, our studies revealed that phytochemicals present in *W. somnifera* inhibited SGLT-2, a glucose transporter, as well as α-amylase and α-glucosidase enzymes. Furthermore, our docking analysis supported *in vitro* findings. These data demonstrate that *W. somnifera* can regulate blood sugar levels and can be used as an alternative traditional medicine for diabetes therapy.

Keywords: Diabetes mellitus, GC-MS analysis, Molecular docking, *Withania somnifera*, α- glucosidase, α-amylase

Diabetes mellitus is a long- term serious chronic condition and a heterogeneous metabolite disorder, where there is a rise in blood glucose level and body can’t produce adequate amount of insulin hormone. Insulin is an essential hormone produced by the pancreas, regulated glucose homeostasis in body. Lack of insulin hormone leads to increased level of blood sugar level inside the body lead to hyperglycaemia, which is the indication of diabetes[1]. Global modernisation, urbanisation and unhealthy lifestyles, lack of exercise and stress has led to increase in diabetes worldwide and is a serious concern for human life expectancy[2]. The current 10th edition of IDF Diabetes confirms that it is the fastest growing health emergencies of 21st century. 537 million people globally are estimated to have diabetes in 2021; by 2030, that figure is expected to rise to 643 million, and by 2045, it is predicted to reach 783 million[3]. If an insulin deficit patient is not diagnosed for a long time and not treated it may lead to life threating conditions and multiple organ failures, dysfunction and damage. The complication may extend up to nerve damage cardiovascular diseases, kidney damage (nephropathy), lower-limb amputation, and eye disease affecting the retina and resulting in visual loss and even blindness[4].

Classification of diabetes is very important aspect, that proper identification and treatment strategies could be adopted according to the types of diabetes. The American Diabetic Association (ADA) proposed and classified diabetes in Type 1, Type 2, gestational diabetes mellitus and other types[5] Type 1 diabetes mellitus also termed as to be Immune mediated diabetes or autoimmune type 1 diabetes is caused due to autoimmune cellular destruction of β-cells of the pancreas and β-cells destruction occurs as

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inflammatory response of T-cells[6]. The symptoms that come with type 1 diabetes are sudden weight loss, extreme tiredness, slow healing of wounds, reoccurring of wounds as well as lack of vision. 90%-95% of patients suffering from diabetes belong to Type 2 diabetes category[7].

About 60% of the world's population uses traditional medicines made from medicinal plants. Although there are various approaches to reduce diabetes and its secondary complications, herbal preparations are preferred because of their few side effects and low cost[8]. A compilation of medicinal plants that have been scientifically proven to have antidiabetic and additional health benefits, along with herbal medications utilized in diabetes treatment. *W. somnifera* (Ashwagandha) is highly esteemed in the Indian Ayurveda medical tradition for its role as a Rasayana, or tonic herb[9]. It is utilized for a range of diseases and particularly as a nerve tonic. Based on these observations, numerous scientific research was conducted, examining in detail its adaptogenic and anti-stress properties[10]. It is anti-inflammatory and anti-arthritic, and has been shown to be beneficial in clinical cases of rheumatism and osteoarthritis. Ashwagandha strongly increases insulin secretion and improves insulin sensitivity in muscle cells. Several studies have shown that giving ashwagandha root powder to diabetic patients can help control and reduce blood sugar levels[11]. Several studies have also shown that taking ashwagandha can be effective in improving and controlling fasting blood sugar levels in adults with stress-related health problems[12]. However, the molecular mechanism by which the phytocompounds present in Ashwagandha control blood sugar levels is not well understood. The present work primarily focused on ashwagandha phytochemical screening, antioxidant activity, and antidiabetic efficacy by analysing important targets using *in vitro* and molecular docking techniques.

Materials and Methods

Sample preparation

The roots of *W. Somnifera L.* were taken from the University of Agriculture Sciences, GKVK, Bangalore, Karnataka, India. A taxonomist from the University of Agricultural Sciences, GKVK, Bangalore, identified and certified the plant root. *W. somnifera* root extract was prepared using methanol and H_2O (1:1), 10 grams of *W. somnifera*root powder was stirred over night with methanol and water. To make sure an effective extraction of bioactive components, particles were separated from liquid extracts with muslin cloth. The filtered liquid extracts were further filtered with Whatman no.1 filter paper. The organic solvent was evaporated to dryness in a rota evaporator at 40°C under reduced pressure. The dried residues were used further analysis[13].

Phytochemical screening

Phytochemical analysis using qualitative analysis tests for flavonoids, steroids, glycosides, tannins, saponins, sugars, aminoacids, coumarins, terpenoids, phenols and quinines were performed as described previously[14, 15].

GC–MS analysis (Gas chromatography-mass spectrometry)

Thermo GC-Trace Ultra Version: 5.0, Thermo MS DSQ II was used to conduct GC-MS analysis on the entire extract. The equipment features a DB 35 - MS Capillary Standard non-polar column with dimensions of 30 mm × 0.25 mm ID × 0.25 µm film. Helium is employed as the carrier gas, with a modest flow rate of 1.0 ml/min. The injector was set to 250°C, and the oven temperature was configured as follows. 60°C for 15 min, then steadily increased to 280°C for 3 min. The components were identified using the Willey and NIST libraries, as well as a comparison of their retention indices. The constituents were identified by comparing them to those available in the computer library (NIST and Willey) attached to the GC-MS instrument, and the findings are summarized[16].

Free radical scavenging capacity studies

DPPH $^+$ radical scavenging activity

The hydrogen-donating capacity of the *W. somnifera* root extract was assessed with slight adjustments to the method. Ascorbic acid was thinned with methanol to create sample solutions containing 5, 10, 20, 50, and 100 µg of sample per milliliter of solution as a positive control. A clean tube was used to pipette one milliliter of 0.25% DPPH, followed by the addition of various concentrations of the sample solution.The mixture was left to incubate at 37°C for 30 min. The UV-Vis spectrophotometer was used to determine the absorption capacity of the reaction solution at 515 nm[17]. The experiment was done in triplicates. The following formula was used to compute the activity of scavenging free radicals:

$$\% \text{ Scavenging} = [(A_0 - A_1) / A_0] \times 100$$

Where A_0 is the absorbance of the control and A_1 is the absorbance of extract/standard taken as Ascorbic acid.

$ABTS^+$ *radical scavenging activity*

The ABTS assay depends on the absorption of light by $ABTS^+$ radicals, and it was carried out using well-established methods described in earlier writings[18]. An antioxidant that is able to provide a hydrogen atom will inactivate a stable free radical, causing a shift in absorption that can be tracked using spectroscopy. The relatively stable $ABTS^+$ radical has a green colour and can be quantified using spectrophotometry at 734 nm. Following the combination of an equal quantity of ABTS with APS and allowing the mixture to rest in darkness at room temperature for 14-16 hours, $ABTS^+$ radical cations were generated. Subsequently, the solution was diluted further with methanol (in a 1:60 ratio) until an absorbance of 1.00 at 734 nm was attained using a spectrophotometer. The ABTS working solution was mixed with varying concentrations of the test sample and the reference standard, up to 50 ml, to reach a final volume of 1ml. The absorbance at 734nm was measured. GraphPad Prism software used for the analysis to ascertain the percent inhibition and IC_{50} values at different doses.The supplied formula was used to compute the percentage inhibition of growth:

$$\%\text{ Inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Sample})}{(\text{OD of Control})} \times 100$$

α -Amylase inhibition assay

The α-amylase inhibition assay was performed with slight modifications as described[19]. The test sample/reference standard was pre-incubated in 12 µL of phosphate buffer (pH 7.0), and 60 µL of enzyme for 10 min at 37°C. 250 µL CNPG3 reagent was mixed with sample and incubated at 37°C for 8 min. In a boiling water bath, the reaction mixture was kept for 2 min to stop the reaction and then cooled. At 405 nm, the absorbance was measured. Control response was also performed without the test material. The inhibition of α-amylase percentage was calculated by using the formula:

$$\%\text{ Inhibition} = \frac{\text{Absorbance (control)} - \text{Absorbance } (\textit{test})}{\text{Absorbance (control)}} \times 100$$

α-Glucosidase inhibition assay

α-glucosidase enzyme hydrolysis of sucrose may be quantified *in vitro* through determining the sugar (Glucose). The α-glucosidase enzyme (2 U/mL) was premixed with 20 µL of 1000 µg/L extract and incubated at 37°C for 5 min. The sample incubated at 37°C by adding the 1 mM p-nitrophenyl glucopyranoside (pNPG) (20 µL) for 20 min in pH 6.8 of phosphate buffer (50 mM). By adding 50 µL of 1 mM sodium carbonate stopped the reaction. The absorbance of enzyme activity (without enzyme, with enzyme) and control of anti-diabetic drug was measured at 405 nm[20].

Computational study

Selection of phytochemicals from W. somnifera root extract for docking study

From the GC-MS analysis, among 16 phytochemicals8 were chosen after obtaining their structure data in SDF format from the PubChem-NCBI database (https://pubchem.ncbi.nlm.nih.gov/). The corresponding structures of these phytochemicals were then transformed into mol files through ACD/Chem sketch software, and then to PDB format using PyMOL molecular graphics system for deeper examination. The structure of Canagliflozin and Dapagliflozin was treated in a way similar to the reference control.

Drug likeliness prediction of phytochemicals from W. somnifera root extract

Through the application of Molinspiration, the drug-like characteristics of plant chemicals are explored (https://www.molinspiration.com/). This approach, guided by the "Lipinski Rule of 5," offers a broad spectrum of chemical features for compounds. The Lipinski rule, which stipulates that a compound should have a molecular weight below 500 Da, fewer than 10 H-bond acceptors, and fewer than 5 H-bond donors. The inclusion of H-bond donors and acceptors within a drug's structure is crucial for its interactions with proteins, distribution, transport across membranes, and solubility in water[21].

Swiss ADME properties

Utilizing SwissADME (http://www.swissadme.ch/)online platform, it's possible to prediction the ADME (Absorption, Distribution, Metabolism, Excretion, and Toxicity) and toxicity characteristics of plant-based phytochemicals. This calculation takes into explanation various factors affecting ADMET, including their interaction with androgen receptors, aromatase, cytochrome P450 3A4 (CYP3A4) enzymes, estrogen receptors, aquatic toxicity in fish, binding to glucocorticoid receptors, absorption by human intestines, interactions with OATP1B1 inhibitors, potential for reproductive toxicity, effects on respiratory systems, and binding to UGT enzymes, which are identified as the most promising compounds for experimental evaluation *via* molecular docking[22].

Identification of protein targets and homology model construction

The proteins, including those obtained by running aprotein (Basic Local Alignment Search Tool) BLAST (https://blast.ncbi.nlm.nih.gov/) search in NCBI, were submitted to the CastP server (http://sts.bioe.uic.edu/) for an analysis of protein structures with evolutionary relevance, utilizing BLAST and HHblits methods[23]. This process offers detailed information on the protein's length, identification number, identity percentage, sequence coverage, and overall score. The CastP calculation server was utilized for the estimation of active site amino acids, following the methods outlined. This was based on the 3D structure of the protein and its measures of surface area and volume[24].

Macromolecule preparation

The macromolecules selected for docking studies are SGLT2, α-Glucosidase & α- amylase and PDB id: 7YNK (Resolution: 3.48 Å), 3WY1 (Resolution: 2.14 Å), & 1B2Y (Resolution: 3.20 Å) (Fig. 1). In type 2 diabetes, increased SGLT-2 increases the kidney's ability to metabolize glucose. This prevents glucose from passing into the urine until plasma glucose levels reach 220 mg/dL, rather than the 180 mg/dL limit[25]. Inhibition of α-glucosidase is effective in improving the metabolic profile of patients with type 2 diabetes and reducing the risk of long-term complications of hyperglycaemia. α-amylase is a calcium metalloenzymes that aids digestion by breaking down polysaccharide molecules into smaller molecules such as glucose and maltose. This enzyme affects postprandial hyperglycaemia and increases blood sugar[26].

Statistical analysis

The data is from three independent experiments as explained in the methodology section. The data is shown as mean ± SD. A $p<0.05$ is considered as a statistically significant.

Results and Discussion

Phytochemical analysis

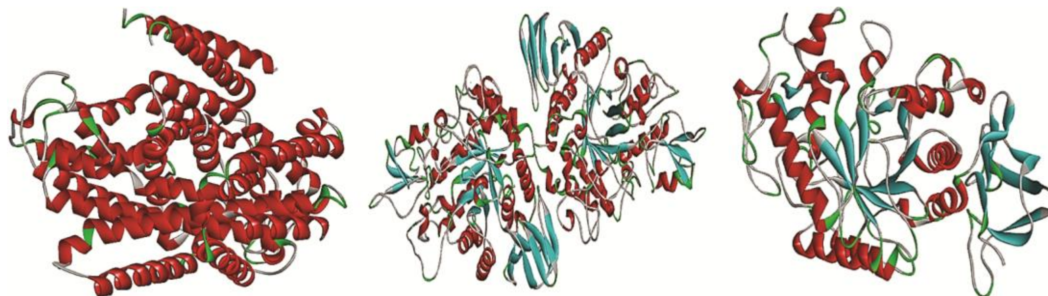
The phytochemical screening analysis by using of different methods the results shows the presence of phytochemicals in Ashwagandha root extract like tannins, glycosides, saponins, flavonoids, steroids, phenols and Coumarins[27] (Table 1).

GC-MS analysis

Glycolaldehyde dimer, 2-Trityl-N-(5-nitrosalicylidene)-3-oxo-4-isoxazolidinamine, 4-Aminoresorcinol, Acetic acid, 5-methylhex-2-yl ester, Propenoic acid, 3, 4-Dichloroatropine, O-Bromoatropine, Hexadecanoic acid, methyl ester, cis-Vaccenic acid, Z,E-7,11-Hexadecadien-1-yl acetate, L-Cysteine S-(triphenyl methyl), 1,5,9,13-Tetradecatetraene, 1,E-8,Z-10-Hexadecanene, alpha-(4-Chlorophenyl)-1,1-biphenyl-2-methyl, 7-Tetradecenal. (Z), and Benzenemethanol present in the Ashwagandha root extract, and these compounds were used further analysis. The GC-MS chromatogram (Fig. 2), and the list of identified compounds were presented in (Table 2).

Table 1 — Qualitative phytochemical analysis of *W. somnifera* extract

Name of the test	Result
Tannins Test	++
Glycosides Test	+++
Saponins Test	++
Flavonoids Test	++
Alkaline Reagent Test	+
Steroid Test	++
Terpenoids Test	+
Phenols	++
Quinone Test	+
Ninhydrin Test	+ (Proline)
Anthocyanin Test	-
Coumarins test	++
Lead Acetate test	++
Benedict's Reagent Test	++
Fehling's Reagent Test	+++



7YNK3WY1 1B2Y

Fig. 1 — Homology model of SGLT2, β-glucosidase & α- amylase

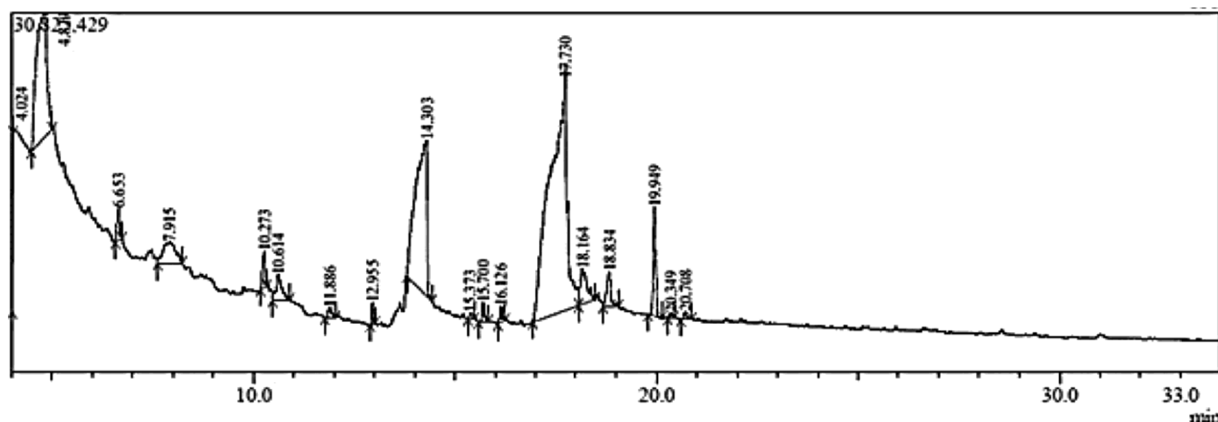


Fig. 2 — GC-MS analysis of *W. somnifera* root extract

Table 2 — The list of identified bioactive compounds from *W. somnifera* root extract

ID	Name	R Time	m/z	Area	Height
1	Glycolaldehyde dimer	4.032	31.00	18961753	5543446
2	2-Trityl-N-(5-nitrosalicylidene)-3-oxo-4-isoxazolidinamine	4.848	97.00	66818072	7105915
3	4-Aminoresorcinol	6.652	125.00	10189597	1769869
4	Acetic acid, 5-methylhex-2-yl ester	7916	43.00	8338219	422665
5	Propenoic acid	10.272	87.00	1952819	368851
6	3, 4-Dichloroatropine	10.610	124.00	2346541	334612
7	O-Bromoatropine	11.886	87.00	219793	80861
8	Hexadecanoic acid, methyl ester	12.955	74.00	809362	259541
9	cis-Vaccenic acid	15.372	55.00	167355	53136
10	Z,E-7,11-Hexadecadien-1-yl acetate	16.128	67.00	504200	173548
11	L-Cysteine S-(triphenyl methyl)-	17.748	165.00	44860236	5452131
12	1,5,9,13-Tetradecatetraene	18.174	67.00	964328	228639
13	1,E-8,Z-10-Hexadecanene	18.833	67.00	2423217	370989
14	alpha-(4-Chlorophenyl)-1,1-biphenyl-2-methyl	19.947	165.00	2050393	625454
15	7-Tetradecenal. (Z)-	-	55.00	---	---
16	Benzenemethanol	20.709	55.00	172373	43480

$DPPH^+$ and $ABTS^+$ radical scavenging activity

Qualitative examination revealed the occurrence of saponins, terpenes, saponins, glycosides, alkaloids, and reducing sugars found in methanolic extracts of Ashwagandha root extract. Total phenolics, flavonoids, and tannins were detected in substantial levels in extracts, as exposed**.** With increasing concentration (5-100 µg/mL), the percentage of $DPPH^+$ and $ABTS^+$ radical scavenging activities increased (Fig. 3A & B). The minimum inhibitory constant value IC_{50} shown for Ashwagandha root extract for $DPPH^+$ 37.8 µg/mL and $ABTS^+$ 34.4 µg/mL. Ascorbic acid and Gallic acid was used as a positive control.

α-amylase inhibition assay

α-amylase is a protein that catalysis the hydrolysis of starch to produce glucose. Controlling this enzyme's catalytic activity lowers glucose synthesis in the postprandial period, which might be a therapeutic advantage for diabetics[28]. The Sample was shown a significant inhibition of α-amylase with IC_{50} value of 92.94 µg/mL. The standard Acarbose showed inhibition of α-amylase with IC_{50} value of 2.30 µg/mL. As depicted in the Figure 4 was shown that the extract of *W. somnifera* effectively inhibits the action of α-amylase enzyme in a dose dependent manner. The percentage inhibition of α-amylase enzyme varied from 14.71% to 77.87% at a concentration range from 3.25 to 200 µg/mL. Overall, this result suggests that the sample has very good anti-diabetic activity. Although the IC_{50} of the positive control Acarbose is lower than that of extracts, the high IC_{50} of extracts isn't unexpected. In comparison to the single component Acarbose, *W. somnifera* extract is combinations of several chemicals.

α-glucosidase inhibition assay

The enzyme α-glucosidase hydrolyzes carbohydrates to glucose in the brush borderlines of

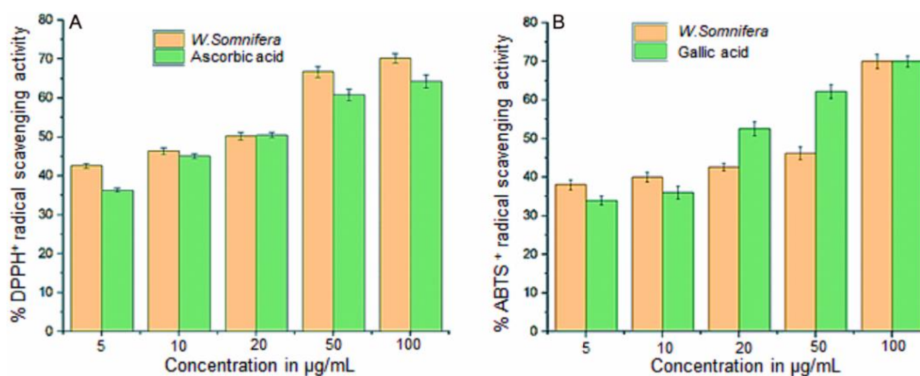


Fig. 3 — Figures 1A and B represent DPPH$^+$ and ABTS$^+$ radical scavenging properties of *W. somnifera* root extract

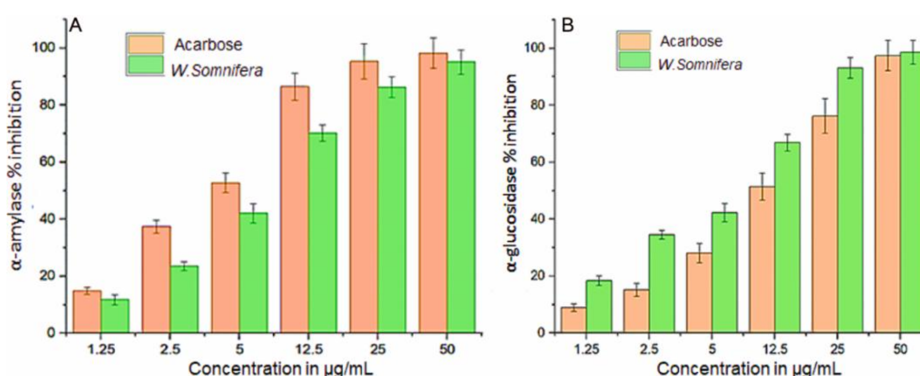


Fig. 4 — (A) α-amylase inhibition of *W. somnifera* root extract; and (B) α-glucosidase inhibition of *W. somnifera* root extract

the gut for absorption. Several studies have revealed that medicinal plant extracts can serve as α-glucosidase inhibitors, implying that they can be used to treat hyperglycemia[29,30]. The effective inhibition of enzyme α-glucosidase was tested for the standard and extract of *W. somnifera*. Different concentrations (3.25, 6.25, 12.5, 25, 50, 100 µg/mL) of extract of *W. Somnifera* were subjected to α-glucosidase inhibitory assay (Fig. 5). α-amylase acts on adsorption process of carbohydrates by hydrolyzing 1,4-glycosidic bonds in polysaccharides (starch, glycogen) to disaccharides, which is followed by α-glucosidase catalyzing the simple carbohydrates to simple sugars, resulting in postprandial hyperglycaemia. As a result, inhibitors of α-amylase and α-glucosidase can help manage diabetes by delaying absorption of carbohydrates and thereby lowering nocturnal levels of blood glucose. This suggests that the sample has very good anti-diabetic activity[31].

In silico studies

Prediction of compounds AMDET properties by using in silico studies

Number of rotatable bonds, AlogP, molecular weight, number of H-bond donors, and number of H-bond acceptors. The pharmacokinetic drug distribution, absorption, metabolism, and excretion are all dependent on the Lipinski rule, which is based on molecular weight, AlogP, number of rotatable bonds, number of H-bond donors, and number of H-bond acceptors. To estimate synthetic molecules' drug-like qualities using reference drugs represented in (Table 3)[32]. The selected compounds are 3,4-Dichloroatropine, Propenoic acid, O-Bromoatropine, alpha-(4-Chlorophenyl)-1,1'-biphenyl-2-methyl, 1,5,9,13-Tetradecatetraene, 5-methylhex-2-yl ester, 2-Trityl-N-(5-nitrosalicylidene)-3-oxo-4-isoxazolidinamine and Benzenemethanol from GC-MS analysis for molecular docking studies based on their pharmacophore and pharmacokinetic studies.

In silico bioactivity study

The biological activity score of synthetic compounds was also evaluated using the Molinspiration Cheminformatics server application (Molinspiration Cheminformatics)[33]. Understanding the active site or binding mechanism through biological activity studies is necessary to comprehend how chemicals behave biologically represented in (Table 4).

Drug research and development must take into account the chemical absorption, distribution,

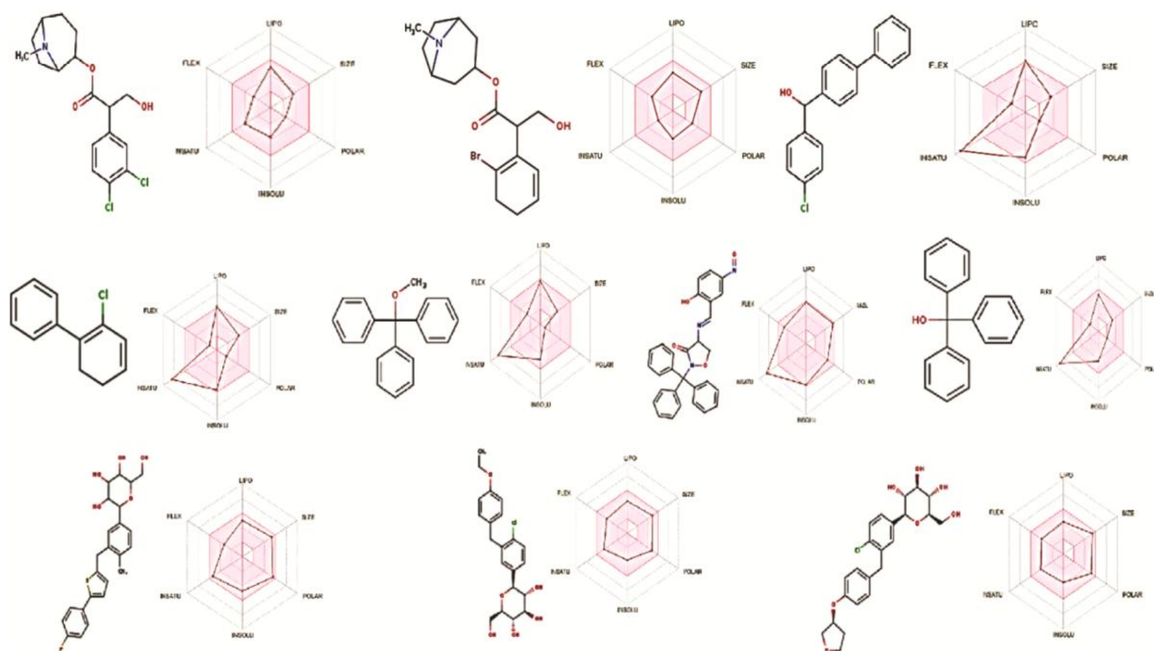


Figure 5 — Represent the bioactive compounds structure from GC-MS analysis for *in silico* studies

Table 3 — Pharmacophore analysis of phytochemicals with reference drug

Compound	miLogP	natoms	noN	noHNH	nrotb
Ligand_1	3.05	23	4	1	5
Ligand_2	3.25	21	3	0	4
Ligand_3	2.79	22	4	1	5
Ligand_4	5.33	21	1	1	3
Ligand_5	3.45	24	1	0	2
Ligand_6	5.14	21	1	0	4
Ligand_7	5.59	36	7	1	7
Ligand_8	4.52	20.23	20	1	3
Canagliflozin	3.92	31	5	4	5
Dapagliflozin	2.60	28	6	4	6

metabolism, excretion, and toxicity (ADMET) processes. A good drug candidate should have appropriate ADMET properties at therapeutic levels in addition to being effective against the therapeutic target. By assessing the toxicity and drug similarity of the compounds chosen for additional investigation using the AdmetSAR tool[34] (Table 5).

Preparation of modelled protein and ligands for docking

By using the BLASTp algorithm and software for comparing primary biological sequence data. BLASTp is used to compare protein or nucleotide sequences to sequence databases. It detects particular regions of similarity between query sequences and database items. The program determines the statistical significance of these matches. BLASTp allows for fast alignment and comparison of a query DNA sequence with a sequence database. Determining the evolutionary and functional relationships between sequences is made easier by it[35] (Tables 6 & 7).

Docking analysis

The homology modelled protein is used for docking. Gasteiger charges and polar hydrogens were given to the macromolecules before to docking, Autodock Vina 4.2 were used to add partial change in order to integrate nonpolar and polar hydrogen atoms. Rotatable bonds were identified and non-polar hydrogen atoms were combined with polar hydrogen atoms for phytochemical compounds in addition to conventional pharmaceuticals for comparison[36,37]. Grid maps and rigid or flexible macromolecules are produced by setting the grid dimension to $40 \times 40 \times 40$ (X=67.914, Y=75.412, Z=65.762) points and spacing to 0.752 Å to facilitate ligand binding (Tables 8-10). The total binding site energy for each grid is determined

Table 4 — Biological activity of phytochemicals with reference drug

Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear Inhibitor	Protease inhibitor	Enzyme inhibitor
Ligand_1	0.53	0.29	-0.01	-0.07	0.17	0.17
Ligand_2	0.10	0.22	-0.36	0.34	0.10	0.11
Ligand_3	0.41	0.28	-0.23	0.25	0.03	0.37
Ligand_4	0.11	0.07	0.10	0.22	-0.09	0.17
Ligand_5	-0.12	-0,24	-0.19	0.05	-0.14	0.15
Ligand_6	-0.16	-0.26	-0.17	0.07	-0.11	0.17
Ligand_7	-0.27	-0.14	-0.19	-0.40	-0.07	0.05
Ligand_8	-0.04	-0.18	-0.01	0.03	-0.13	0.36
Canagliflozin	0.15	-0.21	0.15	0.07	0.02	0.33
Dapagliflozin	0.15	-0.07	-0.05	0.09	0.06	0.25

Table 5 — ADME properties of phytochemicals with reference drug

Compounds	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor	Log K_p (skin permeation)
Ligand_1	High	Yes	No	No	Yes	No	Yes	No	-5.98 cm/s
Ligand_2	High	Yes	No	No	Yes	Yes	Yes	No	-5.61 cm/s
Ligand_3	High	Yes	No	No	No	No	No	No	-6.80 cm/s
Ligand_4	High	Yes	No	Yes	Yes	Yes	Yes	No	-4.38 cm/s
Ligand_5	High	No	No	Yes	Yes	Yes	No	No	-4.54 cm/s
Ligand_6	High	Yes	No	Yes	Yes	No	Yes	No	-4.77 cm/s
Ligand_7	High	No	No	No	No	Yes	No	Yes	-5.85 cm/s
Ligand_8	High	Yes	No	Yes	Yes	No	Yes	No	-5.28 cm/s
Canagliflozin	High	No	Yes	Yes	No	No	Yes	No	-6.72 cm/s
Dapagliflozin	High	No	Yes	No	No	No	Yes	No	-7.13 cm/s

Table 6 — The identification of target active amino acids by Castp analysis

Sl. no	Protein name	Protein code	Area (SA) Å^2	Volume (SA) Å^3	Active amino acids
1.	SGLT2	7YNK	356	456	Gly669, Phe666, Phe670, Val665
2.	α-Glucosidase	7F1N	160	185	Gly375, Gly47, Arg46, Arg45, Leu 475, Asp 52, Thr 373, Leu 379, Glu 421, Asn 423, Glu 476
3.	α- Amylase	1B2Y	234.65	224.065	Leu 165(A), Trp 581(A), Tyr 62, Ile 235, Ala198.

Table 7 — The identification of target protein by Blastp analysis

SI. No.	Target Protein	Length	Sequence ID	% of identity	Sequence Coverage	Total Score
1.	SGLT2	672	NP_003032.1	100%	1 to 672	3517
		676	8HB0_A	100%	5 to 676	3515
		672	prf||1909123A	99%	1 to 672	3508
2.	β- glucosidase	470	NP_001264154.1	100%	1 to 470	2534
		469	NP_066024.1	100%	20 to 469	2431
		469	2ZOX_A	99%	20 to 469	2428
3.	α - amylase	511	NP_001373038.1	100%	1 to 511	2749

Table 8 — The binding affinity of SGLT2 against phytochemicals with reference drug

Phytochemicals with reference drug	Docking score (kcal/mol)	Interacting residue	Type of bond	Bond distance (Å)
Ligand_1	-8.9	His80	H-bond	3.02
		Tyr290	Pi-Alkyl	3.91
		Ala102	Pi-Alkyl	4.35
		Val286	Pi-Alkyl	3.89
Ligand_2	-9.3	His80	H-bond	3.08
		Ser393	H-bond	2.79
		Lys154	Pi-Alkyl	3.85
		Ala102	Pi-Alkyl	3.46
Ligand_3	-8.9	Asp158	H-bond	3.49
		His80	H-bond	3.55
		Ala102	Pi-Alkyl	4.52
Ligand_4	-8.4	Tyr290	Pi-Alkyl	4.97
		Lys145	Pi-Alkyl	4.20
Ligand_5	-7.4	Ser393	H-bond	3.46
		His80	H-bond	3.96
		Gly79	H-bond	3.76
Ligand_6	-3.4	Ser393	H-bond	3.58
		Tyr290	H-bond	3.79
		Asp158	H-bond	3.52
Ligand_7	-8.5	Ser402	H-bond	2.93
		Thr406	H-bond	1.96
		Val430	Pi-Alkyl	3.12
Ligand_8	-9.1	Thr153	H-bond	2.34
		Lys154	H-bond	3.83
		Val157	Pi-Alkyl	4.65
Canagliflozin	-8.3	Ser70	H-bond	1.76
		Ser396	H-bond	2.93
		Tyr290	H-bond	2.97
Dapagliflozin	-7.6	Ser393	H-bond	2.34
		Thr153	H-bond	3.67
		Tyr290	H-bond	2.25

Table 9 — The binding affinity of β- glucosidase against phytochemicals with reference drug

Phytochemicals with reference drug	Docking score (kcal/mol)	Interacting residue	Type of bond	Bond distance (Å)
Ligand_1	-9.1	Glu459	H-bond	2.35
		Trp460	Pi-Alkyl	3.96
		Trp389	Pi-Alkyl	4.28
Ligand_2	-9.2	Tyr341	H-bond	3.53
		Phe226	Pi-Alkyl	4.18
		Ala416	Pi-Alkyl	4.37
Ligand_3	-7.6	Glu210	H-bond	3.28
		Phe468	Pi-Alkyl	4.45
Ligand_4	-7.6	Tyr290	Pi-Alkyl	4.97
		Lys145	Pi-Alkyl	4.20
Ligand_5	-5.5	Phe468	Pi-Alkyl	4.73
		Trp452	Pi-Alkyl	4.26
Ligand_6	-7.5	Val224	Pi-Alkyl	4.78
		Ala426	Pi-Alkyl	4.56
Ligand_7	-11.8	Asn209	H-bond	3.40
		Trp460	H-bond	3.79
		Tyr341	H-bond	3.81
		Phe468	H-bond	3.40
Ligand_8	-8.0	Pr0367	Pi-Alkyl	2.34
		Lys36	Pi-Alkyl	3.83

(*Contd.*)

Table 9 — The binding affinity of β- glucosidase against phytochemicals with reference drug (*Contd.*)

Phytochemicals with reference drug	Docking score (kcal/mol)	Interacting residue	Type of bond	Bond distance (Å)
Canagliflozin	-10.6	Tyr341	H-bond	2.82
		Phe468	H-bond	3.16
		Glu210	H-bond	3.23
Dapagliflozin	-9.3	Trp460	H-bond	2.14
		Tyr341	H-bond	2.25
		Phe468	H-bond	2.16

Table 10 — The binding affinity of α – amylase against phytochemicals with reference drug

Phytochemicals with reference drug	Docking score (kcal/mol)	Interacting residue	Type of bond	Bond distance (Å)
Ligand_1	-7.5	Tyr290	H-bond	2.48
		Leu165	H-bond	3.58
		Asp197	H-bond	3.59
Ligand_2	-8.4	Trp59	Pi-Alkyl	4.84
		Ala198	Pi-Alkyl	4.91
Ligand_3	-7.3	His101	H-bond	3.49
		Thr163	H-bond	3.60
		Asp300	H-bond	2.45
Ligand_4	-9.3	His299	H-bond	3.04
		Trp59	Pi-Alkyl	4.71
Ligand_5	-4.5	His299	H-bond	3.54
Ligand_6	-7.7	Trp59	H-bond	3.21
		Gln36	H-bond	2.24
		Thr163	H-bond	2.26
Ligand_7	-7.6	Arg343	H-bond	2.93
		Val383	H-bond	1.96
Ligand_8	-8.9	Glu414	H-bond	2.34
		Tyr341	H-bond	3.83
		Glu459	Pi-Alkyl	4.65
Canagliflozin	-8.6	Asp300	H-bond	2.76
		Glu233	H-bond	2.83
Dapagliflozin	-8.6	Gln63	H-bond	2.65
		Tyr151	H-bond	2.04
		Glu233	H-bond	2.87

using an equilibrated energy distribution forthe amino acid composition. The configuration file is produced as a text file that can be run in Autodock Vina to assess the target and ligand proteins' ability to bind[38].

Protein-1- SGLT2

The obtained results based on docking studies the *W. somnifera* root extract phytochemical compounds and with reference drugs exhibit more affinity towards the SGLT2, α- glucosidase and α –amylase. The SGLT2 protein ligand complex of ashwagandha root extract compounds is have good binding affinity with a range of -9.6 to -3.5 (kcal/mol) and formed hydrogen bonds with Ser393, His80, Gly79, Tyr290 and Asp158active amino acids. The reference drugs also exhibit binding affinity of -8.3 (kcal/mol) with active amino acids like Ser70, Ser396 and Tyr290 against SGLT2 protein (Fig. 6).

Protein-2- α- glucosidase

The α - glucosidase protein ligand complex of *W. somnifera* root extract compounds are having good binding affinity ranging from -11.8 to -5.5 (kcal/mol) and formed hydrogen bonds with active amino acids such as Asn209, Trp460, Gly79, Tyr341 and Phe468. The reference drugs also exhibit binding affinity of -8.3 (kcal/mol) the active amino acids like Trp460, Tyr341 and Phe468 against α - glucosidase protein (Fig. 7).

Protein-3- α-amylase

The α-amylase protein ligand complex of ashwagandha root extract compounds is having good

binding affinity with ranging from -9.3 to -4.5 (kcal/mol) and formed hydrogen bonds with active amino acids such asTrp59, Gln36, Thr163, Arg343 and Val383.

The reference drug also exhibits binding affinity of -8.6 (kcal/mol) the active amino acids like Asp300, Gln63, Tyr151 and Glu233 against α-amylase protein (Fig. 8).

These results indicate that extracted phytochemicals from ashwagandha root extract exhibiting good diabetic activity.

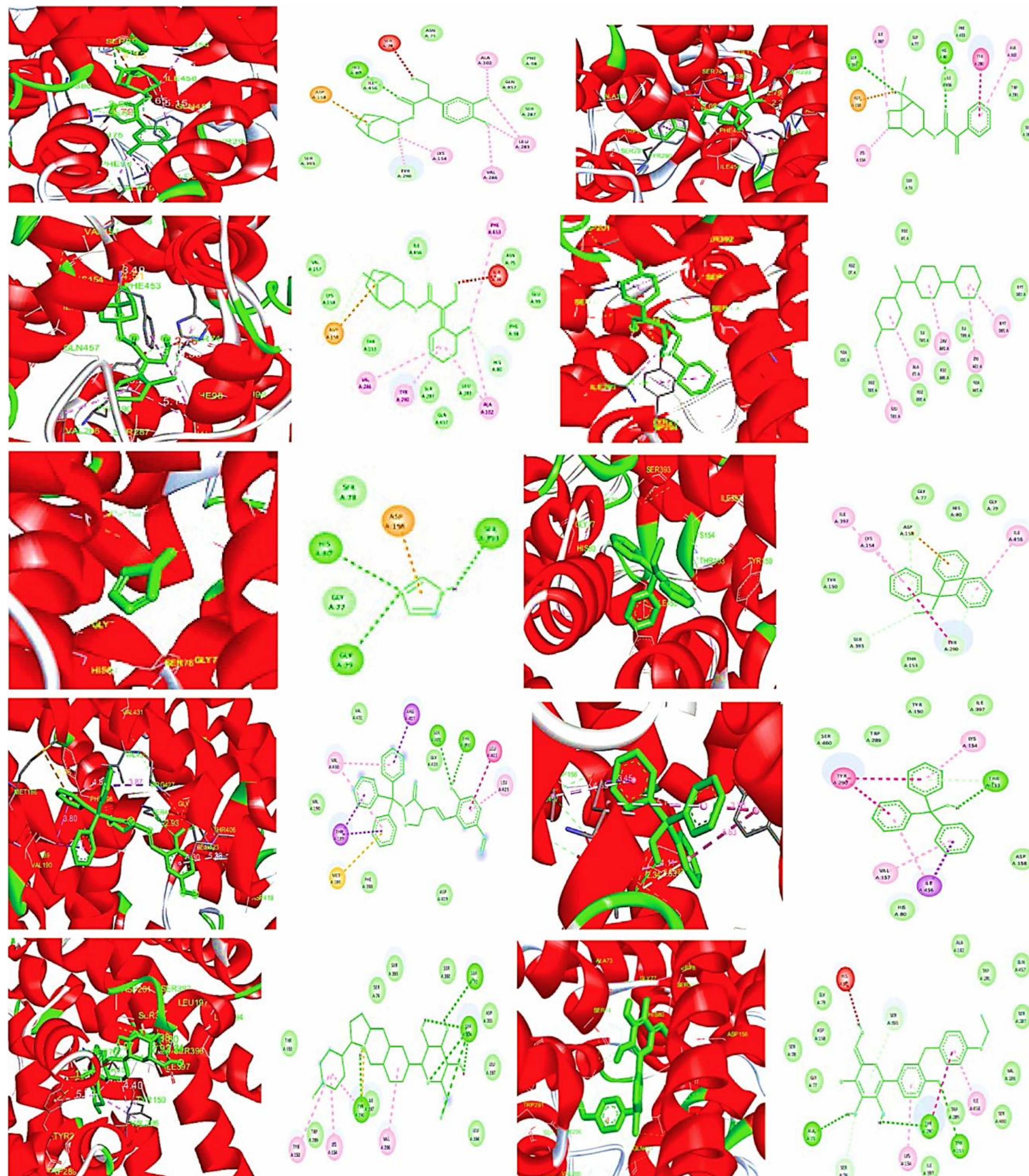


Fig. 6 — 3D and 2D structure of protein-ligand complex of phytochemicals and reference drugs against SGLT2

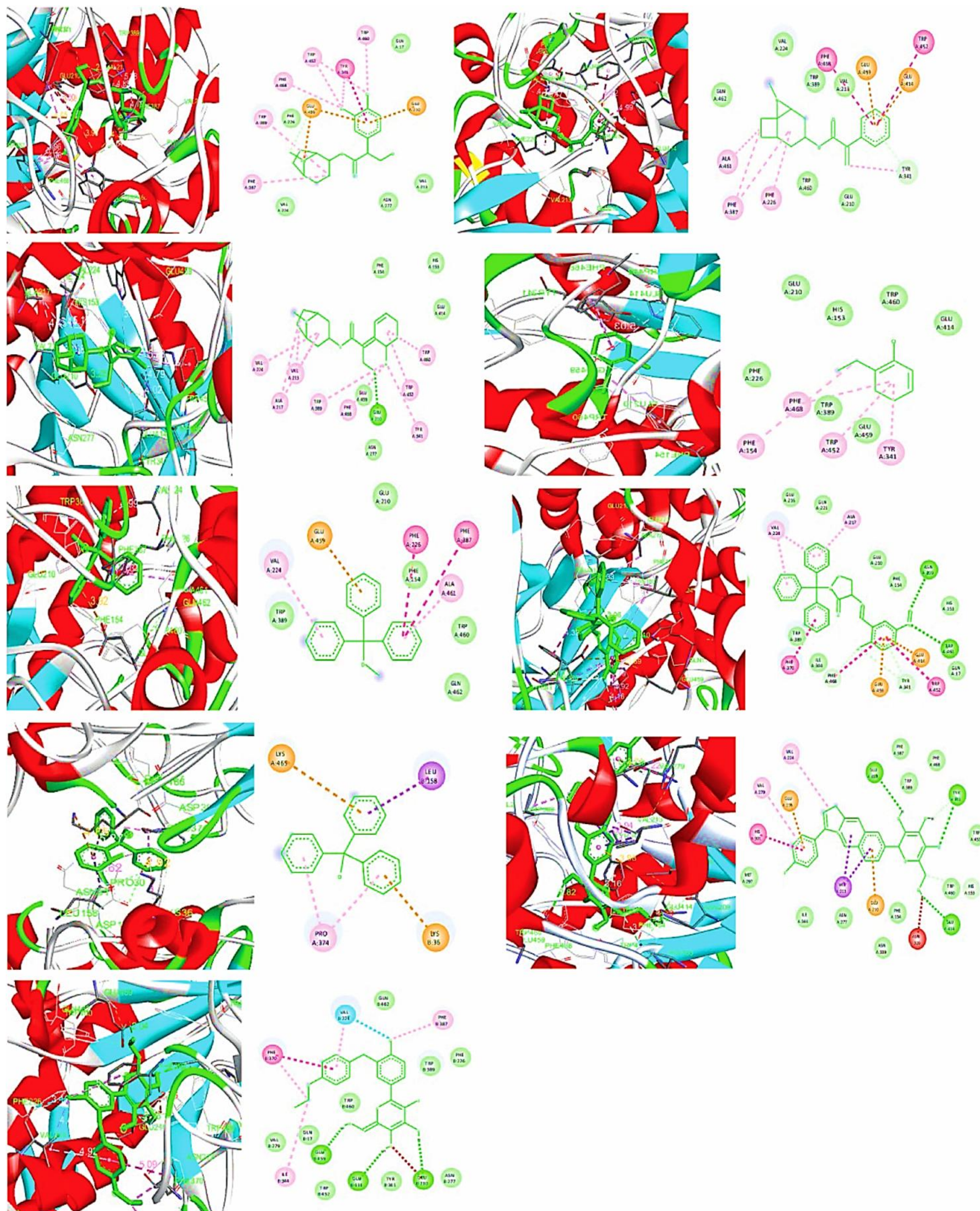


Fig. 7 — 3D and 2D structure of protein-ligand complex of phytochemicals and reference drug against α – glucosidase

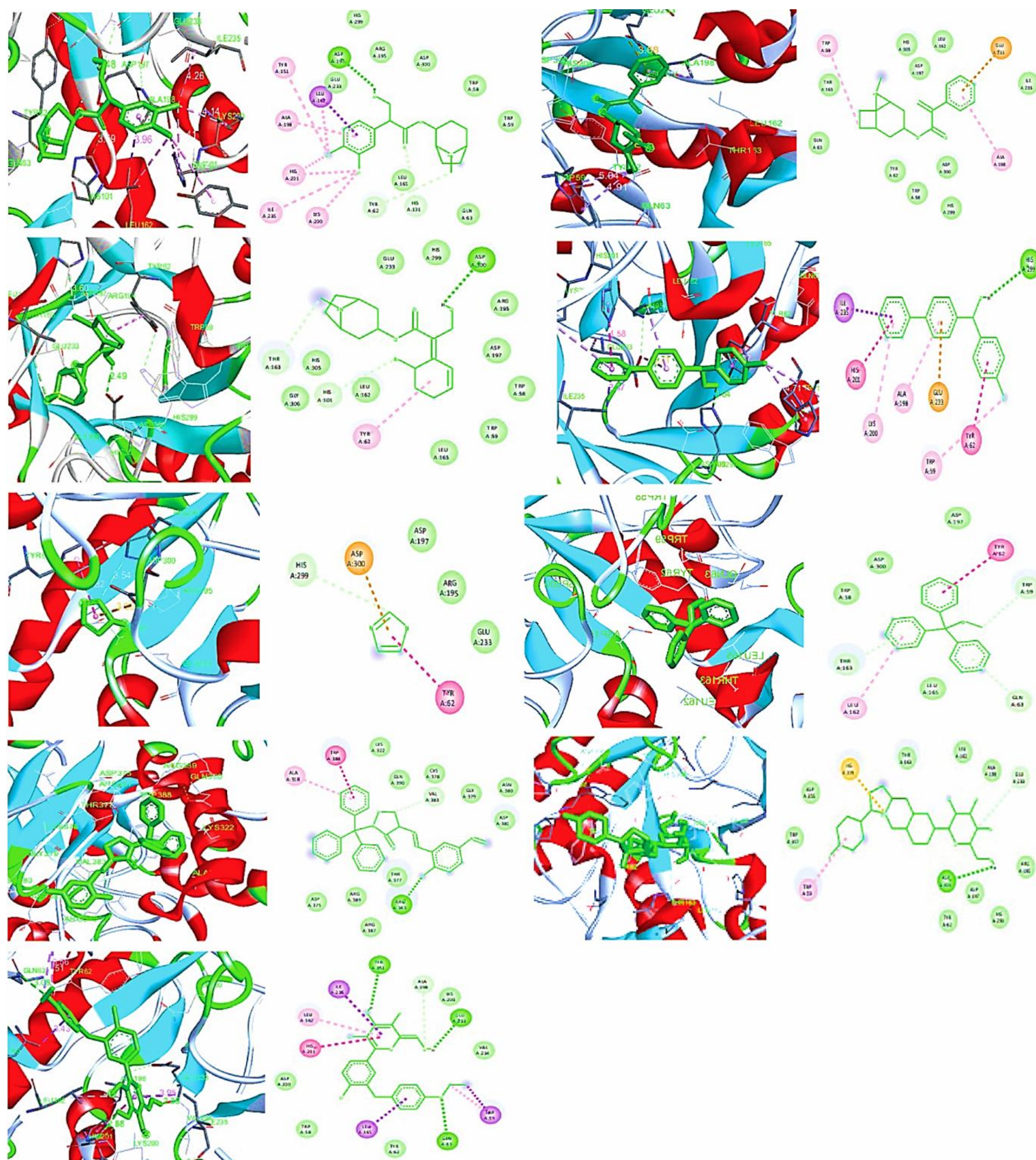


Fig. 8 — 3D and 2D structure of protein-ligand complex of phytochemicals and reference drugs against α- amylase

Conclusion

Insulin is a hormone that regulates blood glucose levels. Hyperglycaemia is a long-term condition caused by the pancreas' inability to produce insulin or the body's inability to adequately use insulin. The fundamental causes of diabetes include oxidative stress and an increase in reactive oxygen species, which can have a considerable impact on other organs. Plants contain natural antioxidants such as tannins, flavonoids, and vitamins C and E, which support β-cell function and lower blood glucose levels. Some studies have showed that ashwagandha

medication resulted in a considerable drop in blood sugar, hemoglobin A1c (HbA1c), blood lipids, and oxidative stress markers.

Several phytochemicals were found after extracting ashwagandha metabolites from the root with methanol and water using GC-MS analysis. A qualitative study confirmed the presence of saponins, terpenes, glycosides, alkaloids, and reducing sugars in ashwagandha solvent extracts. Increasing the concentration (5-100 µg/mL) improved the proportion of $DPPH^+$ and $ABTS^+$ radical scavenging activity. Ashwagandha root extract has a minimal inhibitory constant (IC_{50}) of 37.8 µg/mL for $DPPH^+$ and 34.4 µg/mL for $ABTS^+$. The percentage inhibition of the α-amylase enzyme ranged from 14.71% to 77.87% at concentrations ranging from 3.25 to 50 µg/mL. α-glucosidase delays glucose absorption, aiding in diabetes management. The extract inhibits α-amylase, decreasing nocturnal blood glucose levels, indicating strong anti-diabetic properties. Docking experiments revealed that phytochemical compounds have a higher affinity for SGLT2, α-glucosidase, and α-amylase compared to reference compounds. The phytochemicals have a high binding affinity for all of the identified targets, as evidenced by the formation strong hydrogen bonds between active amino acids. These data indicate that the phytochemicals isolated in our study have favourable impacts on diabetic activity.

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Conflict of interest

All authors declare no conflict of interest.

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