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In Silico Validation Studies of Cyanobacterial Bioactive Compounds Against A-amylase and A-glucosidase Markers in Type 2 Diabetes Mellitus

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Abstract:

Aim: The study aims to assess the binding efficiency of cyanobacterial compounds against key Type 2 Diabetes Mellitus (T2DM) targets, α-amylase and α-glucosidase, using an in-silico approach. Additionally, it aims to design drugs with minimal adverse effects or no toxicity to inhibit the complications and help in the management of T2DM.

Methods: Twenty-five (25) cyanobacterial bioactive compounds were sourced from various cyanobacterial strains *via* the PubChem database. The three-dimensional structures of the target proteins, α-amylase (1KB3) and α-glucosidase (1QOX) were obtained from RCSB PDB and visualized using Discovery Studio Visualizer 3.0. Molecular docking was performed using AutoDock 4.2 and Cygwin.

Results: Studies revealed that Ethyl tumonoate A, Debromoaplysiatoxin, and Scytoscalalrol exhibited higher binding interactions with α-amylase (1KB3), while Ambiguine I Isonitrile, Scytoscalalrol, and Cylindrospermopsin displayed higher binding affinities with α-glucosidase (1QOX) among the tested cyanobacterial bioactive compounds. These compounds exhibited greater binding affinities compared to synthetic drugs like metformin (-7.66 kcal/mol) and acarbose (-8.86 kcal/mol).

Conclusion: Our findings suggest that cyanobacterial bioactive compounds, particularly Ethyl tumonoate A, Ambiguine I Isonitrile, Cylindrospermopsin, and Scytoscalalrol, possess potential binding affinities with T2DM-related targets, making them promising lead compounds for the development of novel drugs with fewer side effects for the management of T2DM and its associated complications.

Keywords: T2DM, Diabetes markers, Cyanobacterial bioactive compounds, Bioinformatics, Molecular docking, α-amylase, α-glucosidase.

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1. INTRODUCTION

A chronic condition of the metabolism of carbohydrates, lipids, and proteins known as diabetes mellitus (DM) is characterized by elevated postprandial and fasting blood glucose levels (hyperglycemia). Diabetes mellitus is caused by either impaired insulin action or insufficient insulin production [1]. Abnormal metabolism of lipids and proteins is a key provider to illness and death in many developing and developed countries, including China, India United States and several other countries. Diabetes mellitus has significant statistical implications. According to current data published by the World Health Organization (WHO) up to March 2013, the incidence of diabetes cases has increased from 153 million patients in 1980 to 347 million persons globally with diabetes mellitus in 2008. (DM). Furthermore, it is estimated that by 2025, this number will have risen to 380 million, accounting for

7.1 percent of the world's adult population [2] and it is supposed to be 500 million up to 2030 [3]. The selection of proper preventive measures is necessary to reduce the disease burden on the health and economy. Although several synthetic drugs exist for the treatment of diabetes mellitus, none of these medications are low-priced or completely effective. Furthermore, prolonged use of these drugs may produce undesirable side effects [4]. As a result, diabetes mellitus is a serious public well-being issue that affects millions of people in high, middle and low-income nations [5], and abnormal metabolism of proteins and lipids is a major contributor to morbidity and mortality in many developed and developing countries, including the United States and India. The insulin functions, including secretion, site of action and action, when altered or defected, are the principal factors responsible for diabetes. The severity of diabetes is connected with the consecutive destruction of microstructures, mainly the retina, nephron, and neurons, affecting eyes, kidneys and nerves. The disease is also associated with several cardiovascular diseases and related complications [6, 7]. Apart from that, oxidative stress also plays a vital role in the development of diabetes mellitus and its complications [4]. The selection of proper preventive measures is necessary to reduce the disease burden on the health and economy. One of the main salivary proteins, alpha-amylase, has been suggested as a sensitive, non-invasive biomarker whose primary function is the enzymatic digestion of carbohydrates by the hydrolysis of starch to sugar and maltose [8]. Treatment for abnormalities in carbohydrate uptake, including diabetes, obesity, dental caries, and periodontal illnesses, includes inhibition of the enzyme α-amylase, which is involved in the digestion of starch and glycogen. It serves as a marker for the diagnosis of diabetes mellitus when it is found to be significantly higher in diabetics than in non-diabetics, leading to excessive glucose production. A few studies have also suggested that reducing the absorption of complex carbs while using a salivary α-amylase inhibitor may help manage blood sugar levels [8]. Therefore, there is a need for additional research to support the earlier results. Thus, there is a need for more studies supporting the previous studies. Alpha-glucosidase was 1.5 times overexpressed in non-insulin-dependent diabetes mellitus patients, which increased postprandial glucose levels, according to reports [9]. The body absorbs monosaccharides and polysaccharides produced by α-amylase and α-glucosidase at varying rates, with monosaccharide components being absorbed very rapidly. It follows that inhibiting α-amylase and α-glucosidase action can delay the release of glucose from complex carbs, modifying the start of postprandial hyperglycemia, making it an excellent target for the treatment or management of type 2 diabetes mellitus [10]. Acarbose, a pseudo-carbohydrate derived from *actinomycetes*, is the α-glucosidase inhibitor that is most frequently administered [11]. In the present years, a quantity of bioactive compounds and their analogues from cyanobacteria with promising antibacterial, antiviral, anticancer, antifungal, antioxidant, anti-inflammatory, antidiabetic and anthel-

mintic potentials have been explored [12].

Novel development for medications in all major disease categories can be found in natural resources, which constitute a significant source. Recently, the main research focus has been on generating novel medications using cyanobacteria [13]. They are present throughout the world, from the Antarctic to hot springs, deserts, soils, salt lakes, and brine water [14]. They serve as models for photosynthetic prokaryotes for the study of numerous metabolites produced by these organisms under such conditions due to their capacity to develop in a variety of ecological conditions, including light, temperature, salinity, alkalinity, and pollution [15-17]. According to studies [18, 19], the biologically active chemicals extracted from cyanobacteria exhibit antibacterial, antiviral, antifungal, enzyme inhibitory, immune-stimulant, cytotoxic, and anti-plasmodium effects. Additionally, cyanobacteria are a good source of additional vital compounds such as vitamins, amino acids, and fatty acids [20]. Biochemically active chemicals have been produced by cyanobacteria like *Microcystis, Anabaena, Nostoc, Spirulina sp.* and, *Oscillatoriaetc* [21]. The secondary metabolites produced by cyanobacteria are novel bioactive constituents used in the production of pharmaceuticals and vital chemicals for agriculture and various fields. It has been discovered that cyanobacterial lipopeptides are intriguing, biochemically active constituents. An enormous 85% of them are bioactive, including cytotoxic (41%), anticancerous (13%), antibiotics (12%), enzyme inhibitors (8%), antiviral (4%) and antifungal (4%) among their prominent properties [22]. The outstanding activity (18%) includes UV-absorbing properties that can be usable as sunscreens, herbicides, antimalarial, antimycotic, antimitotic, anti microalgal and activity that promotes cell differentiation [22].

Through the use of an *in-silico* approach, it is possible to identify lead compounds with the best binding energies and affinities for further study. These methods offer insightful information on the molecular interactions between bioactive constituents and target enzymes [23]. This study aims to investigate the prominent role of bioactive compounds derived from cyanobacteria in the treatment of type 2 diabetes mellitus, concentrating on how they interact with the enzymes α-amylase and α-glucosidase. The use of *In-silico* methods is to examine the affinities and binding energies of the aforementioned cyanobacterial bioactive compounds to the active sites of these enzymes. The research will clarify the inhibitory capability of compounds from cyanobacteria and their ability to control glucose release and carbohydrate digestion, helping to manage type 2 diabetes mellitus [24].

2. MATERIALS AND METHODS

2.1. Software and Tools

The recent study was executed by using bioinformatics tools and software like Auto Dock 4.2, Cygwin, and Discovery Studio 3.0.Operating systems that support 64-bit Windows 10 and 32-bit Windows 7 include biological databases like PubChem (http://pubchem.ncbi.nlm.nih.

gov/), Protein Data Bank (www.rcsb.org/), Molsoft (https://www.molsoft.com/) and Pre ADMET (https://preadmet.webservice. bmdrc.org/).

2.2. Retrieval and Preparation of Targeted Protein Structures

The main source for proteins having experimentally verified structures is the RCSB PDB (Research Collaboratory for Structural Bioinformatics, Protein Data Bank). It contains a large number of 3-dimensional structures that were discovered by techniques like NMR,

X-ray crystallography, *etc*. The PDB was used to retrieve the targeted crystal structure of the proteins PDB ID: 1Kb3 (α- amylase) and 1qOX (α-glucosidase) shown in Figs. (**1** and **2**) from RCSB PDB (https://www.rcsb.org/.). The required hydrogen atoms were added after all water molecules, and crystallographic substructures from the specified protein were taken out to nullify the overall charges for molecular docking purposes. Furthermore, Discovery Studio Visualizer 3.0 was used to visualize the final structure, which shows the 3-D structure of selected proteins.
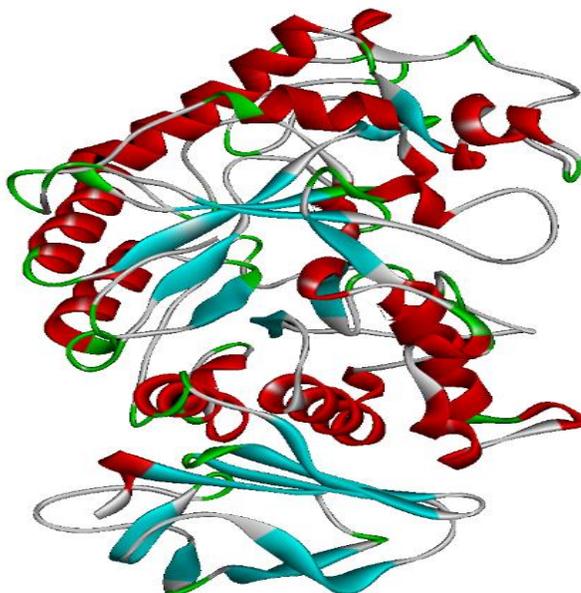
Fig. (1). 3-D structure of α- amylase (PDB ID: 1Kb3).
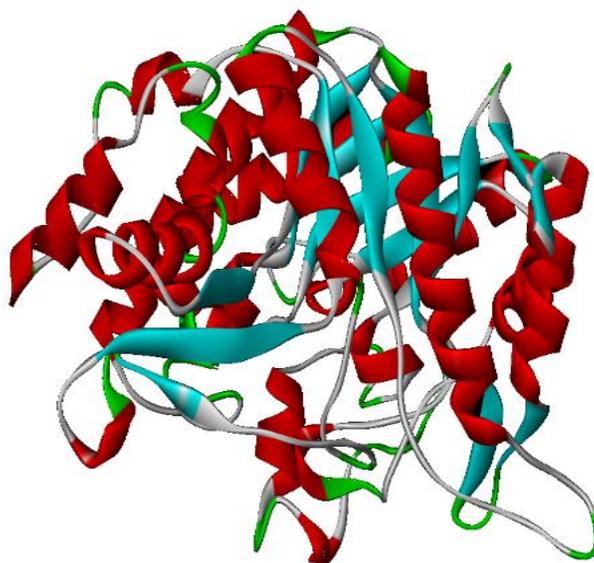
Fig. (2). 3-D structure of α-glucosidase (PDB ID: 1qOX).

2.3. Ligand Library Preparation

All filtered derivatives that were retrieved in 3D sdf format from the PubChem database are included in the compound library. The library includes several hundred compounds that have been screened using Lipinski's rule of five and ADME filters. The drug molecules or chemical compounds' absorption, distribution, metabolism, excretion, and toxicity inside the animal body are represented by the ADME and toxicity profile. The blood-brain barrier, CaCo2, and AMES Toxicity are among the characteristics that make up the ADME profile. The network of blood arteries and tissues known as the blood-brain barrier protects the brain from hazardous constituents. The drug's enormous concentration will make it extremely difficult and even impossible for the human body to survive if it crosses the BBB. Human intestinal absorption (HIA) is a characteristic that shows that medications taken orally are absorbed into the bloodstream through the gastrointestinal tract. For a medication to be therapeutically effective inside the human body, it must have an HIA characteristic. The Ames test is a frequently used method for assessing a substance's genotoxicity by measuring its potential harm to human health through the identification of mutagenic characteristics. This test is defined by its exceptional repeatability and limitless sensitivity. We found 25 compounds with good ADME and toxicity characteristics after filtering.

2.4. Screening and Retrieval of Cyanobacterial and Control Compounds with Chemical Structures

A public chemical database called PubChem (https://pubchem.ncbi.nlm.nih.gov) was developed by the National Library of Medicine (NLM), a division of the National Institutes of Health (NIH) in the United States [25 Hundreds of bioactive compounds from different cyanobacterial strains selected by the literature survey were downloaded from PubChem database for the molecular docking studies and their chemical structures. Selected bioactive compounds filtered based on Lipinski's rule of five (R05) and ADMET profile. Twenty-five cyanobacterial bioactive compounds selected out of 100, namely are - Acarbose (CID: 6918537), Metformin (CID: 4091), Ambiguine B Isonitrile (CID: 16109784), Ambiguine H Isonitrile (CID: 16069590), Ambigol A (CID: 475341), Ambigol B (CID: 475342), Anatoxin-a (CID: 3034748), Aplysiatoxin (CID: 46173823), Carazostatin (CID: 130857), Carbamydocyclophane E (CID: 16216032), Carbamydocyclophane J (CID: 16216032), Curacin A (CID: 5281967), Curacin D (CID: 5281967), Debromoplysiatoxin (CID: 5352033), Nostocarboline (CID: 5326150), Ethyl Tumonoate A (CID: 53493313), Nostocine A (CID: 10749358), Nostocyclyne A (CID: 10088786), Radiosumin (CID: 102506248), Rutin (CID: 5280805), Tanikolide (CID: 5276592), Venturamide B (CID: 16115401) Scytoscalarol (CID: 44605340) and also other compounds with chemical structures retrieved from 'PubChem' database are shown in Table **1**.

Table 1. Chemical structures of antidiabetic drugs and cyanobacterial bioactive compounds with PubChem No. and their source.

S.No.	Compounds Name	PubChem No.	Source of Compounds	Compounds Structure
1	METFORMIN (CONTROL)	4091	*Galega officinalis*	
2	ACARBOSE (CONTROL)	41774	Synthetic drug	
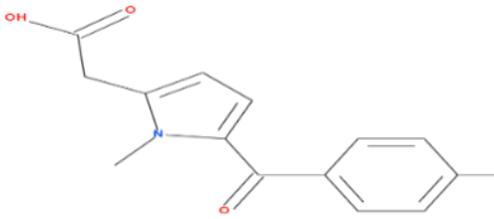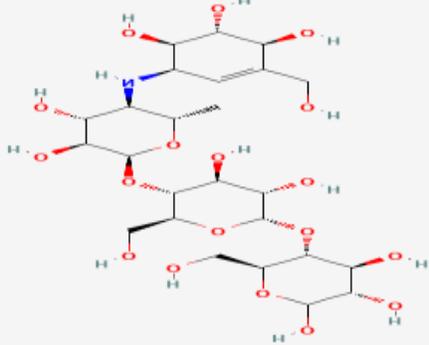
(Table 1) contd.....

S.No.	Compounds Name	PubChem No.	Source of Compounds	Compounds Structure
3	Ambiguine I isonitrile	16109784	*Fischerella sp.*	
4	Ambiguine H isonitrile	16069590	*Fischerella sp.*	
5	Ambigol A	475341	*Fischerella ambigua*	
6	Ambigol B	475342	*Fischerella ambigua*	
7	Anatoxin-a	431734	*Anabaena circinalis*	
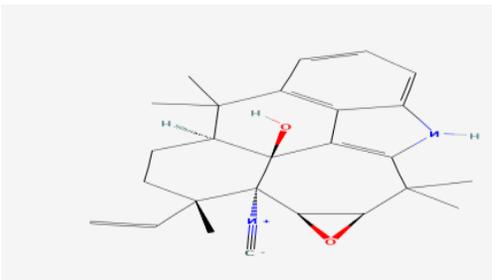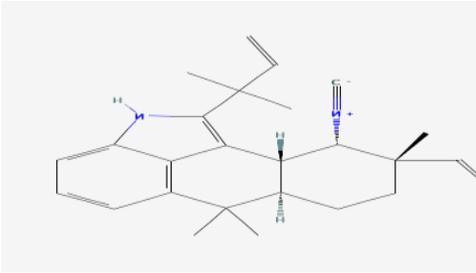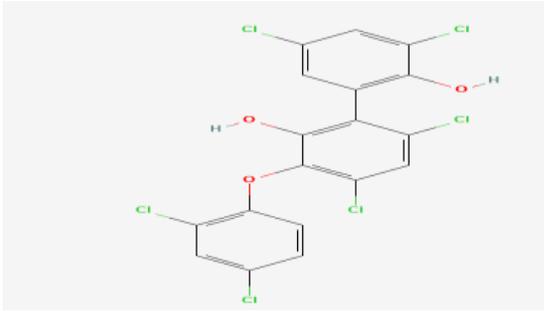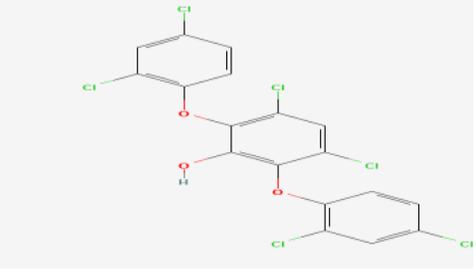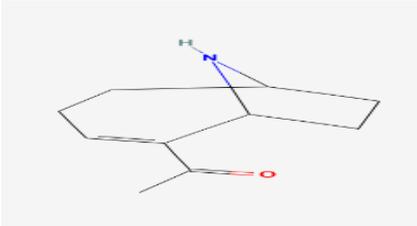
(Table 1) contd.....

S.No.	Compounds Name	PubChem No.	Source of Compounds	Compounds Structure
8	Aplysiatoxin	21672114	*Geitlerinema*	
9	Carazostatin	130857	*Hyella caespitose*	
10	Carbamidocyclophane E	16216032	*Nostoc sp.*	
11	Carbamidocyclophane J	122206459	*Nostoc sp.*	
12	Curacin D	10546404	*Lyngbya sp.*	
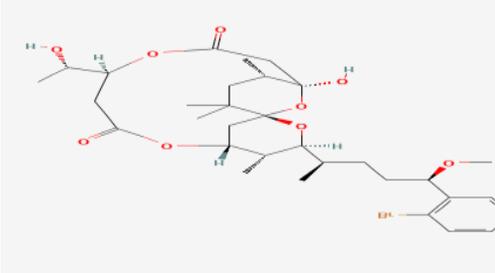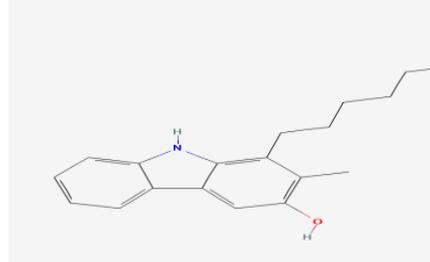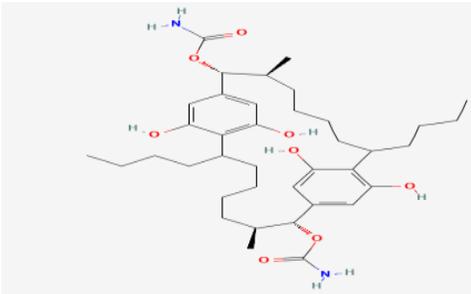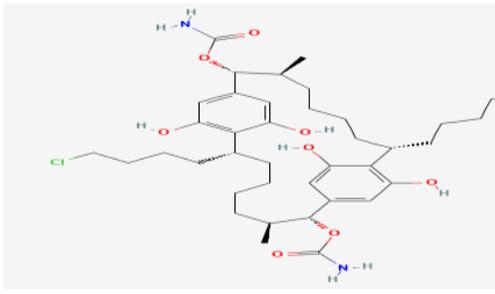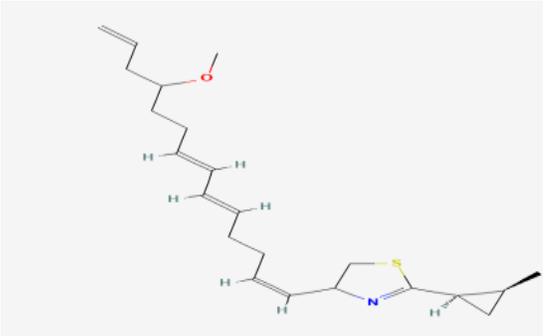
(Table 1) contd.....

S.No.	Compounds Name	PubChem No.	Source of Compounds	Compounds Structure
13	Curacin A	5281967	*Lyngbya sp.*	
14	Cylindrospermopsin	42628600	*Cylindrospermopsis sp.*	
15	Debromoaplysiatoxin	5352033	*Lyngbya majuscule*	
16	Ethyl Tumonoate A	53493313	*Oscillatoria margaritifera*	
17	Nostocarboline	5326150	*Nostoc sp.*	
18	Nostocine A	10749358	*Nostoc spongiae forme*	
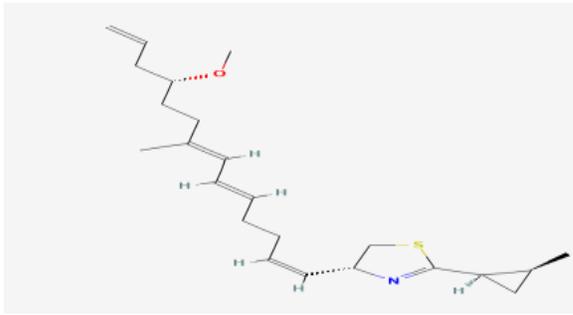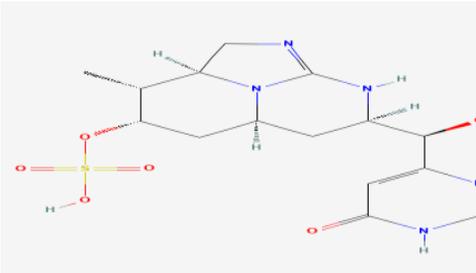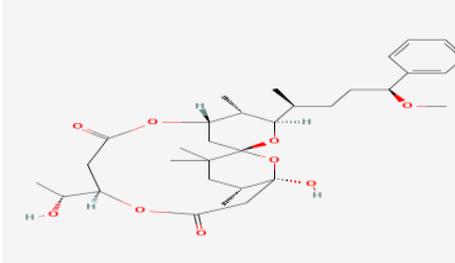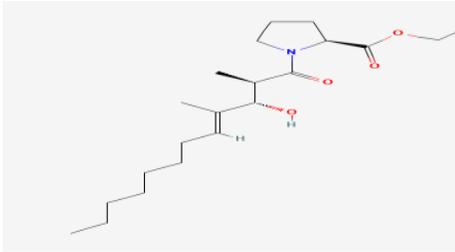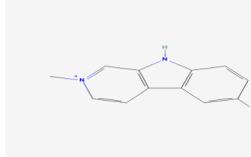
(Table 1) contd.....

S.No.	Compounds Name	PubChem No.	Source of Compounds	Compounds Structure
19	Nostocycline A	10088786	*Nostoc sp.*	
20	Radiosumin	10812638	*Plectonema radiosum*	
21	Rutin	5280805	*Gracilaria dendroides*	
22	Scytoscalalrol	44605340	*Scytonema pseudohofmanni*	
23	Tanikolide	5276592	*Lyngbya majuscula*	
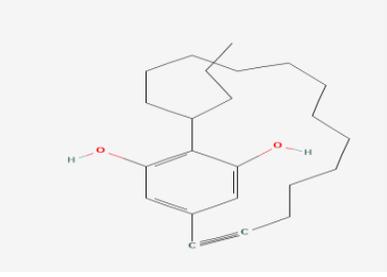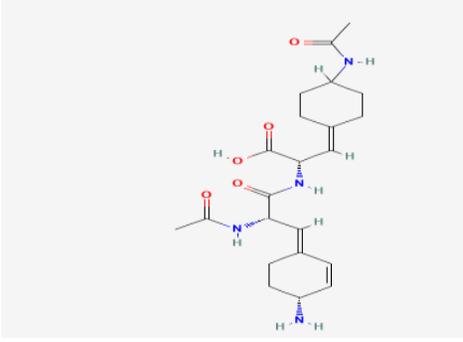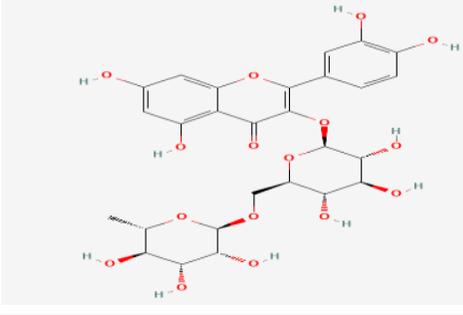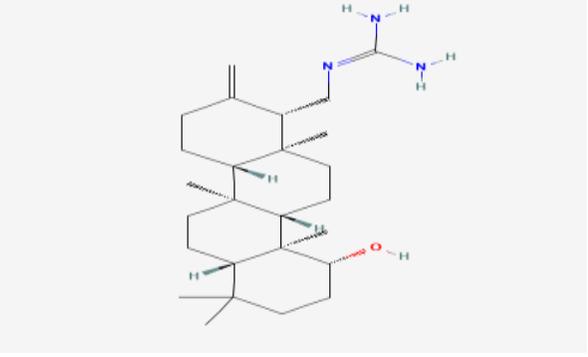
(Table 1) contd.....

S.No.	Compounds Name	PubChem No.	Source of Compounds	Compounds Structure
24	Tjipanazole J	15705741	*Tolypothrix tjipanasensis*	
25	Toyocamycin	11824	*Tolypothrix tenuis*	
26	Tubercidin	6245	*Plectonema radiosum*	
27	Venturamide B	16115401	*Oscillatoria sp.*	
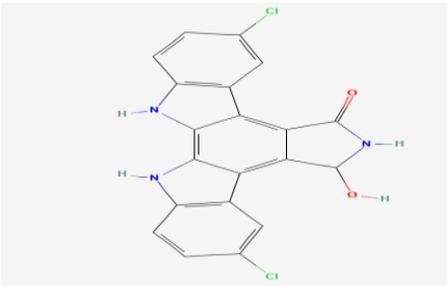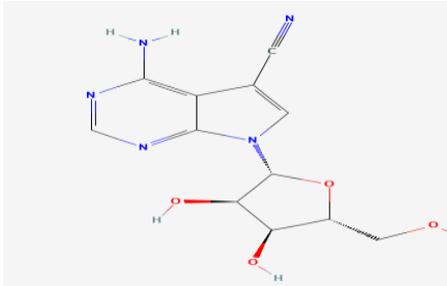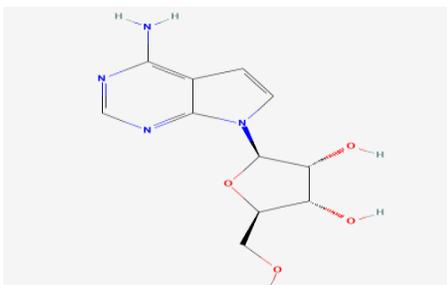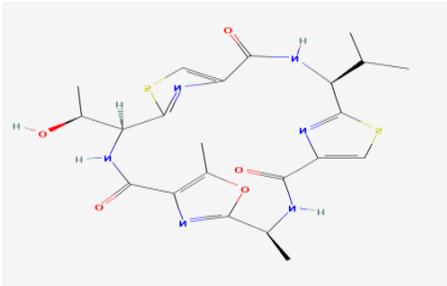
2.5. Prediction of ADME and Toxicity Profile

All 100 of the resulting compounds were subsequently approved to go through ADME and toxicity filtration after the Lipinski filter had been applied. All 100 cyanobacterial compounds' ADMET properties were predicted using the preADMET tool, and only candidates that favoured the values of the BBB, CaCo2, HIA, and AMES toxicity and Mouse/Rat carcinogenicity parameters were chosen. Out of 100 compounds, 25 were found to have useful ADMET profiles after the ADMET screening. The same preADMET web server was used to evaluate the toxicity profile of the 25 compounds that were chosen.

2.6. Molecular Docking using AutoDock 4.2 and Cygwin

The bioinformatics tools AutoDock 4.2 and Cygwin

were used to evaluate and identify the lowest free binding energy of the selected cyanobacterial compounds along with 2 reference compounds against the targeted protein α- amylase (1Kb3) and α-glucosidase (1qOX) which is associated with type 2 diabetes mellitus.

3. RESULTS AND DISCUSSION

3.1. Drug-likeness Properties of Cyanobacterial Compounds

The main purpose of Lipinski's rule of five, commonly referred to as the thumb rule, is to assess the drug-likeness of a chemical compound in order to establish whether or not a compound with a particular pharmacological activity is orally active in humans. For a molecule to function as a medication and demonstrate good permeability through cell membranes, the log P value must be lower than 5. Other criteria for this rule state that the number of hydrogen bond acceptors must be ≤ 10 (The sum of Os and Ns), the number of hydrogen bond donors must be ≤ 5 (The sum of OHs and NHs), and molecular weight should be <500Da. If the number of violations are equal to 1 or no violation, it states that the compound can simply bind to the target

receptor [26]. Drugs are excluded from further screening if more than two parameters are out of the standard range [27]. Out of 100 screened compounds from cyanobacteria, only 25 compounds were filtered through Lipinski's Rule of five and were taken further for ADMET profiling. The list of filtered compounds is given in Table **2**.

3.2. ADMET Evaluation of Filtered Compounds

According to Table **3**, 25 filtered compounds from Lipinski's rule of five were used in the ADMET investigation. Studies show that poor pharmacokinetics and toxicity are the main reasons for expensive late-stage drug development failures, and it is now generally recognized that these factors should be taken into account as early as feasible in the drug discovery process. The number of compounds for which preliminary data on absorption, distribution, metabolism, excretion (ADME), and toxicity (T) are required has greatly expanded as a result of combinatorial chemistry and high-throughput screening [27]. PreADMET, a tool that is accessible online (https://preadmet.webservice.bmdrc.org), evaluates ADME attributes.

Table 2. Drug likeness properties of filtered bioactive compounds from cyanobacteria along with control drug.

S. No.	Name of the Compounds	Drug Likeness Properties				Drug- Likeness Score
		LogP	Molecular Weight	Hydrogen Bond Donor	Hydrogen Bond Acceptor	
A	METFORMIN (CONTROL)	-1.00	129.10	5	2	-0.82
B	ACARBOSE (CONTROL)	-4.58	645.25	14	19	0.40
1	Ambiguine I Isonitrile	6.19	402.50	2	2	-1.1
2	Ambiguine H Isonitrile	7.58	372.26	1	1	-0.78
3	Ambigol A	8.36	485.00	2	3	-0.14
4	Ambigol B	8.16	485.00	1	3	-0.48
5	Anatoxin-a	1.53	165.23	1	1	-1.42
6	Aplysiatoxin	4.93	670.24	3	10	0.32
7	Carazostatin	6.83	295.40	2	1	-0.41
8	Carbamydocyclophane E	8.71	670.90	8	8	0.82
9	Carbamydocyclophane J	8.71	670.90	8	8	0.82
10	Curacin A	6.51	373.60	0	3	0.35
11	Curacin D	6.51	373.60	0	3	0.35
12	Debromoplysiatoxin	4.33	592.70	3	10	0.47
13	Nostocarboline	3.36	217.67	1	0	-0.94
14	Ethyl Tumonoate A	5.08	367.50	1	4	-0.60
15	Nostocine A	-0.92	151.13	1	4	-1.16
16	Nostocyclyne A	8.36	342.50	2	2	-0.08
17	Radiosumin	-1.95	432.50	6	6	-0.21
18	Rutin	-1.55	610.50	10	16	0.91
19	Scytoscalalrol	4.83	415.7	5	2	-0.47
20	Tanikolide	5.34	284.40	1	3	-0.95
21	Tjipanazole J	5.94	290.7	2	0	-1.31
22	Tolyporphin	4.08	742.8	4	12	0.02
23	Toyocamycin	-1.02	291.26	5	7	0.53
24	Tubercidin	-0.92	266.25	5	6	0.54
25	Venturamide B	1.75	518.60	4	10	-0.58

Abbreviation: Log P- partition coefficient.

Table 3. ADMET profiling of filtered cyanobacterial bioactive compounds using Pre ADMET server.

S. No.	Name of The Compound	BBB	HIA	Caco2	AMES Toxicity	Carcinogenicity Mouse/rat
1	Ambiguine I Isonitrile	7.45316**	92.849612	23.9099	Mutagen	Positive/ Positive
2	Ambiguine H isonitriles	16.5872	97.046579	48.6152	Mutagen	Positive/ Negative
3	Ambigol A	13.4989	95.546983	46.5271	Mutagen	Negative/Positive
4	Ambigol B	14.9377	96.842893	56.983	Mutagen	Negative/Positive
5	Anatoxin-a	0.612474	95.398255	30.5172	Mutagen	Positive/ Negative
6	Aplysiatoxin	0.62309	93.927517	21.7641	Non-Mutagen	Negative/Negative
7	Carazostatin	15.6199	93.267025	45.6263	Mutagen	Negative/Negative
8	Carbamidocyclophane E	6.6225	78.754511	20.4097	Mutagen	Negative/Negative
9	Carbamidocyclophane J	6.54959	85.988582	21.0478	Mutagen	Negative/Negative
10	Curacin D	3.20083	97.506753	31.6657	Mutagen	Positive/ Negative
11	Curacin A	5.7525	97.546286	35.2846	Non-Mutagen	Positive/ Negative
12	Cylindrospermopsin	0.0412124	69.533771	21.0569	Mutagen	Negative/Positive
13	Debromoaplysiatoxin	1.92282	96.111782	33.1237	Non-Mutagen	Negative/Negative
14	Ethyl Tumonoate A	0.239313	90.065359	24.9041	Mutagen	Positive/Negative
15	Nostocarboline	5.18456	97.910575	16.7822	Mutagen	Positive/Negative
16	Nostocine A	0.114421	78.765338	11.6462	Mutagen	Positive/Positive
17	Nostocycline A	18.0699	93.610401	47.7531	Mutagen	Negative/Negative
18	Radiosumin	0.0598323	63.980144	20.8546	Mutagen	Positive/Positive
19	Rutin	0.0285642	2.861176	7.91267	Non-Mutagen	Negative/Negative
20	Scytoscalarol	0.75716	92.010946	21.0215	Mutagen	Negative/ Positive
21	Tanikolide	7.06301	94.877159	46.7781	Non-Mutagen	Negative/Negative
22	Tolyporphin J	0.067607	82.169793	0.808384	Non-Mutagen	Positive/Positive
23	Toyocamycin	0.112285	46.547342	5.36832	Mutagen	Negative/ Positive
24	Tubercidin	0.360937	65.797783	5.22828	Mutagen	Negative/Negative
25	Venturamide B	0.0561416	56.779147	9.19956	Mutagen	Negative/ Positive

Abbreviations: BBB- Blood brain barrier; HIA-Human intestinal absorption; Caco-2- heterogenous human epithelial colorectal adenocarcinoma; M- mutagen; C-carcinogen (rat/mouse).

A total of 25 ligands from Cyanobacteria were selected out of 100 for the molecular docking investigation. After molecular docking investigation of selected ligands out of 25 top 3 compounds, namely, Ethyl Tumonoate A, Debromoaplysiatoxin and Scytoscalarol, showed highly significant interaction with α-amylase listed in Table **4** and also out of 25 ligands top 3 compounds namely, Ambiguine I isonitrile, Cylindrospermopsin and Debromoaplysiatoxin showed excellent binding interaction with α-glucosidase listed in Tables **5** and **6**.

The molecular docking interaction results reveal the free binding energy of α-amylase was found with Metformin (reference compound) concluded 10 amino acid residues, namely, Ile 235, Glu 233, Ala 193, Asp 197, Asp 96, Tyr 62, Trp 58, His 299, Asp 300, His 305 (Fig. **3**); with Ehtyl Tumunoate A concluded 10 amino acid residues- Glu 171, Asp 206, Gly 205, Pro 204, Trp 203, Asn 137, Lys 140, Asp 159, Arg 158, Thr 155 (Fig. **4**); with Debromo-aplysiatoxin concluded 07 amino acid residue- Pro 241, Lys 243, Ala 154, Asp 246, Gly 205, Trp 203, Arg 158 (Fig. **5**); with Scytoscalarol concluded 05 amino acid residues- Thr 155, Asp 159, Lys 140, Trp 203, Glu 171 (Fig. **6**). The values of free binding energy for Metformin with α-amylase, Ehtyl Tumunoate A with α-amylase, and Scytoscalarol with α-amylase docking scores originated were –7.66, –9.28, –7.97, -7.14 kcal/mol respectively, although the conforming values of inhibition constant (Ki) were expected to be 2.43, 0.5765, 1.43, 5.80 µM separately listed in Table **5**.

Figs. (**3**-**6**): Docked structure of selected top three leads and Metformin, a control drug, with the active site residues of α-amylase.

Table 4. Binding energy of docked cyanobacterial bioactive compounds and metformin (control) with protein (α-amylase).

S. No.	Name of Compounds	Free Binding Energy Kcal/mol	Inhibition Constant Ki (µM)
0	METFORMIN (Control)	-7.66	2.43
1	Ethyl Tumonoate A	-9.28	0.15765
2	Debromoaplysiatoxin	-7.97	1.43
3	Scytoscalarol	-7.14	5.80

(Table 4) contd.....

S. No.	Name of Compounds	Free Binding Energy Kcal/mol	Inhibition Constant Ki (µM)
4	Ambigol A	-4.93	241.67
5	Ambigol B	-5.21	152.01
6	Ambiguine H isonitriles	-4.95	235.76
7	Tanikolide	-3.28	3910
8	Tjipanazole J	-5.63	75.05
9	Tolyporphin J	-6.07	35.63
10	Toyocamycin	-5.27	116.27
11	Tubercidin	-4.62	411.82
12	Venturamide B	-5.02	209.47
13	Nostocarboline	-4.45	550.91
14	Nostocine A	-4.56	493.97
15	Nostocycline A	-6.21	28.07
16	Radiosumin	-5.2	153.42
17	Rutin	-4.39	606.76
18	Carbamidocyclophane E	-4.13	940.81
19	Carbamidocyclophane J	-3.34	3.58
20	Curacin D	-6.31	23.89
21	Curacin A	-3.23	4270
22	Ambiguine B isonitrile	-5.17	162.87
23	Anatoxin-a	-5.00	217.36
24	Aplysiatoxin	-5.49	95.04
25	Carazostatin	-5.20	155.48

Table 5. Active sites residues in the receptor of top three cyanobacterial bioactive compounds and metformin with Binding energy (receptor: α-amylase).

S. No.	Name of Compounds	Free Binding Energy Kcal/mol	Interacting Residues in the Receptor
0	METFORMIN (CONTROL)	-7.66	Ile 235, Glu 233, Ala 193, Asp 197, Asp 96, Tyr 62, Trp 58, His 299, Asp 300, His 305
1	Ethyl Tumonoate A	-9.28	Glu 171, Asp 206, Gly 205, Pro 204, Trp 203, Asn 137, Lys 140, Asp 159, Arg 158, Thr 155
2	Debromoaplysiatoxin	-7.97	Pro 241, Lys 243, Ala 154, Asp 246, Gly 205, Trp 203, Arg 158
3	Scytoscalarol	-7.14	Thr 155, Asp 159, Lys 140, Trp 203, Glu 171

Table 6. Binding energy of docked cyanobacterial bioactive compounds and acarbose (control) with protein (α-glucosidase).

S. No.	Name of Compounds	Free Binding Energy Kcal/mol	Inhibition Constant Ki (µM)
0	ACARBOSE (control)	-8.86	0.31974
1	Ambiguine I isonitriles	-9.46	0.11641
2	Scytoscalarol	-7.5	3.2
3	Cylindrospermopsin	-7.17	5.51
4	Rutin	-2.01	33410
5	Ambiguine H isonitriles	-4.97	226.79
6	Tanikolide	-5.05	198.48
7	Tjipanazole J	-6.18	29.62
8	Toyocamycin	-6.59	14.68
9	Venturamide B	-5.79	57.3
10	Debromoaplysiatoxin	-4.94	241.16
11	Ethyl Tumonoate A	-3.47	2870
12	Nostocarboline	-4.51	497.92
13	Nostocine A	-4.57	445.18
14	Nostocycline A	-4.97	227.14

(Table 6) contd.....

S. No.	Name of Compounds	Free Binding Energy Kcal/mol	Inhibition Constant Ki (µM)
15	Radiosumin	-4.88	267.02
16	Carbamidocyclophane J	-2.42	16750
17	carbamidocyclophane c	-2.42	16780
18	Ambigol A	-5.82	54.22
19	Ambigol B	-6.55	15.72
20	Anatoxin-a	-6.10	33.65
21	Aplysiatoxin	-4.52	490.20
22	Carazostatin	-6.07	35.76
23	Carbamidocyclophane E	-2.94	7.04
24	Curacin D	-4.70	1.03
25	Curacin A	-4.24	776.45
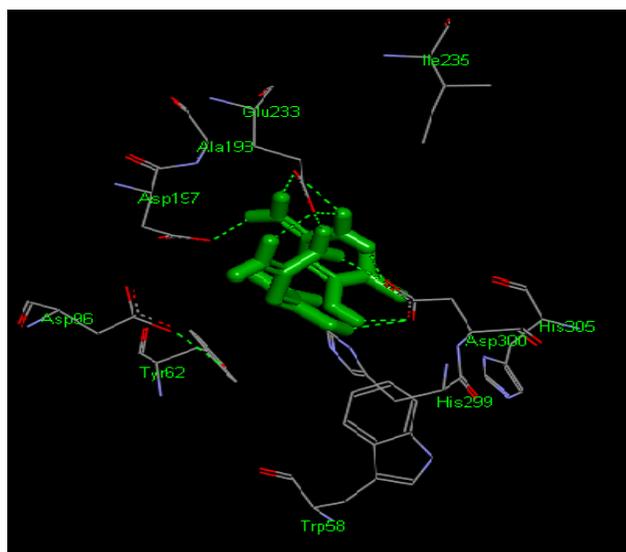
Fig. (3). Docked structure of metformin and α-amylase.
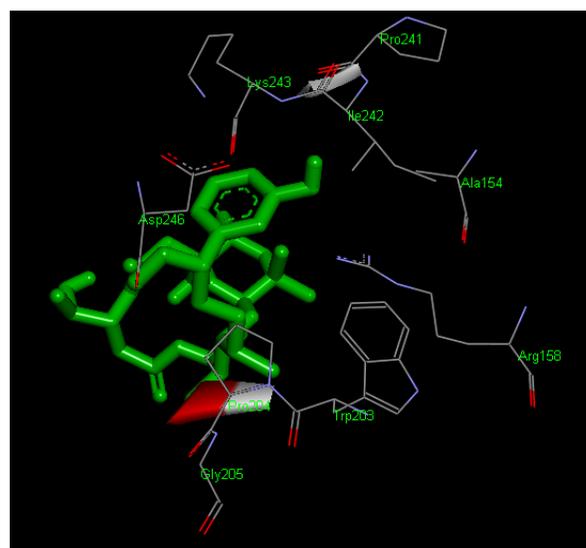
Fig. (5). Docked structure of debromoaplysiatoxin and α-amylase.
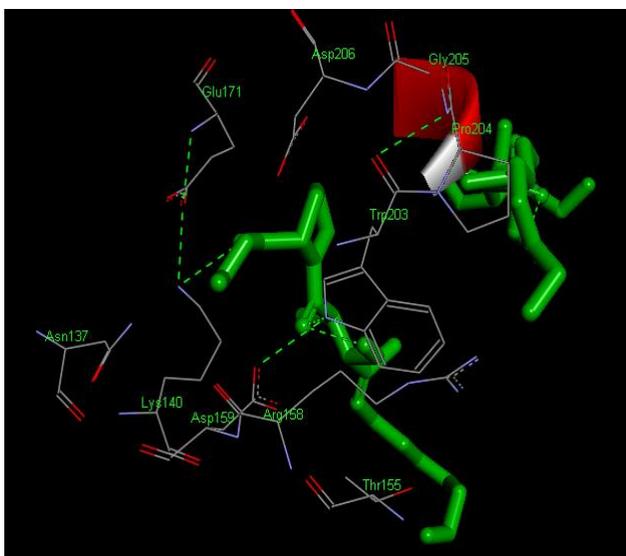
Fig. (4). Docked structure of ethyl tumonoate A and α-amylase.
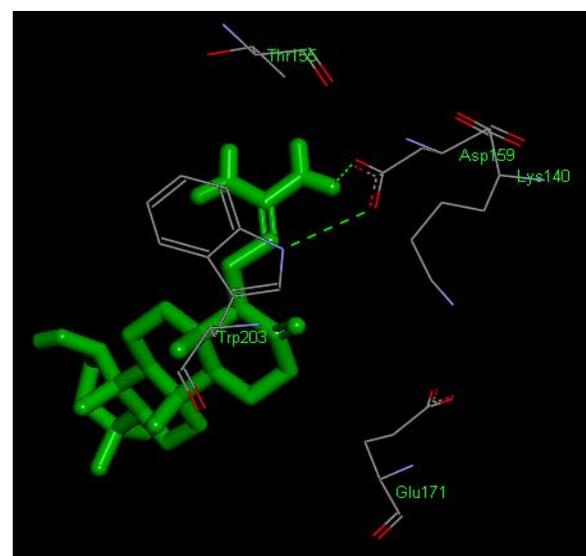
Fig. (6). Docked structure of scytoscalarol and α-amylase.
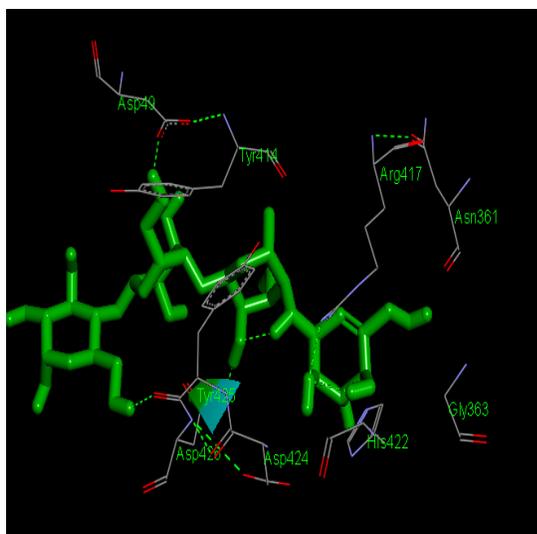
Fig. (7). Docked structure of acarbose and α-glucosidase.
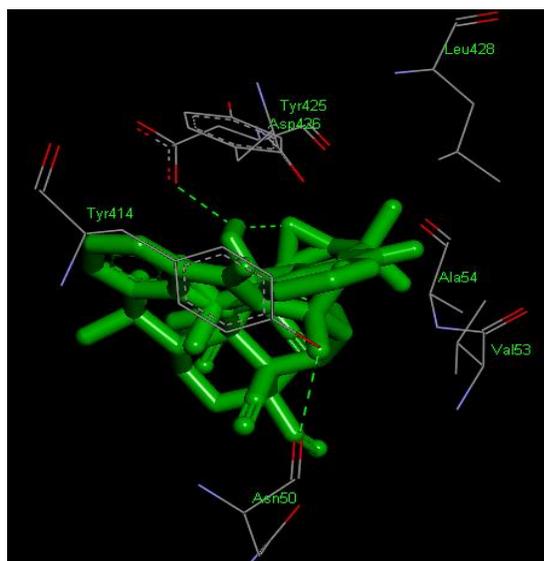
Fig. (8). Docked structure of ambiguine I isonitriles and α-glucosidase.

Similarly, the molecular docking interaction revealed that the free binding energy of α-glucosidase was found with Acarbose (reference compound) showed 09 amino acid residues-Asp 49, Tyr 414, Arg 417, Asn 361, Tyr 425, Asp 426, Asp 424, His 422, Gly 363 (Fig. **7**); with Ambiguine I Isonitrile showed 07 amino acid residues-Leu 428, Tyr 425, Asp 426, Tyr 414, Ala 54, Val 53, Asn 50 (Fig. **8**); with Scytoscalarol showed 09 amino acid residues - Asp 49, Gly 415, Tyr 414, Asp 426, Val 53, Tyr 425, Asp 424, Arg 417, Leu 428 (Fig. **9**); with Cylindrospermopsin showed 11 amino acid residues - Arg 417, His 422, Asn 361, Leu 364, Val 423, Gly 415, Tyr 414, Asp 424, Tyr 425, Thr 427, Asp 426 (Fig. **10**). The values of free binding energy for Acarbose with α-glucosidase; Ambiguine I

Isonitrile with α-glucosidase; Scytoscalarol with α-glucosidase; Cylindrospermopsin with α-glucosidase are "–8.86, –9.46, –7.5, -7.17 kcal/mol respectively, although the conforming values of inhibition constant (Ki) were expected to be 0.31974; 0.1164; 3.20; 5.51 μM which are separately listed in Table **7**.
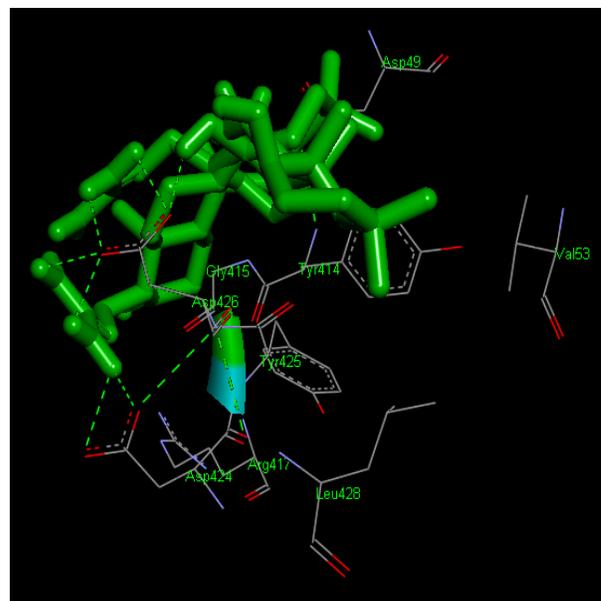
Fig. (9). Docked structure of scytoscalarol and α-glucosidase.

Figs. (**7**-**10**): Docked structure of selected top three leads and Acarbose, a control drug, with the active site residues of α-glucosidase.
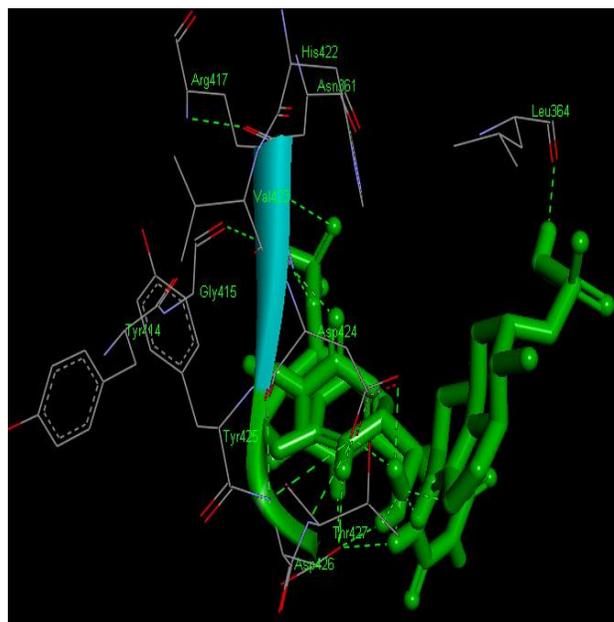
Fig. (10). Docked structure of cylindrospermopsin and α-glucosidase.

Table 7. Active sites residues in the receptor of top three cyanobacterial bioactive compounds and metformin with Binding energy (receptor: α-glucosidase).

S. No.	Name of Compounds	Free Binding Energy Kcal/mol	Interacting Residues in the Receptor
0	ACARBOSE (Reference compound)	-8.86	Asp 49, Tyr 414, Arg 417, Asn 361, Tyr 425, Asp 426, Asp 424, His 422, Gly 363
1	Ambiguine I isonitriles	-9.46	Leu 428, Tyr 425, Asp 426, Tyr 414, Ala 54, Val 53, Asn 50
2	Scytoscalarol	-7.5	Asp 49, Gly 415, Tyr 414, Asp 426, Val 53, Tyr 425, Asp 424, Arg 417, Leu 428
3	Cylindrospermopsin	-7.17	Arg 417, His 422, Asn 361, Leu 364, Val 423, Gly 415, Tyr 414, Asp 424, Tyr 425, Thr 427, Asp 426

The resultant compounds were also found to be suitable with noble druggable character, as per Lipinski's rule of five and ADEMT profile properties. Therefore, this potent compound could be a possible drug applicant for treating type 2 diabetes mellitus [28]. The resulting cyanobacterial compounds are subjected to in-vitro analysis where phytochemical evaluation, anti-oxidant activity, anti-diabetic activity and anti-inflammatory activity. Current progress in the understanding of the biology of T2DM has resulted in an increasing number of therapies and remedies that are permitted or in clinical development for this disorder [29]. Some compounds have exhibited very interesting results and successfully reached Phase II and Phase III clinical trials [30].

CONCLUSION

Diabetes mellitus is a global metabolic disorder that is alarmingly on the rise. The prolonged use of currently available synthetic medications offers only a temporary solution and comes with undesirable side effects. The resurgence of the natural medicine field holds promise, and there is a strong call for solutions to diabetes from natural sources. Recently, there have been reports highlighting cyanobacteria as a valuable source of bioactive compounds, including Ambiguine I Isonitrile, Cylindrospermopsin, Scytoscalalrol, Ambigol A, Ambigol B, Anatoxin-a, Aplysiatoxin, Carazostatin, Carbamydocyclophane E, Carbamydocyclophane J, Curacin A, Curacin D, Debromoplysiatoxin, Nostocarboline, Ethyl Tumonoate A, Nostocine A, Nostocyclyne A, Radiosumin, Rutin, Scytoscalarol, Tanikolide, and Venturamide A.

Furthermore, these bioactive compounds from cyanobacteria hold immense potential in research and offer a limitless outlook for the development of new drug candidates. In this study, molecular docking analysis has unveiled that certain cyanobacterial bioactive compounds, such as Ambiguine I Isonitrile, Cylindrospermopsin, Scytoscalalrol, and Ethyl Tumonoate A, exhibit strong potential as candidates for the development of novel drugs targeting α-amylase and α-glucosidase enzymes in the treatment of type 2 diabetes mellitus. Further experimental in-vitro and in-vivo studies are needed to confirm the therapeutic effectiveness of these compounds in the development of innovative antidiabetic medications.

LIST OF ABBREVIATIONS

T2DM   =   Type 2 Diabetes Mellitus

DM   =   Diabetes Mellitus

WHO   =   World Health Organization

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

The data and supportive information are available within the article.

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CONFLICT OF INTEREST

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