



Research Article

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**MOLECULAR DOCKING ANALYSIS OF BIOACTIVE COMPONENTS FROM
Phyllanthus niruri FOR ITS ANTIDIABETIC POTENTIAL**

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ABSTRACT

Diabetes is a chronic metabolic disease characterized by elevated levels of blood glucose (or blood sugar), which, over time, leads to damage to the heart, blood vessels, eyes, kidneys, and nerves. This study aimed at identify novel inhibitors of human salivary amylase (HSA) from methanol leaf extract of *Phyllanthus niruri* for the treatment of Type II Diabetes mellitus. This study aimed at identifying novel inhibitors of human salivary amylase (HSA) from methanol leaf extract of *Phyllanthus niruri* for the treatment of Type II Diabetes mellitus. The fresh sample of *Phyllanthus niruri* was extracted, screened for phytochemicals, analysed using Gas Chromatography-Mass Spectrometry (GC-MS) technique and then docked with Autodock 4.0 program. The results of the phytochemical analysis of the methanol extract of *Phyllanthus niruri* revealed the presence of alkaloids, tannins, flavonoids, anthocyanins, cardiac glycosides, and phenols, while saponins, steroids, quinones and terpenoids were not detected. The GC-MS result revealed a total of thirty-one (31) compounds. Based on the docking results, CID_312822 had the best binding energy of -9.22 kcal/mol, followed by CID_616782 with a binding energy of -8.42 kcal/mol, and CID_41687, which has a binding energy of -6.37 kcal/mol. All compounds were non-toxic except CID_5363335, which had a high tumorigens with low mutagenicity and irritability. In conclusion, the results of this study highlight the possible health benefits of methanol leaf extract of *Phyllanthus niruri* and lay a foundation for further investigation into its antidiabetic use.

Keywords: *Phyllanthus niruri*; Diabetes Mellitus; Human Salivary Amylase; Molecular Docking

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of chronic hyperglycemia characterized by disturbances to carbohydrate, protein, and fat metabolism resulting from absolute or relative insulin deficiency with dysfunction in organ systems [1]. This disease has shown a tremendous increase in prevalence with a demographic transition in its epidemiology in recent years. Populations previously unaffected or minimally affected by DM are now reporting soaring prevalence figures, which poses a real challenge to health financing by governments and nongovernmental organizations. The latest prevalence figure published by the International Diabetes Federation (IDF) is 425 million persons living with DM worldwide, with nearly 50% of these undiagnosed [2]. The developing economies of Africa

and Asia contribute a significant fraction of this figure. There is also a rising burden from the complications of DM alongside the ever-increasing prevalence of the disease [3].

α -Amylase (E.C. 3.2.1.1) are enzymes that catalyze the hydrolysis of α -1,4-glucosidic linkages in starch and other related polysaccharides. These enzymes are ubiquitous in both the plant and animal kingdoms. In humans, amylase is present in both salivary and pancreatic secretions and is produced by two different loci, AMY1 and AMY2, respectively. Considerable sequence similarity exists between the salivary enzyme and α -amylases from other sources, especially human and porcine pancreas. Amylases are monomeric calcium-binding proteins with a single polypeptide chain folded into three domains, and belong to the family of proteins that harbor the (β/α) 8 β -barrel topology first identified in the triose phosphate isomerase [4].

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defense against insects, fungi, diseases, and herbivorous mammals. Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. The annual global export value of 50,000 to 70,000 types of plants with suspected medicinal properties was estimated to be US\$2.2 billion in 2012 [5]. Traditional and modern medicine both benefit from the use of medicinal plants. Research has demonstrated the true usefulness of these plants, which serve as the principal source of healthcare for 80% of the rural population [6]. Since ancient times, people from many continents, but especially those in Africa, have employed plants as sources of cures to heal a wide range of maladies. Even with the notable advancements in synthetic organic pharmaceutical goods over the 20th century, more than 25% of prescribed medications in developed nations are derived, either directly or indirectly, from plants [7]. Still, there is a dearth of research on plants utilized in traditional medicine [8].

Phyllanthus niruri (*Chanca piedra*) is a widespread tropical plant commonly found in coastal areas, known by the common names gale of the wind, stonebreaker or seed-under-leaf. It is a relative of the spurges, belonging to the genus *Phyllanthus* of the family *Phyllanthaceae* and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers, which are often flushed with red. The fruits are tiny, smooth capsules containing seeds [9]. *Phyllanthus niruri*. It grows 50–70 cm (20–28 in) tall and bears ascending herbaceous branches. The bark is smooth and light green. It bears numerous pale green flowers which are often flushed with red. The fruits are tiny, smooth capsules containing seeds *Phyllanthus niruri* has a long history in herbal medicine systems in every tropical country where it grows. For the most part, it is employed for similar conditions worldwide. Its main uses are for many types of biliary and urinary conditions including kidney and gallbladder stones; for hepatitis, colds, flu, tuberculosis, and other viral infections; liver diseases and disorders including anemia, jaundice and liver cancer; and for bacterial infections such as cystitis, prostatitis, venereal diseases and urinary tract infections. It is also widely employed for diabetes and hypertension as well as for its diuretic, pain-relieving, digestive stimulant, antispasmodic, fever reducing, and cellular protective properties in many other conditions [9]. This study is aimed at identifying novel inhibitors of human salivary amylase (HSA) from methanol leaf extract of *Phyllanthus niruri* for the treatment of Type II Diabetes mellitus.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents used in the research were purchased from BDH Chemicals Ltd. Poole, England, and are of analytical grade.

Sample Collection and Identification

Fresh leaf of *Phyllanthus niruri* sample was collected from a garden behind Federal Government College Maiduguri, Borno State and identified by a Botanist at the Department of Biological Sciences and voucher sample (Vet253B26) was deposited at the Veterinary Pharmacology Laboratory herbarium, University of Maiduguri, Nigeria.

Preparation of the Plant Extract

The sample was dried under a shed (*Phyllanthus niruri*) was and then ground into a powdered form using a mortar and pestle. Two hundred grams (200 g) of the sample was extracted using the cold maceration method with methanol and distilled water at a proportion (70/30) for 72 hours. The extract was evaporated to dryness under reduced pressure using rotary evaporator.

Preliminary Phytochemical Screening

The phytochemical screening of the methanol leaf extract of *Phyllanthus niruri* was carried out using the methods of [10].

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

The phytocompounds of the methanol leaf extract of *Phyllanthus niruri* were identified using Gas Chromatography-Mass Spectrometry (GC-MS). The 7890A gas chromatograph system (Agilent 19091-433HP, USA) and mass spectrophotometer were used to perform the GC-MS analysis. The system was equipped with an HP-5 MS fused silica column (5% phenyl methyl siloxane 30.0m 250 m, film thickness 0.25 m), which was interfaced with a 5675C Inert MSD with Triple-Axis detector. As the carrier gas, helium gas was adjusted to a flow rate of 1.0 ml/min. Other GC-MS conditions include a 1 l injector in split mode with a split ratio of 1:50 and an injection temperature of 300 °C. Ion source temperature is 250 °C, interface temperature is 300 °C, pressure is 16.2 psi, and out time is 1.8 mm. The column temperature increased from 36 °C to 150 °C at a rate of 4 °C/min after starting at 36 °C for 5 minutes. The temperature was raised to 250°C.

Molecular Docking Analysis

The physicochemical and pharmacokinetic properties of the bioactive compounds of *Phyllanthus niruri* was conducted using data warrior tool whereas, the docking studies was performed with the help of Autodock 4.0 program. To evaluate drug-likeness, Lipinski's Rule of Five was applied, considering parameters such as molecular weight (≤ 500 Da), hydrogen bond donors (≤ 5), hydrogen bond acceptors (≤ 10), and LogP (≤ 5). The ADME profile, including gastrointestinal (GI) absorption, blood-brain barrier (BBB) penetration, cytochrome P450 (CYP) metabolism, and renal clearance, was analyzed. Toxicity risks were predicted. Molecular docking simulations were carried out using AutoDock Vina, assessing ligand-protein interactions based on binding affinity and hydrogen bonding with key active site residues.

The three-dimensional (3-D) structures of the human salivary amylase (PDB: 1SMD) enzyme and the ligands (phytocompounds) used in this research were obtained from Research Collaboratory for Structural Bioinformatics (RCSB) PDB database and Pubchem respectively. ADT (AutoDock Tools), Graphical User Interface program was used for energy minimization, while protein and ligand preparation and grid box creation were completed using Graphical User Interface program AutoDock Tools (ADT). AutoDock saved the prepared file in PDBQT format. AutoDock/ Vina was employed for docking using protein and ligand information along with grid box properties in the configuration file. AutoDock/ Vina employs an iterated local search global optimizer[15,16]. Throughout the docking procedure, both the protein and ligands were considered as rigid. The results of less than 1.0 Å in positional root-mean-square deviation (RMSD) were clustered together and represented by the result with the most favorable free energy of binding. The pose with the lowest energy of binding or binding affinity was extracted and aligned with the receptor structure for further analysis [15,16].

RESULTS

Phytochemical Analysis of Compounds Obtained from Methanol Extract of *Phyllanthus niruri*

The results of the phytochemical analysis of the methanol extract of *Phyllanthus niruri* revealed the presence of alkaloids, tannins, flavonoids, anthocyanins, cardiac glycosides, and phenols. However, saponins, steroids, quinones, and terpenoids were absent. (Table 1).

Table 1: Phytochemical Constituents of Methanol Extract of *Phyllanthus niruri*

Phytochemicals	Inference
Alkaloids	+
Tannins	+
Flavonoids	+
Saponins	-
Anthocyanins	+
Steroids	-
Quinones	-
Terpenoids	-
Cardiac glycosides	+
Beta cyanins	+
Phenols	+

Key: + = Presence; - = Absence

GC-MS Result

The results of the GC-MS analysis (Table 4.3) revealed several compounds from the methanol extract of *Phyllanthus niruri*. GC-MS chromatogram indicated the presence of 24 compounds (Table 3). The compounds determined under Peak 2 are 1 H-Indole, 3, 3'-[1-(4-pyridinyl)-1, 2-ethanediyl] bis [2-ethyl while Peak 3 consists of N-Dimethylaminomethyl-tert. -butyl-isopropylphosphine. Similarly, compounds obtained in Peak 4 is Octane, 1-(ethenylthio) - and Cyclohexanone, 4-hydroxy-, while Peak 5 is 1H-Imidazole, 4, 5-dihydro-2-methyl-. Also, compounds obtained in Peak 6 are 2-Butene, 2-nitro- and 1,3-Benzodioxol-2-one, hexahydro-, cis, the compounds obtained in Peak 7 is 1H-Imidazole, 4,5-dihydro-2-methyl- while peak 8 is Oxazole, 4,5-dihydro-2,5-dimethyl and peak 9 is Benzeneacetic acid, 2-hydroxy-, methyl ester while peak10 are 1-Allyl-cyclohexane-1,2-diol and 6,10,14-Trimethyl-pentadecan-2-ol, compounds obtained in peak 11 include 10-Heneicosene (c,t), 2-Dodecanol, compounds of peak 12 include 1-Allyl-cyclohexane-1,2-diol, Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl), compounds of peak 14 include 2-Heptadecenal, compound of peak 21 include 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester), compound obtained in peak 22 include 1-Decanol, 2-hexyl-, compound obtained in peak 23 include 4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester), compounds obtained in peak 24 include 1-Hexyl-2-nitrocyclohexane, Cholestan-3-ol, 2-methylene-, (3 β ,5 α)- (Campesterol), compound obtained in peak 24 include 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, phytol.

Table 2: Properties of Phyto-Compounds from Methanol Extract of *Phyllanthus niruri*

S/N	PubChem ID	Compound	Formula	Molecular Weight	Retention Time(min)	Peaks
1	CID_616782	1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl] bis [2-ethyl-	C ₂₇ H ₂₇ N ₃	393	8.621	2
2	CID_546995	N-Dimethylaminomethyl-tert.-butyl-isopropylphosphine	C ₁₀ H ₂₄ NP	189	9.822	3
3	CID_283728	Octane, 1-(ethenylthio)-	C ₁₀ H ₂₀ S	172	10.125	4
4	CID_543706	Cyclohexanone, 4-hydroxy-		114	10.125	4
5	CID_10798	1H-Imidazole, 4,5-dihydro-2-methyl-	C ₄ H ₈ N ₂	84	12.048	5
6	CID_5364398	2-Butene, 2-nitro-	C ₄ H ₇ NO ₂	101	12.66	6

7	CID_697893	1,3-Benzodioxol-2-one, hexahydro-, cis-	C ₇ H ₁₀ O ₃	142	12.66	6
8	CID_10798	1H-Imidazole, 4,5-dihydro-2-methyl-	C ₄ H ₈ N ₂	84	13.37	7
9	CID_543562	Oxazole, 4,5-dihydro-2,5-dimethyl-	C ₅ H ₉ NO	99	15.435	8
10	CID_89719	Benzeneacetic acid, 2-hydroxy-, methyl ester	C ₉ H ₁₀ O ₃	166	17.329	9
11	CID_534396	1-Allyl-cyclohexane-1,2-diol	C ₉ H ₁₆ O ₂	156	17.965	10
12	CID_530418	6,10,14-Trimethyl-pentadecan-2-ol	C ₁₈ H ₃₈ O	270	17.965	10
13	CID_5364553	10-Heneicosene (c,t)	C ₂₁ H ₄₂	294	18.554	11
14	CID_25045	2-Dodecanol	C ₁₂ H ₂₆ O	186	18.554	11
15	CID_534396	1-Allyl-cyclohexane-1,2-diol	C ₉ H ₁₆ O ₂	156	21.112	12
16	CID_41687	Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl)-	C ₂₀ H ₄₀	280	21.112	12
17	CID_5363335	2-Heptadecenal	C ₁₇ H ₃₂ O	252	22.405	14
18	CID_534521	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	C ₇ H ₉ FN ₂ O ₂	172	29.46	21
19	CID_95337	1-Decanol, 2-hexyl-	C ₁₆ H ₃₄ O	242	29.786	22
20	CID_534521	4-Fluoro-1-methyl-5-carboxylic acid, ethyl(ester)	C ₇ H ₉ FN ₂ O ₂	172	31.234	23
21	CID_544017	1-Hexyl-2-nitrocyclohexane	C ₁₂ H ₂₃ NO ₂	213	32.023	24
22	CID_312822	Cholestan-3-ol, 2-methylene-, (3 β ,5 α)-(Campesterol)	C ₂₈ H ₄₈ O	400	32.023	24
23	CID_5366244	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C ₂₀ H ₄₀ O	296	32.247	25
24	CID_5280435	Phytol	C ₂₀ H ₄₀ O	296	32.247	25

Physiochemical Properties

All the compounds obtained from GC-MS analysis were further filtered for physicochemical properties (molecular $\leq$ 500 Da, number of hydrogen bond acceptor $\leq$ 10, number of hydrogen bond donor's $\leq$ 5, $\log P \leq 5$ and $\log S \leq 5$). Out of the Thirty-one (31) compounds screened, only (18) had desirable physicochemical properties. This include: CID_616782, CID_546995, CID_283728, CID_543706, CID_10798, CID_5364398, CID_697893, CID_10798, CID_543562, CID_89719, CID_534396, CID_530418, CID_5364553, CID_25045, CID_534396, CID_41687, CID_5363335, CID_534521, CID_95337, CID_534521, CID_544017, CID_312822, CID_5366244, CID_5280435.

Table 3: Physiochemical Properties of Compounds Isolated from Methanol Extract of *Phyllanthus niruri*

S/N	PubChem ID	Molecular weight (≤ 500)	Number of HBA (≤ 10)	Number of HBD (≤ 5)	MolLogP (≤ 5)	Drug likeness
1	CID_616782	393.532	3	2	6.0935	1.456
2	CID_546995	189.282	1	0	2.2356	-18.174
3	CID_283728	172.335	0	0	4.125	-20.988
4	CID_543706	114.143	2	1	0.4915	-2.7749
5	CID_10798	84.1215	2	1	-0.388	3.7798
6	CID_5364398	101.105	3	0	0.862	-10.035
7	CID_697893	142.153	3	0	0.7777	-5.4682
8	CID_10798	84.1215	2	1	-0.388	3.7798

9	CID_543562	99.1325	2	0	0.0802	1.1851
10	CID_89719	166.175	3	1	1.225	-6.8927
11	CID_534396	156.224	2	2	1.3691	-10.136
12	CID_530418	270.499	1	1	6.5175	-8.8795
13	CID_5364553	294.564	0	0	9.3586	-24.054
14	CID_25045	186.337	1	1	4.4994	-24.047
15	CID_534396	156.224	2	2	1.3691	-10.136
16	CID_41687	280.538	0	0	7.3351	-3.8753
17	CID_5363335	252.440	1	0	6.402	-27.622
18	CID_534521	172.158	4	0	0.7781	-1.9363
19	CID_95337	242.445	1	1	6.176	-22.455
20	CID_534521	172.158	4	0	0.7781	-1.9363
21	CID_544017	213.320	3	0	3.1361	-21.133
22	CID_312822	400.688	1	1	7.4008	-8.1908
23	CID_5366244	296.537	1	1	7.4212	-3.7661
24	CID_5280435	296.537	1	1	7.4212	-3.7661

Table 4: Pharmacokinetic Properties of Compounds Isolated from Methanol Extract of *Phyllanthus niruri*

S/No.	Compound name	Mutagens	Tumorigens	Reproducibility	Irritant
1	CID_616782	None	none	none	none
2	CID_546995	None	none	none	none
3	CID_283728	None	none	none	none
4	CID_543706	none	none	none	none
5	CID_10798	none	none	none	none
6	CID_5364398	none	none	none	none
7	CID_697893	none	none	none	none
8	CID_10798	none	none	none	none
9	CID_543562	none	none	none	none
10	CID_89719	none	low	low	none
11	CID_534396	none	none	none	low
12	CID_530418	none	none	none	none
13	CID_5364553	none	none	none	none
14	CID_25045	none	none	none	none
15	CID_534396	none	none	none	low
16	CID_41687	none	none	none	none
17	CID_5363335	low	high	none	low
18	CID_534521	none	none	none	none
19	CID_95337	none	none	none	none
20	CID_534521	none	none	none	none
21	CID_544017	none	none	none	none
22	CID_312822	none	none	none	none
23	CID_5366244	none	none	none	none
24	CID_5280435	none	none	none	none

Docking Scores

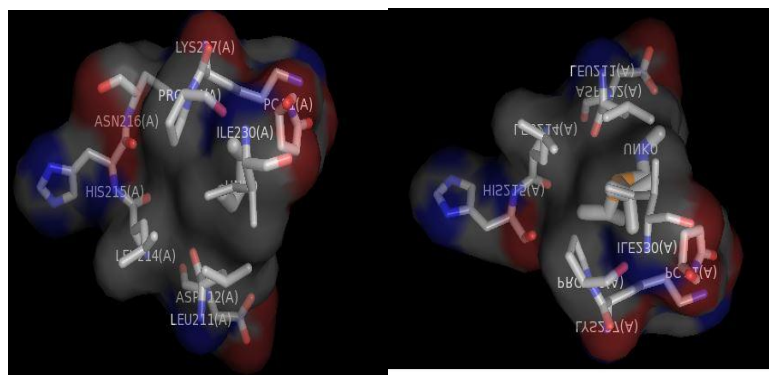
The molecular docking analysis of the selected compounds revealed that CID_312822 exhibited the most favorable binding energy at -9.22 kcal/mol, followed by CID_616782 with -8.42 kcal/mol and CID_41687 with -6.37 kcal/mol. Several other compounds demonstrated moderate binding affinities, including CID_544017 (-5.37 kcal/mol),

CID_530418 (-5.09 kcal/mol), CID_534396 (-5.00 kcal/mol), CID_534396 (-4.99 kcal/mol), CID_697893 (-4.92 kcal/mol), CID_5280435 (-4.90 kcal/mol), CID_5366244 (-4.89 kcal/mol), CID_546995 (-4.80 kcal/mol), CID_89719 (-4.72 kcal/mol), CID_5363335 (-4.43 kcal/mol), CID_95337 (-4.39 kcal/mol), CID_25045 (-4.25 kcal/mol), CID_283728 (-4.15 kcal/mol), CID_543706 (-4.03 kcal/mol), and CID_5364553 (-4.03 kcal/mol). These results, as summarized in Table 5, indicate that CID_312822 demonstrated the highest binding affinity, suggesting its potential as the most promising candidate for further investigation.

Table 5: Docking Scores and Residues Involved in H-Bond Formation

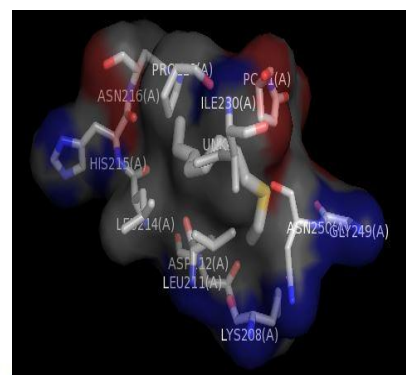
S/No.	Compound Id	Docking score (kcal/mol)	Residues involve in H-bonds	Distance (Å)
1	CID_616782	-8.42	0	0
2	CID_546995	-4.80	0	0
3	CID_283728	-4.15	0	0
4	CID_543706	-4.03	Pro228	2.70
5	CID_10798	-2.91		
6	CID_5364398	-3.23		
7	CID_697893	-4.92	Asn216	2.76
8	CID_10798	-2.91		
9	CID_543562	-3.36		
10	CID_89719	-4.72	Leu211	2.53
11	CID_534396	-4.99	Pro228	3.13
			Leu211	2.64
12	CID_530418	-5.09	Asp212	2.64
13	CID_5364553	-4.03	0	0
14	CID_25045	-4.25	Leu214	2.73
15	CID_534396	-5.00	Pro228	3.09
			Leu211	2.67
16	CID_41687	-6.37	0	0
17	CID_5363335	-4.43	0	0
18	CID_534521	-3.37		
19	CID_95337	-4.39	Asp212	2.70
20	CID_534521	-3.39		
21	CID_544017	-5.37	0	0
22	CID_312822	-9.22		
23	CID_5366244	-4.89	Asn216	3.00
24	CID_5280435	-4.90	Gly249	2.83
			Tyr2	3.23

Structure of protein-ligand complex of Residues involved

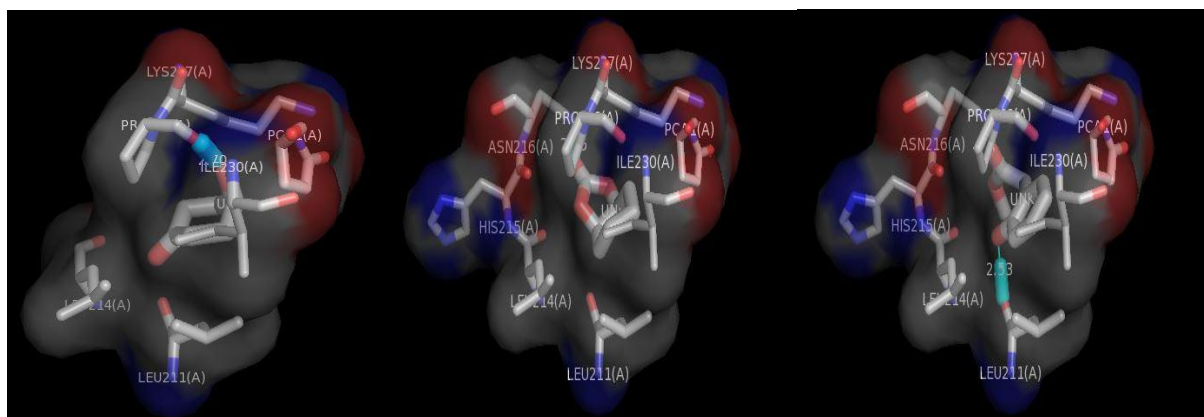


(a) CID_616782

(b) CID_546995



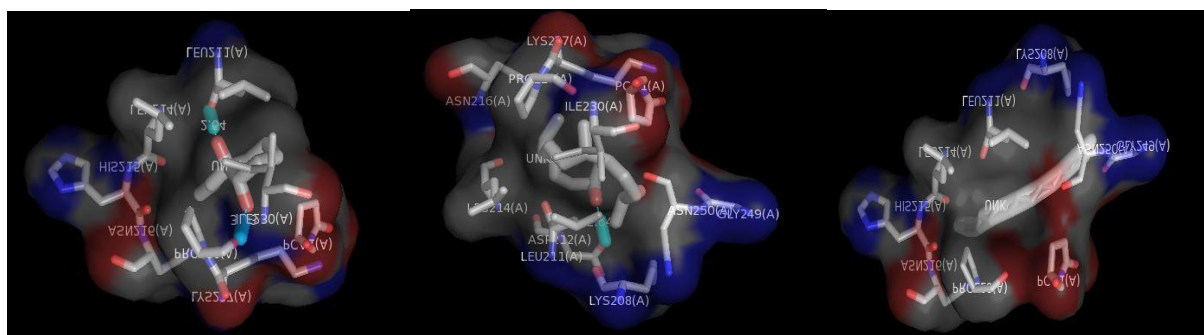
(c) CID_283728



(d) CID_543706

(e) CID_69789

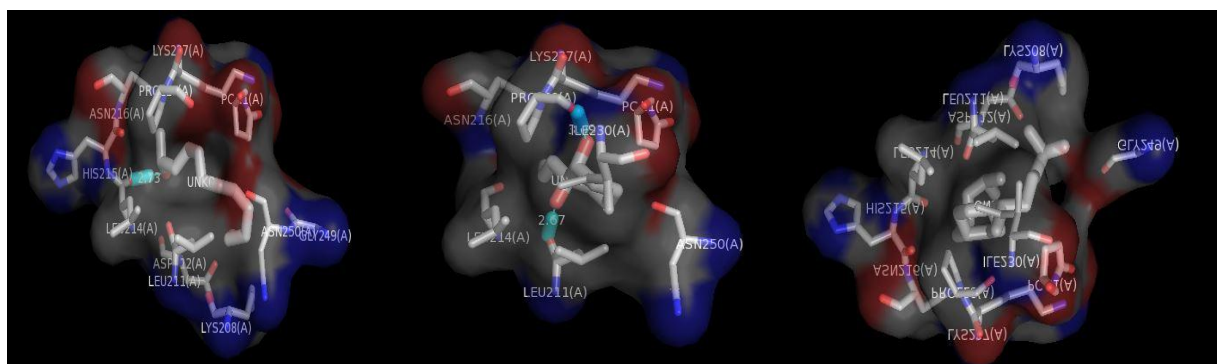
(f) CID_89719



(g) CID_534396

(h) CID_530418

(i) CID_5364553



(j) CID_25045

(k) CID_534396

(l) CID_41687

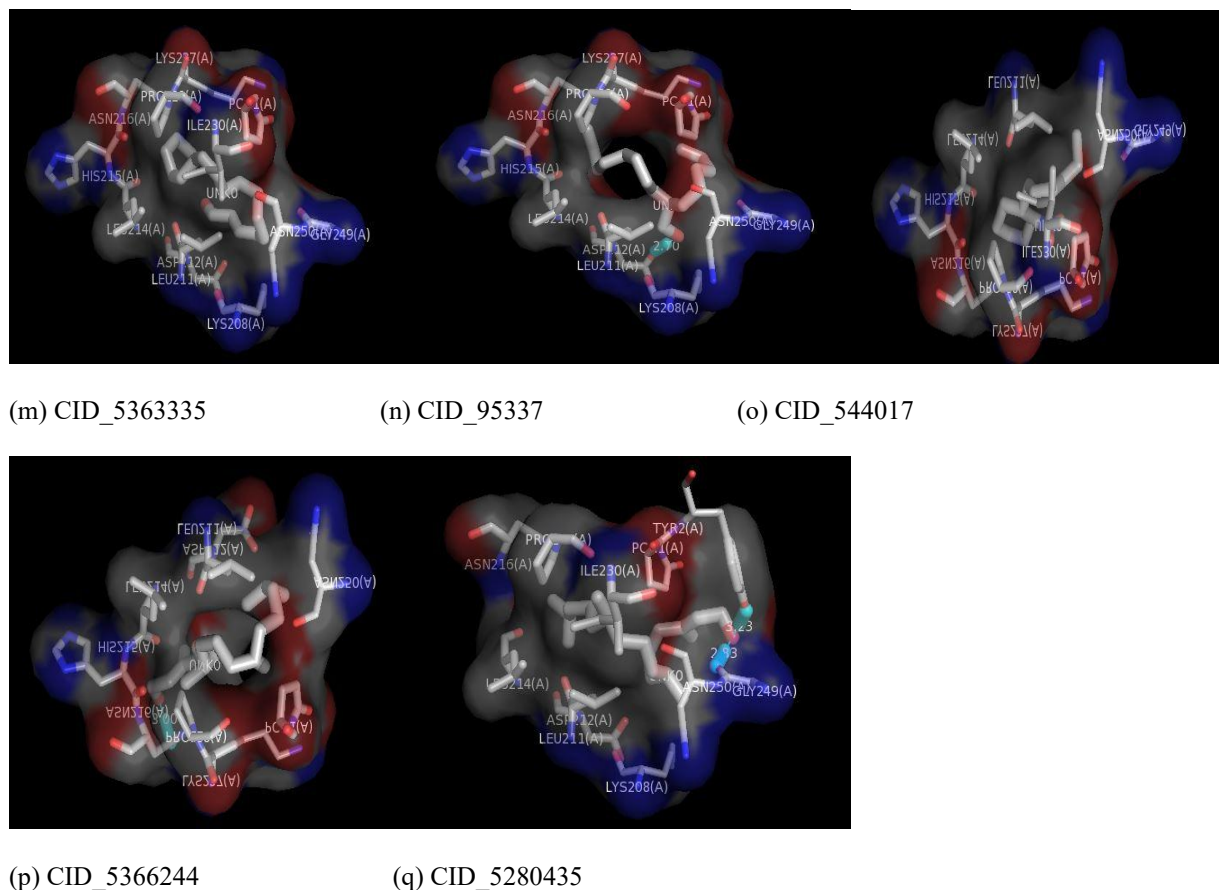


Fig. 5 (a-q): 3 D Protein-ligand interactions of human salivary amylase (has) enzyme with bioactive compounds from *Phyllanthus niruri*.

Discussion

Phytochemicals, which are naturally occurring bioactive compounds in plant-derived foods, are recognized for their antioxidant properties. These compounds play a crucial role in neutralizing free radicals, thereby preventing oxidative damage to cellular structures such as membranes, proteins, and DNA [17]. Phytochemical screening of the methanol leaf extract of *Phyllanthus niruri* confirmed the presence of alkaloids, tannins, flavonoids, anthocyanins, cardiac glycosides, and phenols, whereas saponins, steroids, quinones, and terpenoids were absent. This composition highlights the extract's potential pharmacological activity, with the absence of certain constituents possibly affecting its specific biological applications and efficacy [12]. Gas Chromatography-Mass Spectrometry (GC-MS) analysis identified 31 compounds, which were then evaluated based on Lipinski's Rule of Five (molecular weight ≤ 500 Da, hydrogen bond acceptors ≤ 10 , hydrogen bond donor ≤ 5 , $\text{LogP} \leq 5$) to determine their drug-likeness [19]. Of these, 18 compounds met the criteria and were subsequently subjected to molecular docking analysis to assess their binding free energy with the target protein.

The molecular docking study revealed that CID_312822 (Cholestan-3-ol, 2-methylene-, (3 β ,5 α)-(Campesterol) had the most favorable binding energy (-9.22 kcal/mol), followed by CID_616782 (1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl] bis [2-ethyl-]: (-8.42 kcal/mol) and CID_41687 (Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl): (-6.37 kcal/mol). Binding energy measures the strength and stability of the interaction between a ligand and its target protein, with more negative values indicating stronger and more stable interactions [07]. A lower binding energy signifies a higher affinity for the target, suggesting that the ligand has greater potential to bind effectively and inhibit the protein's function. Several factors contribute to variations in binding affinity among the

compounds, including molecular structure, hydrogen bonding interactions, hydrophobic interactions, and steric compatibility within the binding site [9]. For instance, CID_312822 (Cholestan-3-ol, 2-methylene-, (3 β ,5 α)-(Campesterol), which demonstrated the highest binding affinity, likely engages in strong hydrogen bonding and hydrophobic interactions with key amino acid residues in the binding pocket, enhancing the stability of the protein-ligand complex. In contrast, compounds with moderate or lower binding affinities may exhibit fewer hydrogen bonds or encounter steric hindrances that weaken their interactions.

The docking analysis further indicated specific hydrogen bonding interactions between certain compounds and key residues. CID_530418 (6,10,14-Trimethyl-pentadecan-2-ol): (-5.09 kcal/mol) formed a hydrogen bond with Asp212 at a distance of 2.64Å, whereas CID_534396 (1-Allyl-cyclohexane-1,2-diol): (-5.00 kcal/mol) established hydrogen bonds with Pro228 (3.09Å) and Leu211 (2.67Å). Such interactions contribute to the stability of the ligand-protein complex and may influence their potential biological activity [20]. Comparing these findings with previous studies on *in silico* antidiabetic activity helps validate the results and establish their relevance. Prior research has demonstrated that natural compounds with strong binding affinities, particularly those capable of forming multiple hydrogen bonds with essential active site residues, possess potential antidiabetic effects [9]. Notably, flavonoids and phenolic compounds have been reported to effectively interact with enzymes involved in glucose metabolism, such as alpha-amylase and alpha-glucosidase, suggesting their ability to regulate postprandial glucose levels [16]. The significant binding affinities of CID_312822 (Cholestan-3-ol, 2-methylene-, (3 β ,5 α)-(Campesterol) and CID_616782 (1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl] bis [2-ethyl-) support these findings, indicating their possible therapeutic relevance. The toxicity evaluation showed that most compounds were non-toxic, except for CID_5363335 (2-Heptadecenal), which exhibited high tumorigenic potential but low mutagenicity and irritability. These findings are crucial in determining the drug-likeness of the compounds and guiding further research on their pharmacological safety [21].

CONCLUSION

The molecular docking results suggest that CID_312822 (Cholestan-3-ol, 2-methylene-, (3 β ,5 α)-(Campesterol), CID_616782 (1H-Indole, 3,3'-[1-(4-pyridinyl)-1,2-ethanediyl] bis [2-ethyl-), and CID_41687 (Cyclohexane, 1-(1,5-dimethylhexyl)-4-(4-methylpentyl) demonstrate the highest binding affinities, making them promising candidates for further investigation in the development of antidiabetic drugs. The findings emphasize the importance of molecular interactions in drug discovery and underscore the necessity of validating *in silico* predictions through comparisons with existing literature to establish the therapeutic potential of bioactive compounds. Therefore, the bioactive compounds from methanol leaf extract of *Phyllanthus niruri* may be considered suitable inhibitors of human salivary amylase (HSA) for the treatment of Type II Diabetes mellitus.

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