An experimental and computational approach to evaluate the antidiabetic activity of Guggul gum by inhibition of a common diabetes target

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Research Article

Keywords: Antidiabetic, Antioxidant, Commiphora wightii, Molecular docking, Pancreatic α-amylase, SwissADME

Posted Date: September 26th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3383828/v1

Abstract

Background

In recent years, plant formulations with antidiabetic and antioxidant properties have gained popularity due to their lower cost and lesser side effects. Guggul gum is one such formulation that is already being used in curing arthritis, lowering cholesterol, and in weight management. The present study explored the antioxidant and antidiabetic properties of the aqua-ethanolic guggul gum extract (*Commiphora wightii*) using *in vitro* assays and *in silico* techniques. To predict the inhibition, GCMS-identified compounds were docked to the Human pancreatic α-amylase (PDB ID: 1HNY) in *in silico* studies. The inhibition of alpha-amylase enzyme (a common diabetes target) has been further evaluated at an *in vitro* level to show a hypoglycemic role of the compounds.

Results

The extract showed a good amount of phenolic (5.14 ± 0.011 mg) and flavonoid (0.66 ± 0.023 mg) content along with a promising free radical scavenging activity of 41.96 ± 4.02% at the highest concentration (9.6 mg/ml). For the *in silico* studies, the drug-likeliness of the GCMS-identified bioactive compounds of the extract was evaluated using SwissADME. Out of 6 compounds, 3 showed permissible values for LIPO, FLEX, INSATU, INSOLU, POLAR, and SIZE suggesting them as a potential candidate for antidiabetic drugs. In molecular docking studies, out of 6 GCMS-identified compounds, three showed binding energy (BE) more than the standard drug acarbose indicating better inhibition. This was further confirmed by *in vitro* analysis where the pancreatic α-amylase inhibitory activity of the extract and the standard drug (acarbose) at an IC_{50} value of 4.17 ± 1.26 mg/ml and 3.69 ± 0.89 mg/ml respectively, were comparable.

Conclusion

The results demonstrated Guggul gum as a potential alternative to commercial antidiabetic drugs. However, the isolation of the identified compounds could be done in the future for *in vivo* studies that can substantiate the extract's significant role in diabetes management.

Background

Type 2 Diabetes Mellitus (T2DM) is a group of metabolic disorders in which the body either resists the effects of insulin or simply fails to produce enough insulin that is required to maintain the normal levels of glucose in the body [1]. The raised glucose level in T2DM induces free radicals in the body and destabilizes the balance with the body's natural antioxidant system. Oxidative damage caused to pancreatic β-cells can further worsen glucose metabolism [2, 3].

Several commercial drugs are available in the markets that are being used to manage this disorder and one such drug is acarbose. This starch blocker slows the digestion of carbohydrates in the body by inhibiting pancreatic alpha-amylase and thus controls the post-prandial glucose levels. The activity of the pancreatic α-amylase can be correlated to elevated postprandial glucose levels as this enzyme acts on dietary starch to convert it into glucose leading to post-prandial hyperglycemia (PPHG) [4, 5]. Acarbose inhibits the enzyme by mimicking the transition state

of the starch (substrate) with the amine linkage [6]. Thus, this makes α-amylase a common diabetes target whose inhibition is beneficial in T2DM management.

Although acarbose is extensively used in managing T2DM, it is not free from undesirable side effects. For instance, this drug has been reported to increase hypoglycemia risk when given in combination with other oral antidiabetic drugs like sulfonylureas [7]. The drug could even lead to adverse gastrointestinal symptoms like abdominal pain, flatulence, and diarrhoea [8]. Due to these significant side effects of commercial drugs, plant formulations, with known antioxidant activities have proved to be a safer option.

The Indian scriptures related to Ayurveda have mentioned innumerable herbal formulations that are currently being used by the masses in the treatment of several diseases caused due to free radical damage [9, 10]. These plants have also been reported to block the absorption of dietary carbohydrates and thus prevent PPHG [11]. One such traditional herbal product is Guggul gum obtained from *Commiphora wightii*. The oleo-gum resin of this plant exudes as a result of injury and has enormous benefits, making it a quintessential part of the Indian Ayurvedic System [12]. The gum constituents, like triterpenoids, steroids, flavonoids, polyphenols, sugars, amino acids, etc., can cure disorders like gout, obesity, rheumatoid arthritis, chronic cough, asthma, facial paralysis, diabetes, liver diseases, etc. [13].

The present study aims to evaluate the antioxidant and antidiabetic properties of the aqua-ethanolic extract of Guggul gum obtained from *Commiphora wightii.* For evaluation of antioxidant properties, the total phenolic and flavonoid contents of the extract were determined followed by an interpretation of the extract's radical scavenging activity. This was followed by an evaluation of the antidiabetic capacity of the extract. In the first phase, drug-likeliness (using *SwissADME*) and molecular docking studies (using *AutoDock 4.2*) for the GCMS-identified compounds were performed. This was supported by an *in vitro* antidiabetic study of the extract in the next phase. In both these *in silico* and *in vitro* studies, inhibition of a common diabetes target, α-amylase, was considered to find ideal bioactive compounds to form substantial grounds for *in vivo* studies in the future.

Materials & Methods

Plant materials and reagents

The aqua-ethanolic extract of guggul gum (*Commiphora wightii*) was procured from SV Agrofood, Maharashtra (Batch No: SVA/GE/202010). Porcine Pancreatic -amylase (PPA), Folin-Ciocalteu reagent, and acarbose were purchased from Sigma-Aldrich Corporation, USA. DPPH (2,2-diphenyl-1-picrylhydrazyl) was obtained from SRL Diagnostics, India. DNSA (3,5 Di-nitro salicylic acid) was purchased from HiMedia Laboratories, Mumbai, India. The remaining chemicals used were of analytical grade.

Qualitative and quantitative analysis of guggul extract

Fourier-transform Infrared Spectroscopy (FTIR) analysis

The FTIR was done for molecular profiling of the sample to identify the organic, inorganic, and polymeric groups present in the sample. A standard method was used to identify the chemical bonds present in the molecule, where about 2 mg of the sample was dried and ground with 200 mg of potassium bromide [14].

Gas Chromatography-Mass Spectroscopy (GCMS) analysis

The gas chromatography-mass spectrometry (GCMS) analysis provides the type of bioactive compounds in the extract [15]. To identify various phytocompounds, 1 µl of the extract was injected into the Rxi5 silicon column. For GC, the temperature for the column oven was set to 45^0C, and the ion source temperature was set to 270^0C. The solvent cut time was 2.55 min. while the total flow was 20.8 ml/min. The flow control pressure was set at 31.0 kPa. For the MS parameters, the start and end time was 2.55 min and 33.50 min respectively. The m/z was in the range of 40–700 and the scan speed was set at 2500 scans/second. The peaks represented the m/z ratio of the solvent fractions. The peaks of the corresponding compounds were compared with the National Institute Standard and Technology (NIST) database.

Evaluation of total phenolic and flavonoid content

A slightly modified “Folin-Ciocalteu” (FC) reagent method described by Wolfe et al. [16] was used to determine the total phenolic content (TPC) in the extract. A small amount of the extract was mixed with equal volumes of FC reagent (diluted with water in a 1:10 ratio) and sodium carbonate (0.7 M). After a quick vortex, the tubes were allowed to stand for 20 min. at room temperature. After color development, the absorbance was read at 760 nm using a UV-vis spectrophotometer. A similar experiment was performed with gallic acid as standard.

For the estimation of the total flavonoid content (TFC), an aluminium chloride ($AlCl_3$) colorimetric assay was performed [17]. To 0.5 ml of samples/standard, 150 µl of 5% sodium nitrate and 2.5 ml of distilled water were added. To this solution, 300 µl of 10% $AlCl_3$ was added after 5 min. A min. later 1 mL of NaOH (0.001M) and 550 µl distilled water were added to the mixture. The setup was left undisturbed for 15 min. at room temperature. The absorbance was read at 510 nm using a UV-vis spectrophotometer. The experiment was repeated for standard by replacing gum extract with quercetin.

Evaluation of antioxidant activity (DPPH radical scavenging assay)

As stated above, plants are a rich source of antioxidants, allowing them to quench the reactive oxygen species (ROS). To evaluate the free radical scavenging activity of the gum, a DPPH radical scavenging assay was performed [18]. The polyphenolic compounds of most plant extract can donate hydrogen atoms and thus decolorize the methanolic solution of DPPH. The gum extract (concentration range: 1.2–9.6 mg/ml) was mixed with 0.1 mM DPPH in a 3:2 ratio. The reaction mixture was quickly vortexed and kept in the dark for 30 min. at room temperature. Later, the absorbance of the mixture was read at 517 nm using a UV-vis spectrophotometer. The reaction was repeated by replacing the test samples with standard/positive control (Ascorbic acid). The following equation was used to calculate the percentage of DPPH radical scavenging activity:

$$\%DPPHRadicalScavengingActivity = \frac{Abs(Control) - Abs(Sample)}{Abs(Control)} \times 100$$

Here, Abs (Control) = Absorbance for negative control (distilled water)

Abs (Sample) = Absorbance for Guggul gum extract/Ascorbic acid

The methanolic solution of DPPH produces a violet/ purple color which turns into shades of yellow color in the presence of antioxidants from the extract or standard. The intensity of the yellow color is directly proportional to the antioxidant capacity of the extract.

Antidiabetic activity evaluation: In silico

For the evaluation of the antidiabetic activity of the extract at an *in* silico level, the inhibition capacity of GCMS-identified compounds towards pancreatic α- amylase was evaluated. This was done through Molecular docking studies. Before that, the drug-likeliness of each of the compounds was evaluated.

Drug-likeliness of bio-active compound

For a rapid appraisal of the drug-likeliness of the major GCMS-identified compounds, bioavailability radar was generated using the SwissADME tool (SwissADME). The radar plot bears six axes each of which depicts an important bioavailability property namely, Lipophility (LIPO), Size, Polarity (POLAR), Insolubility (INSOL), Insaturation (INSATU), and Flexibility (FLEX). The LIPO for an ideal drug-like compound should range between − 0.7 and + 5.0 while it should weigh between 150 and 500 g/mol. The total polar surface area (TPSA), depicting the POLAR, should be between 20–130 $Å^2$, solubility (log S) should not be more than 6 and the value for saturation should not be less than 0.25. Finally, in terms of flexibility, not more than 9 rotatable bonds should be present [19].

Molecular docking studies

For molecular docking studies, the x-ray 3D structure of Human pancreatic α amylase (HPA) was downloaded from the RCSB protein data bank with a PDB ID: 1HNY [20]. After downloading, the ligands, hetatoms, and water were removed, and then polar hydrogens and Kollman charges were added to the protein using Autodock 4.2 [21]. The 3D structures of ligands were downloaded from the PubChem database. For protein energy minimization an online open-source minimization server “YASARA” (YASARA - Yet Another Scientific Artificial Reality Application) was used. Similarly, the energy for ligand was also minimized using OpenBabel (ver. 3.1.1-x64). The molecular docking was performed using Autodock 4.2 merged with the MGLTools Package (Version 1.5.7). The active sites of the protein were chosen as the grid center and the grid box of size 46 x 78 x 54 (for x, y, and z) was set at 10.44, 48.919, and 17.614 Å (for x, y, and z). The docking scores were generated in a file (.log) and were defined as binding energy (kcal/mol). The ligand binding sites and ligand-protein interaction were developed using Biovia Discovery Studio 2021 v21.1.0.20298 [22].

Antidiabetic activity evaluation: In vitro (3,5 Di-nitro salicylic acid (DNSA) method)

To validate the results of molecular docking studies, the antidiabetic activity of the guggul extract was evaluated at an *in vitro* level. For this, the -amylase inhibitory activity of the guggul extract was performed using the chromogenic DNSA method. DNSA is an aromatic compound that has the potential to react with starch and produce an intermediate compound that can absorb light at 540 nm. This colorimetric reagent was prepared by adding up the DNSA solution and $C_4H_4O_6KNa{\cdot}4H_2O$ (sodium potassium tartrate solution or “*Rochelle salt*”) at 96 mmol concentration [23]. The total reaction mixture was composed of 0.5 ml sodium phosphate buffer (0.02M, pH 6.9), PPA solution (0.04 units), and Guggul extract at varying concentrations (0.8–6.4 mg/ml). This mixture was pre-incubated for 10 min. at 37^o C. Quickly after this, 0.5 ml of 1% v/v starch solution was added to this mixture followed by incubation at the same conditions. To terminate the reaction, 1 ml of DNSA was added to the reaction mixture followed by quick incubation in a boiling water bath. The mixture was cooled down and its absorbance was read at 540 nm [24]. The process was repeated for acarbose (positive control/standard), a known -amylase inhibitor. The following formula was used to calculate the percentage inhibition in the enzyme activity:

$$\%Inhibitionofenzymeactivity = \frac{Abs\,(Control) - Abs\,(Sample)}{Abs\,(Control)} \times 100$$

Here, Abs (Control) = Absorbance for negative control (distilled water)

Abs (Sample) = Absorbance for Guggul gum extract/Acarbose

Results

Qualitative and quantitative analysis of guggul extract

An elaborative data of the different functional groups along with their frequency and intensity is depicted in the spectrum in Fig. 1. The spectrum showed major absorption band peaks at 3413.60 cm^{-1} due to O-H stretching (alcohol), at 2927.41 cm^{-1} due to N-H stretching (amine salt), at 1649.81 cm^{-1} due to C = N stretching (imine/oxime), 1456.60 cm^{-1} due to C-H bending (aromatic compound), at 1034.35 cm^{-1} due to S = O stretching (sulphoxide) and at 851.22 cm^{-1} due to C-Cl stretching (halo compound). The extract showed TPC as 5.14 ± 0.011 mg GAE/g of crude extract and TFC as 0.66 ± 0.023 mg QE/g of crude extract (Table 1). The reference compounds used were gallic acid and quercetin, respectively. The concentration values were calculated by using the standard curve equation obtained by preparing a graph of the varying concentrations of the standards used against the absorption value.

Table 1
Polyphenol contents of aqua-ethanolic extract of Guggul gum

S. No.	Polyphenols	Mean absorbance	Concentration in extract
1.	Phenolics	0.5136 ± 0.11 (760 nm)	5.14 ± 0.011 mg GAE/g of crude extract
2.	Flavonoids	0.03 ± 0.02 (510 nm)	0.66 ± 0.023 mg QE/g of crude extract

GAE = Gallic acid equivalent, QE = Quercetin equivalent. The values are means of three replicates with standard deviations (mean ± SD).

The GCMS analysis revealed 16 identified compounds. Out of these compounds, 6 were selected based on their reported biological activities and area %. These compounds were *Acetic acid, ethoxy-, 1-methylethyl ester*, *Ethane, 1,1-diethoxy-*; *10-(Tetrahydro-pyran-2-yloxy)-tricyclo[4.2.1.1(2,5)]decan-9-ol*; *Diisooctyl phthalate*; *1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-*, *[S-(E,Z,E,E)]-* and *(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene*. Table 2 summarises the compounds along with their molecular formula, molecular weight, retention time, area %, and reported biological properties. The 5th and 6th compounds in the GCMS analysis represent diterpenoids like Cembrene and Neocembrene respectively.

Table 2
Major compounds identified in GC-MS analysis

Peak #	Compound name	Molecular Formula	Molecular weight (g/mol)	Retention time (min)	Area%	Reported biological activities
1	Acetic acid, ethoxy-, 1-methylethyl ester	$C_7H_{14}O_3$	146	3.033	3.15	–
2	Ethane, 1,1-diethoxy-	$C_6H_{14}O_2$	118	4.339	51.76	o Antioxidant and antimicrobial activity [25]. o Antimicrobial activity [26].
3	10-(Tetrahydro-pyran-2-yloxy)-tricyclo[4.2.1.1(2,5)]decan-9-ol	$C_{15}H_{24}O_3$	252.35	17.515	0.32	–
4	Diisooctyl phthalate	$C_{24}H_{38}O_4$	390	20.539	0.73	o Proliferation of primary osteoblasts [27]. o Antioxidant and immunopromoting activity [28]. o Antimicrobial activity [29]. o Antioxidant and free radical scavenging activity [30].
5	1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	$C_{20}H_{32}$	272.5	23.164	1.08	o Also known as Cembrene. o Estrogenic and antioxidant activity [31]. o Antidiabetic [32]. o Anticholinesterase activity [33].
6	(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene	$C_{20}H_{32}$	272.5	23.584	0.77	o Also known as Neocembrene or Cembrene A. o Cyclooxygenase enzyme inhibition and antiproliferative activity [34]. o Apoptotic activity [35].

Evaluation of antioxidant activity (DPPH radical scavenging assay)

The % DPPH radical scavenging activity of the extract in comparison to the standard used (ascorbic acid) is depicted in Fig. 2. The % radical scavenging activity of the extract ranged between 4.73 ± 0.83% and 41.95 ± 4.02%. Also, it is evident from the graph that with increasing concentration of the extract (range: 1.2 to 9.6 mg/ml) the value for % scavenging activity was also increased. Hence, it can be noted that the extract can scavenge free radicals effectively at higher concentrations.

Antidiabetic activity evaluation: In silico

The bioavailability radar plots depicting the drug-likeliness of the GCMS-identified compounds are shown in Fig. 3. The pink area in the bioavailability radar represents the optimal level of each of the six physiochemical properties considered. In this study, out of six identified compounds, three compounds, *viz. Acetic acid, ethoxy-, 1-methylethyl ester, Ethane, 1,1-diethoxy-* and *10-(Tetrahydro-pyran-2-yloxy)-tricyclo[4.2.1.1(2,5)]decan-9-ol*, showed good bioavailability as the values of all its six physiochemical properties are included in the pink area of bioavailability radar. The other two compounds, 1,3,6,10-Cyclotetradecatetraene,3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]- and (R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene showed slight out of range value of lipophilicity. For all six compounds, the molecular weight, TPSA value, and saturation levels were in the desired range.

The results of the molecular docking analysis are summarized in Table 3. The standard (acarbose) showed a binding energy (BE) of -6.02 kcal/mol. While the 6 major GCMS-identified compounds (*Ligand 1–6*) showed BE in the range of -4.46 to -7 kcal/mol. The post-docking analysis also revealed that out of 6 identified compounds in GCMS, 3 compounds (*viz. Ligand 4*: Diisooctyl phthalate; *Ligand 5*: 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-,[S-(E,Z,E,E)]- and *Ligand 6*: (R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl) cyclotetradeca-1,5,9-triene) have shown more negative BE as compared to the control (i.e. acarbose). The BEs of *Ligands 1,2 and 3* were slightly less negative than the control in the range of -5.98 to -4.46 kcal/mol. Various types of bond formation (*viz. hydrogen and hydrophobic*) are shown in Fig. 4 (for acarbose) and Figs. 5 & 6 (for ligands 1–6). The standard has shown 4 H-bonds and 1 hydrophobic bond. Maximum 3 H-bonds and 7 hydrophobic bonds were present in the case of ligands.

Table 3
Molecular docking results of human pancreatic α-amylase (HPA) with various phytochemicals of aqua-ethanolic extract of Guggul gum identified in GC-MS analysis

Ligand name	Ligand bound with alpha-amylase	PubChem Compound CIDs	Binding energy (kcal/mol)	Reference RMSD (Å)	Hydrogen-bond interaction	Hydrophobic bonds
Control	Acarbose	41774	-6.02	46.61	ASP:300, HIS:305, LYS:200; **Carbon hydrogen-** GLY:306	**Alkyl-** ILE:235
Ligand 1	Acetic acid, ethoxy-, 1-methylethyl ester	547730	-5.71	49.66	LYS:200, HIS:201; **Carbon hydrogen-** ASP: 197	**π-alkyl-** HIS:201, TYR: 151; **Alkyl-** LYS:200, ILE:235
Ligand 2	Ethane, 1,1-diethoxy-	7765	-4.46	49.61	LYS:200	**π-alkyl-** HIS:201, TYR:151; **Alkyl-** LYS:200, ILE:235
Ligand 3	10-(Tetrahydro-pyran-2-yloxy)-tricyclo[4.2.1.1(2,5)]decan-9-ol	559088	-5.98	49.81	GLN:63, ASP:300	**π-alkyl-** HIS:101, TRP: 59; **Alkyl-** LEU: 162, LEU: 165, TYR: 62
Ligand 4	Diisooctyl phthalate	33934	-6.06	45.40	LYS:200, HIS:201	**π-alkyl-** ILE:235, TYR:62; **Alkyl-** LEU:162, ALA:198, HIS:299; **π-sigma-** TYR:62, ILE:235; **π-cation-** GLU:233; **π-anion-** HIS:201
Ligand 5	1,3,6,10-Cyclotetradecatetraene,3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-	6436662	-6.89	46.69	N/A	**π-alkyl-** TRP:59, HIS:299, LEU:165, HIS:305; **Alkyl-** LEU:165; **π-sigma-** TYR:62

Ligand name	Ligand bound with alpha-amylase	PubChem Compound CIDs	Binding energy (kcal/mol)	Reference RMSD (Å)	Hydrogen-bond interaction	Hydrophobic bonds
Ligand 6	(R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene	5281384	-7.00	47.03	N/A	**π-alkyl-** LEU:162, LEU:165; **Alkyl-** LEU:165; **π-sigma-** TRP:59

Antidiabetic activity evaluation: In vitro

To support the *in silico* studies, the *in vitro* assay was performed. The % inhibition of the reference drug (acarbose) and the extract against α-amylase were calculated and listed in Table 4. At a concentration of 0.8 mg/ml, the % inhibition of the extract was 16.20 ± 1.44%. This %inhibition rose to 76.40 ± 0.92% at a concentration of 3.2 mg/ml and then it declined. A similar trend was observed in the case of when the standard (acarbose) was used. The IC_{50} values were also calculated for both acarbose and the extract which were 3.69 ± 0.89 mg/ml and 4.17 ± 1.26 mg/ml, respectively. So, the assay revealed that, with increasing concentration, the % inhibition value also increased. However, a sharp decline was observed at higher concentrations.

Table 4
Antidiabetic activity evaluation of the extract: *In-vitro* α-amylase inhibitory activity using Di-nitro salicylic (DNSA) Method

S. No.	Concentration (mg/ml)	% Inhibition (Acarbose)	% Inhibition (Extract)
1.	0.8	24.62 ± 1.16	16.20 ± 1.44
2.	1.6	31.44 ± 0.89	46.70 ± 1.09
3.	3.2	76.84 ± 0.29	76.40 ± 0.92
4.	4.8	71.06 ± 0.41	56.81 ± 0.95
5.	6.4	49.65 ± 0.91	45.03 ± 1.58
IC_{50}		**3.69 ± 0.89 mg/ml**	**4.17 ± 1.26 mg/ml**

The values are means of three replicates with standard deviations (mean ± SD).

Discussion

The quest for a compound that demonstrates superior antidiabetic and antioxidant properties compared to traditional allopathic medications continues to pose a formidable challenge. This brings to light the role of polyphenolic compounds that have been known to manage free radical-associated diseases, like diabetes, efficiently due to the presence of hydroxylated groups that scavenge ROS by chelating metal ions [36]. Polyphenols (flavonoids and phenolic compounds) are crucial secondary metabolites of plants and a good source of natural antioxidants. The antioxidants can scavenge ROS and protect the body against free-radical-mediated diseases like diabetes mellitus (DM) [37, 38]. The guggul gum extract used in the present study has shown the presence of ample amounts of such compounds that have been reported to show antioxidant and antidiabetic activities. The FTIR study suggested the presence of alcohol, alkanes, amines, nitro groups, alkyl halides, and aromatic functional groups in

the aqua-ethanolic extract of guggul. The analysis has also shown the presence of aromatic compounds (1456.60 cm^{-1}) and benzene derivates (709.04 cm^{-1}) in the extract. Usually, all polyphenolic compounds have a typical chemical structure in which an aromatic ring with various substitutes is present. Also, the concentration of these compounds per gram of the crude extract is quite high as reported in the current study using TPC and TFC values.

The GCMS analysis further confirmed the presence of different phytoconstituents in the aqua-ethanolic extract of guggul gum. This powerful separation (GC) and identification (MS) technique was used to identify phytochemical compounds that might have conferred the extract with antioxidant and antidiabetic activities. Cembrene has been reported to show antidiabetic activity and both of these compounds could even suppress the activity of the cholate-activated rate of human pancreatic IB phospholipase A2 (PLA2) which in turn is responsible for the absorption of fat and cholesterol in the gastrointestinal tract [39]. Hence, this extract also proves to be beneficial in controlling lipid abnormalities associated with diabetes. Hyperglycemia leads to glucose autoxidation and protein glycosylation. These traits play a pivotal role in elevating free radicals' levels, leading the body into oxidative stress. Nitric oxide and other free radicals can easily damage the pancreatic beta-cells leading to inflammation [40, 41]. The plant extracts with polyphenolic contents have the potential to scavenge free radicals and thus exhibit a protective effect on the pancreatic tissues [42]. The qualitative and quantitative phytochemical analysis of the extract has already shown ample presence of phenols and flavonoids depicting antioxidant properties and thus can be used as a viable option in the reversal of ROS-induced pancreatic cell damage. These results were further corroborated by the DPPH radical scavenging activity of the extract where at higher concentrations, the extract successfully scavenged free radicals. The scavenging activity of the extract could be attributed to the GCMS-identified compounds like Ethane, 1,1-diethoxy- and Diisoctyl phthalate that have already been reported to show strong antioxidant activity. Flavonoids showing antioxidant properties are suggested to play a pivotal role in DM management. These compounds could minimize hyperglycemia's deleterious effects and enhance depleted glucose metabolism [43, 44].

The concept of drug-likeliness is an important consideration in the early phases of drug design to select compounds with desirable bioavailability. Drug-likeliness defines the similarity between a drug-like compound and the drug itself. The more drug-likeliness, the more chance of any compound being transformed into a drug [45]. As most of the compounds have shown higher drug-likeliness properties, the extract could be used as an alternative and valuable source of anti-diabetic drugs. The bioavailability radar of the compounds shows that they have optimal values for most of the physiochemical properties and hence can readily reach their intended biological destinations.

As already stated, α-amylase is an enzyme that aids digestion by breaking starch into maltose and glucose, thus increasing the concentration of these biomolecules in blood. The enzyme also causes post-prandial elevation of glucose levels making it an acceptable therapeutic target for DM management [5]. Acarbose is a known oral antidiabetic drug and inhibitor of this enzyme. In the present study, for the evaluation of the antidiabetic activity of the extract, the inhibitory activity of the GCMS-identified compounds was compared to that of acarbose. For this, molecular docking was chosen to predict the binding efficiency of the ligands. The more negative the value of the BE of a ligand, the more its affinity towards the enzyme and hence better inhibition [46]. In the present study, the ligands either showed more negative or slightly less negative BE than the control depicting its enhanced affinity towards HPA. The X-ray crystal structure of HPA (PDB ID:1HNY) and previously reported kinetic studies have revealed that the active sites of this enzyme had ASP:197, GLU: 233, and ASP: 300 as the major amino acid residues [20]. In another co-crystallisation experiment of HPA with carbohydrate inhibitor acarbose (PDB ID: 1B2Y) several H-bonds between HPA and acarbose in the active site of the enzyme were shown. The prime amino acid residues involved in the binding of acarbose with HPA were THR:163, GLN:63, HIS:305, TRP:59, ARG: 195, HIS: 299, ASP: 300, HIS: 101, ASP: 197, HIS: 201, GLU: 233, LYS: 200 and GLY: 306. These experimental studies also revealed that when starch is

hydrolyzed by HPA, then ASP:197 shows a nucleophilic reaction, GLU: 233 acts in acid–base catalysis and ASP:300 is the key residue involved in the optimization of the orientation of the substrate [47]. In the present study also, the acarbose and ligands 1–6 (compounds of the guggul extract) have shown similar kind of interactions with the enzyme through H-bonds and other hydrophobic bonds (*viz.* π-*sigma*, alkyl, π-alkyl, and π-π-stacked). The major amino acid residues that were involved in forming bonds with acarbose were ASP:300, HIS:305, LYS:200, GLY:306, and ILE: 235 (Fig. 4). This result is consistent with previous molecular docking studies where acarbose has shown similar interactions when docked with 1HNY [48, 49]. The primary amino acids of the enzyme that participated in forming H-bonds with the ligands were ASP:300, ASP: 197, HIS:201, GLY: 306, and GLN:63 (Figs. 5 & 6**)**. In Ligand 4, two H-bonds were present involving residues LYS:200 and HIS: 201. Apart from the conventional H-bonds, the higher BE could also be attributed to the hydrophobic bonds formed by the ligands with HPA which ranged from 4–9 in all the ligands showing more negative BE than acarbose. In the ligands 5 and 6 (i.e., Cembrene and Neocembrene), there were no H-bonds present but many π -alkyl, π -sigma, and alkyl interactions were involved. These bonds largely involve charge transfer and thus aid in the firm intercalation of the drug at the binding site of the enzyme [50].

The *in vitro* antidiabetic analysis further substantiated the *in silico* studies' results. The results confirmed that the extract at a higher concentration was not able to inhibit the enzyme, thus delimiting its antidiabetic potential at a concentration higher than 3.2 mg/ml. The results of this assay also revealed that in comparison to the standard commercial drug (i.e., acarbose) the extract can also inhibit half of the maximum biological activity of the enzyme at a slightly higher concentration. With inhibition of α-amylase, there would be less conversion of starch into glucose, and thus a controlled post-prandial glucose level would be maintained.

Therefore, based on the aforementioned findings, it can be inferred that the extract has shown the presence of polyphenolic compounds which might have conferred antioxidant and antidiabetic properties to the extract. Due to this, the extract has even shown the potential to inhibit the activity of a common diabetes target, α-amylase. The rising concerns of high prices, significant hypoglycemia, and weight gain associated with commercial antidiabetic drugs are making them a less favorable choice among diabetic patients. However, the complete dependency of a diabetic patient on herbal supplements cannot be achieved. This compels them to concomitantly consume the herbal supplements with other antidiabetic drugs leading to pharmacodynamic and pharmacokinetic interactions. So, although the extract has shown potential antidiabetic activity at an *in silico* and *in vitro* level there is a need to evaluate its performance in animal models and further at the clinical level.

Conclusion

In the present study, the presence of ample amounts of phenolic (5.14 ± 0.011 mg) and flavonoid (0.66 ± 0.023 mg) compounds suggests that the extract could be used in combating the oxidative stress induced due to T2DM. Out of 6 GCMS-identified compounds, 3 have even shown optimal values of LIPO, FLEX, INSATU, INSOLU, POLAR, and SIZE, indicating their high drug likeliness. The extract has even shown credible antidiabetic effects by inhibiting the pancreatic α-amylase enzyme both at *in-silico* and *in vitro* levels. At the *in silico* level, out of 6 compounds, three showed BE more than the standard drug acarbose indicating better inhibition of HPA with significant amounts of hydrogen and hydrophobic bonds. This was further supported by *in vitro* analysis where the extract was able to inhibit half of the maximum biological activity of the enzyme at a slightly higher concentration than the standard drug (acarbose) (IC_{50} value of 4.17 mg/ml and 3.69 mg/ml respectively). Thus, it could be concluded that the extract can be used by diabetic patients as a good alternative to the available commercial drugs in T2DM management. However, precautions are mandatory as the phytochemicals of the extract can alter the pharmacokinetics and pharmacodynamics of other concomitantly administered antidiabetic drugs/agents. Further, comprehensive

research could also be done to isolate and differentiate the active compounds of the extract and explore its antidiabetic properties at an *in vivo* level.

Abbreviations

GCMS: Gas Chromatography-Mass Spectroscopy

α-amylase: alpha-amylase

PDB ID: Protein Data Bank Identification

mg: milligram

ml: milliliter

IC_{50}: Half maximal inhibitory concentration

LIPO: Lipophility

FLEX: Flexibility

INSATU: Insaturation

INSOLU: Insolubility

POLAR: Polarity

BE: Binding energy

T2DM: Type 2 Diabetes Mellitus

PPHG: Post-prandial hyperglycemia

PPA: Porcine Pancreatic -amylase

DPPH: 2,2-diphenyl-1-picrylhydrazyl

DNSA: 3,5 Di-nitro salicylic acid

µl: microliter

^{0}C: degree Celsius

min: minute/s

kPa: kilopascal

m/z: mass to charge ratio

NIST: National Institute Standard and Technology

FC: Folin-Ciocalteu

TPC: Total Phenolic Content

M: Molar

Nm: nanometre

UV-vis: Ultra violet-visible

TFC: Total Flavonoid Content

%: percentage

$AlCl_3$: Aluminium chloride

ROS: Reactive oxygen species

mM: millimolar

Abs: absorbance

mol: Mole

TPSA: Total polar surface area

$Å^2$: square angstroms

HPA: Human pancreatic α amylase

YASARA: Yet another scientific artificial reality application

Å: Angstroms

Kcal/mol: kilocalories per mole

mmol: millimoles

pH: potential of hydrogen

v/v: volume by volume

GAE: Gallic acid equivalent

g: grams

QE: Quercetin equivalent

H-bonds: Hydrogen bonds

PLA2: phospholipase A2

ASP: Aspartic acid

GLU: Glutamic acid

THR: Threonine

GLN: Glutamine

HIS: Histidine

TRP: Tryptophan

ARG: Arginine

LYS: Lysine

GLY: Glycine

lLE: Isoleucine

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and material

The manuscript has no associated data. All the data has been included in the published article.

Competing interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Authors' contributions

SJ: Compilation, Writing: Original draft preparation, Methodology, Investigation, Data Collection; **SC**: Writing: Review & Editing, Supervision, Conceptualization, Validation; **NG & MKS**: Validation, Writing: Review & Editing

Funding

We acknowledge the financial support from the Council of Scientific and Industrial Research (CSIR), New Delhi, India to Ms. Shalini Jain (CSIR-SRF (NET) File No. 09/1180(0003)/2019-EMR-I).

Acknowledgments

The infrastructural financial support under the CURIE programme from the WISE-KIRAN division of the Department of Science and Technology, New Delhi, India to IIS (deemed to be University), Jaipur, India (File No. DST/CURIE-02/2023/IISU) is gratefully acknowledged.

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Figures

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Figure 1

FTIR spectrum of guggul gum extract (*Commiphora wightii*) showing the presence of various functional groups.

Image not available with this version

Figure 2

DPPH radical scavenging activity of guggul gum extract compared with ascorbic acid (standard antioxidant) in terms of free radical percentage inhibition. Results are expressed in Mean ± SD (n=3).

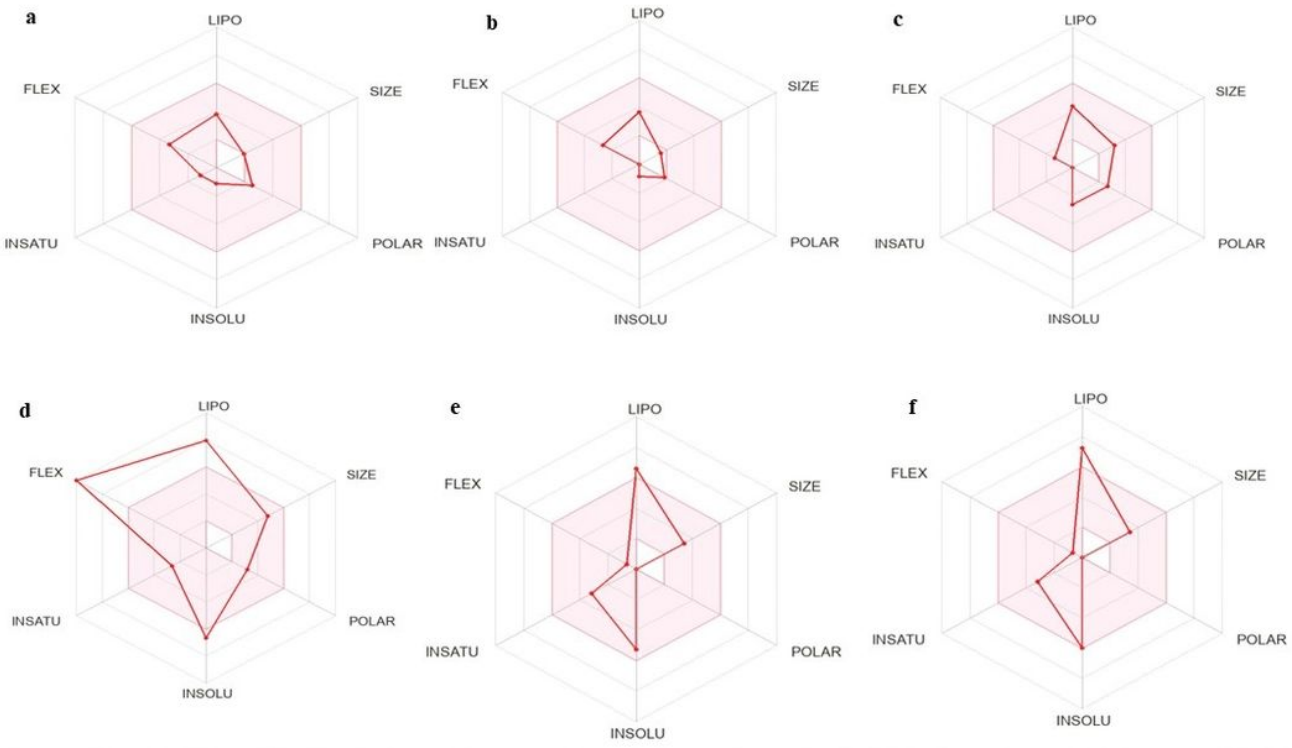

Figure 3

Bioavailability radar plots depicting drug-likeliness of the compounds: **a.** Acetic acid, ethoxy-, 1-methylethyl ester, **b.** Ethane, 1,1-diethoxy-, **c.** 10-(Tetrahydro-pyran-2-yloxy)-tricyclo[4.2.1.1(2,5)]decan-9-ol, **d.** Diisooctyl phthalate, **e.**

1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-, **f.** (R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene. The colored zone is the suitable physicochemical space for oral bioavailability. Here LIPO: Lipophility; FLEX: Flexibility; INSATU: Insaturation; INSOLU: Insolubility; POLAR: Polarity.

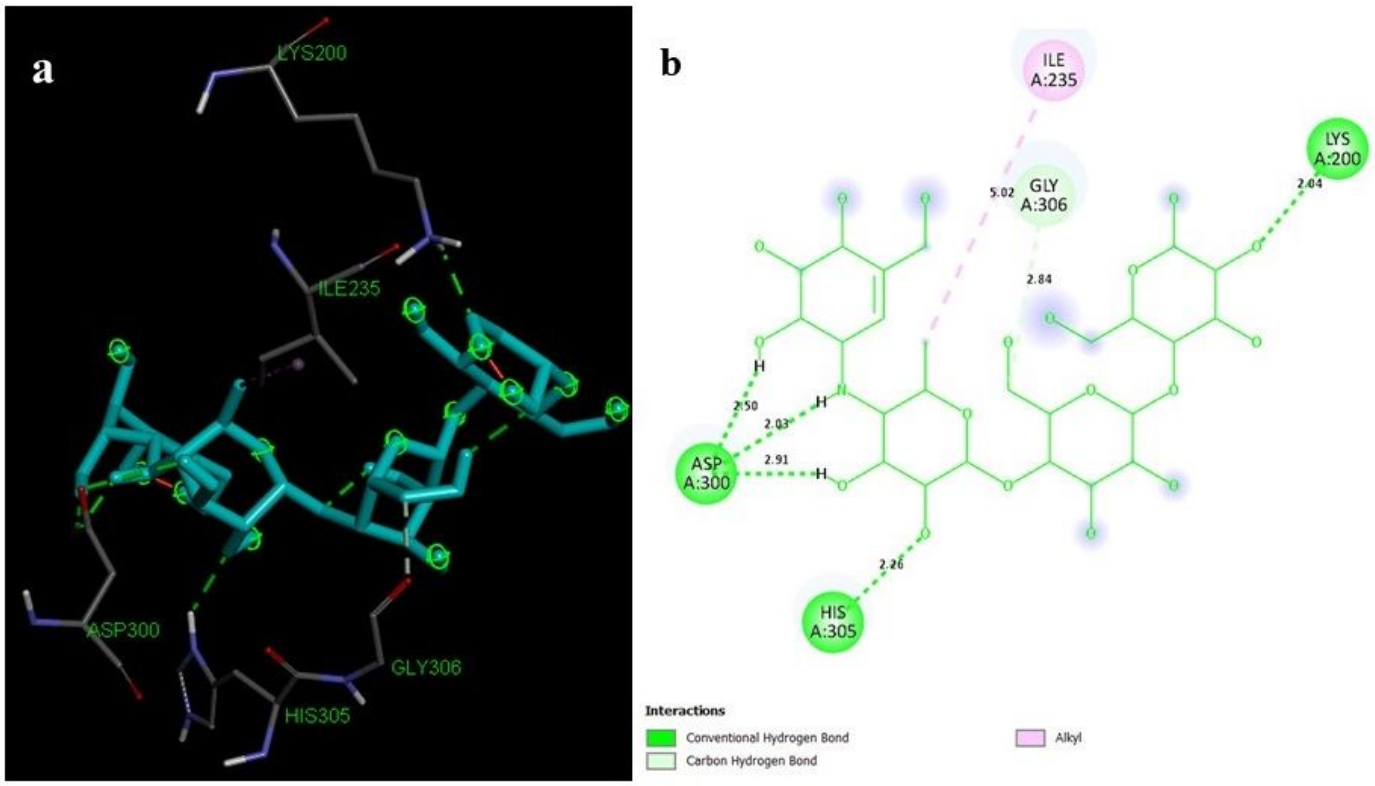

Figure 4

Structure of Human pancreatic α-amylase (1HNY) docked to the active sites of a known inhibitor: Acarbose (Control) **a.)** 3D structure **b.)** 2D structure. Green dotted lines show the conventional H-bonds and pink-dotted lines show the alkyl-bond. Images are retrieved from Discovery Studio 2021 (version v21.1.0.20298).

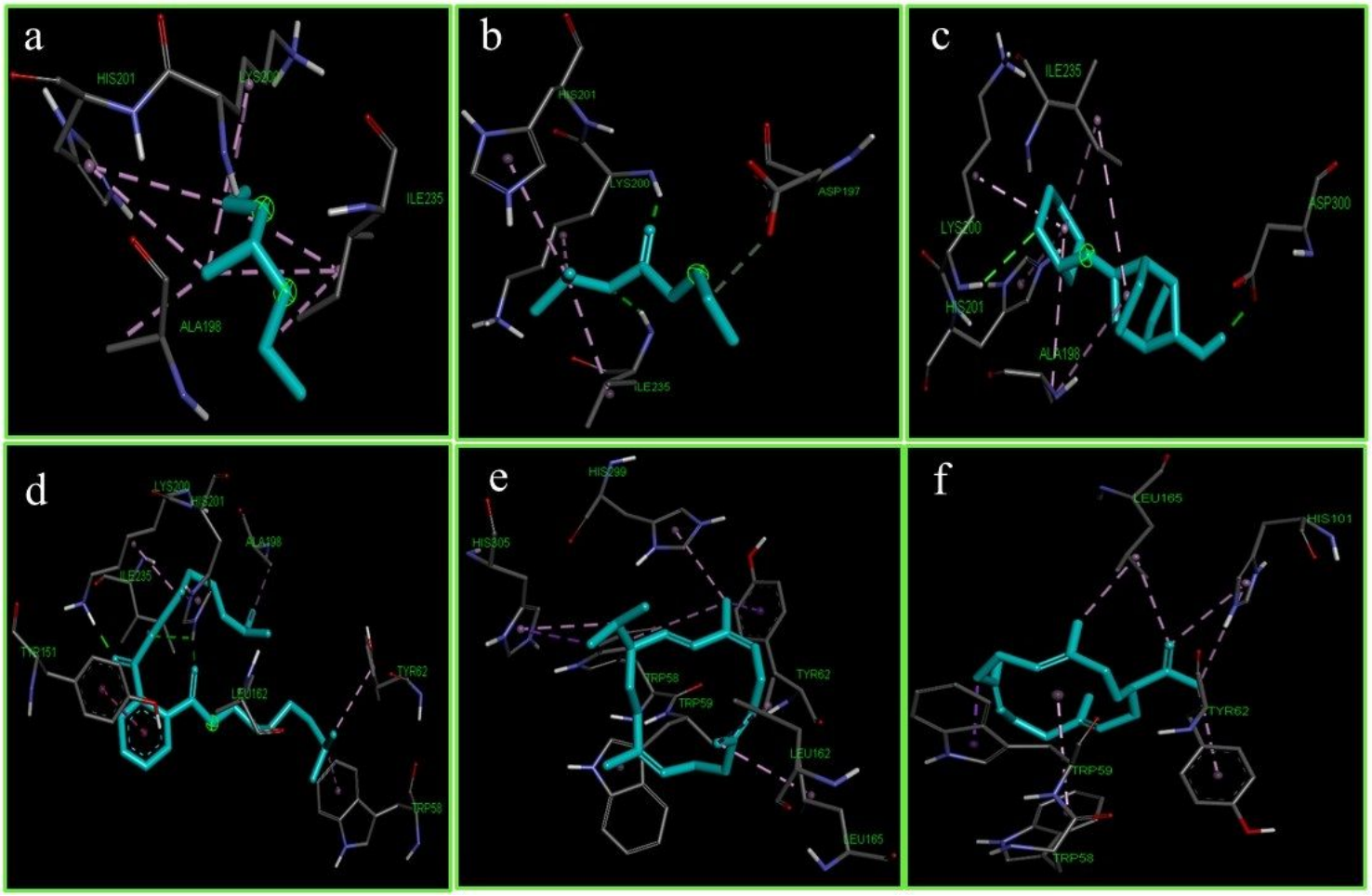

Figure 5

3D structure of Human pancreatic α-amylase (PDB ID:1HNY) docked to various ligands (in cyan color): **a. Ligand 1:** Acetic acid, ethoxy-, 1-methylethyl ester, **b. Ligand 2:** Ethane, 1,1-diethoxy-, **c. Ligand 3:** 10-(Tetrahydro-pyran-2-yloxy)-tricyclo[4.2.1.1(2,5)]decan-9-ol, **d. Ligand 4:** Diisooctyl phthalate, **e. Ligand 5:** 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-, **f. Ligand 6:** (R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene. Images are retrieved from Discovery Studio 2021 (version v21.1.0.20298).

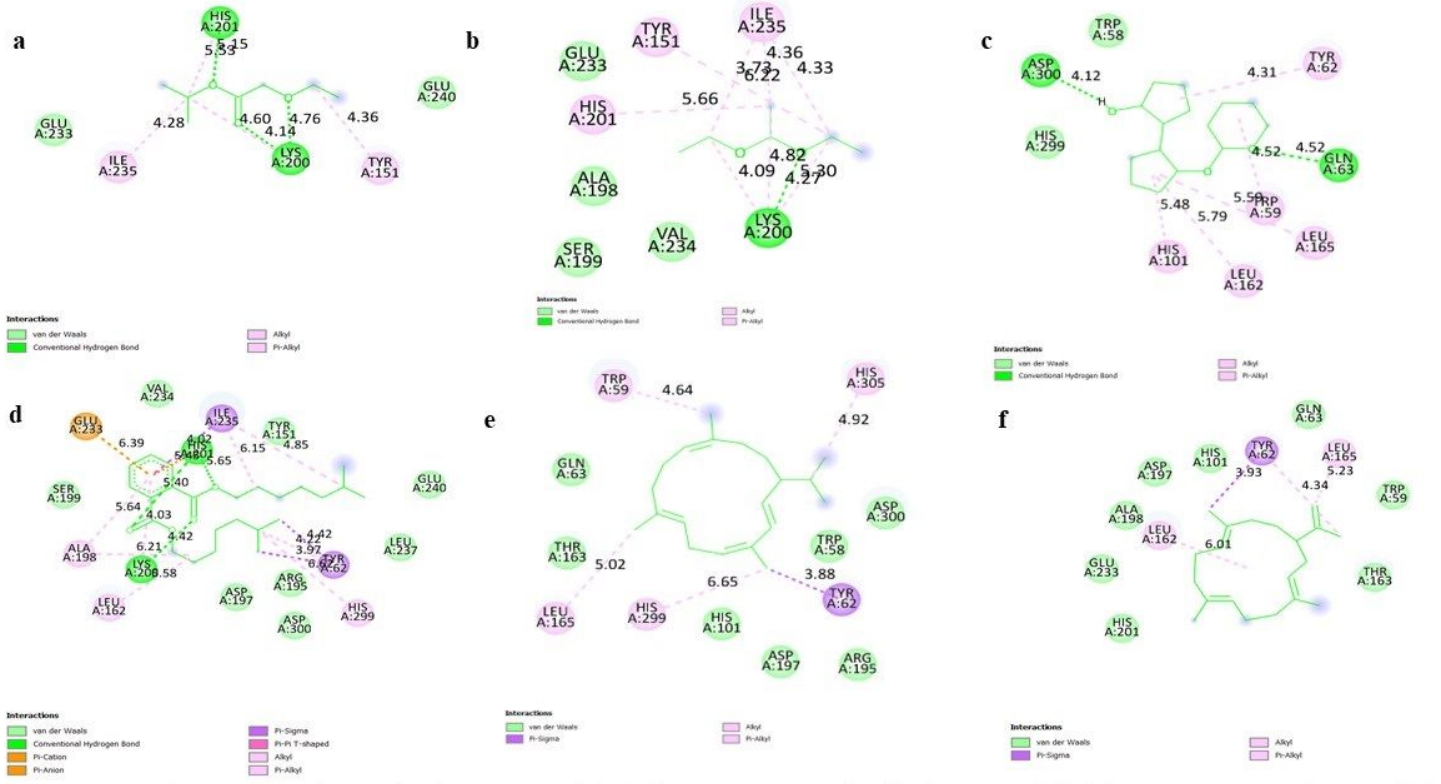

Figure 6

2D structure of Human pancreatic α-amylase (PDB ID: 1HNY) docked to: **a. Ligand 1:**Acetic acid, ethoxy-, 1-methylethyl ester, **b. Ligand 2:** Ethane, 1,1-diethoxy-, **c. Ligand 3:** 10-(Tetrahydro-pyran-2-yloxy)-tricyclo[4.2.1.1(2,5)]decan-9-ol, **d. Ligand 4:** Diisooctyl phthalate, **e. Ligand 5:** 1,3,6,10-Cyclotetradecatetraene, 3,7,11-trimethyl-14-(1-methylethyl)-, [S-(E,Z,E,E)]-, **f. Ligand 6:** (R,1E,5E,9E)-1,5,9-Trimethyl-12-(prop-1-en-2-yl)cyclotetradeca-1,5,9-triene. Images are retrieved from Discovery Studio 2021 (version v21.1.0.20298). Green dotted lines show the conventional H-bonds, pink-dotted lines show the alkyl-bond and the lavender dotted lines depict the π-σ bonds.