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GC-MS Characterization and Molecular docking analysis of candidate compounds derived from aqueous extract of seed of *Phaseolus vulgaris* L. to identify anti-Diabetic potential

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Abstract:

Herbal drugs are used for the treatment of diseases and disorders with its less side effects, easy availability and low cost. Several bioactive compounds have been isolated from aqueous extract of seed of *Phaseolus vulgaris* L. and were taken for screening. This study aimed to evaluate molecular interactions of selected diabetes mellitus (DM) targets with bioactive compounds isolated from aqueous extract of seed of *Phaseolus vulgaris* L**.** In this article, screening of the best substances as bioactive compounds is achieved by molecular docking analysis with selected DM target proteins i.e., aldose reductase (AR). In this study, five phytochemicals of aqueous extract of seed of *Phaseolus vulgaris* L. were selected from GC- MS (5,12-Naphathacenequinone, 4- Isopropylbenzyl alcohol, Decanoic acid, 1,4- Benzenedicarboxylic acid, and 1,4-Dimethoxybenzene) were analyzed for the inhibition activity against Aldose Reductase (1US0) receptor of anti-diabetic activity using. The binding energy for each compound was chosen with the targets using AutoDock 4.2. The binding energy of -11.21, -4.99kcal/mol all values was shown for bioactive compound. Out of five compounds, 5, 12-naphathacenequinone, and 4-isopropylbenzyl alcohol were observed as most suitable ligands for management of diabetes mellitus**.**

1. Introduction

Diabetes mellitus (DM) is recognized as a metabolic disorder which results from the defect in insulin secretion and action [1]. According to World Health Organization, an estimated 422 million adults are living with diabetes mellitus (WHO 2016) [2]. In 2013, 381 million persons have been shown to have diabetes according to the International Diabetes Federation ("Simple treatment to curb diabetes") [3] and the number is projected to double by 2030.

Diabetes Mellitus is a cluster of metabolic disorder, an illness of hyperglycemia in which person grieves from disorders like failure of pancreas to produce insulin or insensitivity of cells towards insulin (insulin resistance). Diabetes Mellitus (DM) was previously called as "Non-insulin dependent diabetes mellitus" (NIDDM) [4].

Principal symptoms of DM are polyuria (recurrent urination), polydipsia (augmented thirst) and polyphagia (amplified hunger). Common explanations of Type 2 DM are lifestyle changes, obesity (defined as body mass index greater than 30), absence of physical activity, extreme body weight, deprived diet and anxiety [5]. There are numerous synthetic drugs available such as meglitinides, biguanides, sulfonylureas, thiazolidinediones, α-glucosidase inhibitors, dipeptidyl peptidaseIV inhibitors for treatment of DM [6, 7].

Today, researchers emphases primarily on finding of effective, low side effect and innocent therapeutic drugs to treat of DM [8]. Medicinal plants contain chemical groups (e.g., Phenolic acids, Flavonoids, Triterpenoids, Alkaloids and Carbohydrates) that hold strong antidiabetic properties, which can normalize blood glucose level. In traditional medicine, numerous medicinal plants were used that avoid difficulties in organization of Diabetes Mellitus.

Thus, the current research was intended to comprehend GC-MS Characterization and Molecular docking analysis of candidate compounds derived from aqueous extract of seed of *Phaseolus vulgaris* L. to identify anti-Diabetic potential.

2. Materials and Methods

2.1 GC – MS ANALYSIS

GC-MS analysis was dispensed on a Perkin Elmer Turbo Mass photometer (Norwalk, CTO6859, and USA) which includes a Perkin Elmer Auto sampler XLGC. The column used was Perkin Elmer Elite - five capillary column mensuration 30m × zero.25mm with a film thickness of 0.25mm composed of 95% Dimethyl polysiloxane. The carrier gas used was noble gas at a rate of flow of zero.5ml/min. 1µl sample injection volume was utilized. The inlet temperature was maintained as 250°C. The kitchen appliance temperature was programmed at first at 110°C for four min, then an increase to 240°C. And then programmed to increase to 280°C at a rate of 20°C ending with a 5 min. Total run time was 90 min. The MS transfer line was maintained at a temperature of 200°c. The source temperature was maintained at 180°c.

GCMS was analyzed using electron impact ionization at 70eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and processing were dispensed by Turbo-Mass OCPTVS-Demo SPL package.

2.2.1 Identification of chemical constituents

Interpretation on spectrum GC-MS was conducted mistreatment the information of National Institute customary and Technology (NIST) having quite sixty two,000 patterns. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library. Measurement of peak areas and processing were dispensed by Turbo-Mass OCPTVS-Demo SPL software package**.**

2.2 Molecular Docking Studies

2.2.1. Ligand Preparation

The structures of five phytochemicals namely 1,4 – Benzenedicarboxylic acid, Decanoic acid, 4-isopropylbenzyl alcohol, 1,4 – Dimethoxybenzene and 5,12 – Naphthacenequinone (figure 1) used in this study were found out from GC-MS result . These phytochemicals satisfied Lipinski‟s rule of five and ADME properties. The structures were takes as input for docking program in Autodock 4.0.

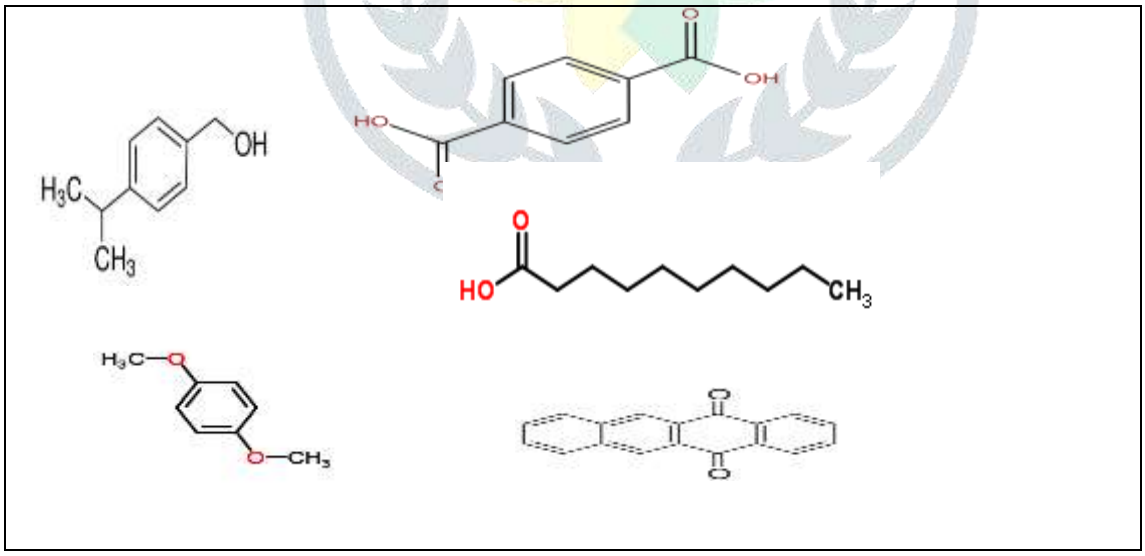


Figure1. Phytochemicals structures present *Phaseolus vulgaris L.*

2.2.2. Protein receptor Preparation:

The Insulin receptors Aldose Reductase, was imported from the Protein Data Bank (PDB) (http://www.rcsb.org/) shown in Figure .All the PDB was loaded in the Molegro virtual docker (MVD) with

the removal of all water molecules. The standard Molegro algorithm was utilized for rendering the missing charges, protonation states, and assigning of polar hydrogen to the receptor

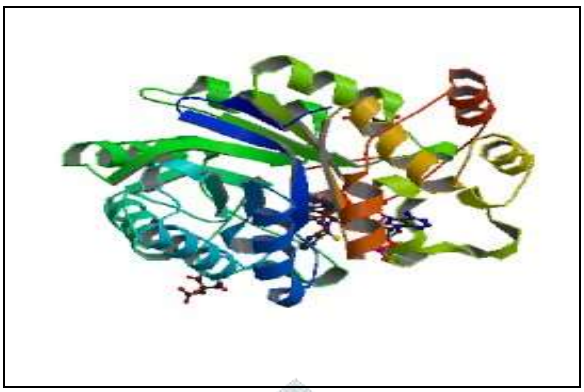


Figure 2: 3-dimensional structure of Aldose Reductase retrieved from PDB.

2.2.3. Docking program

Auto Dock calculations were performed in several steps: 1) preparation of coordinate files using Auto Dock Tools, 2) precalculation of atomic affinities using Auto Grid, 3) docking of ligands using Auto Dock and 4) analysis of results using Auto Dock Tools. The primary method for conformations searching is a Lamarckian Genetic Algorithm [9] and Auto Dock is shown to be an effective tool capable of quickly and accurately predicting binding conformations and binding energies of ligands with macromolecular targets [10, 11].

Docking is performed for protein and ligand molecules using one of several search methods. The most efficient method is Lamarckian genetic algorithm (LGA), but traditional genetic algorithms and simulated annealing are also available. For various systems, AutoDock is repeated several times to produce various docked conformations and analysis of binding energy and consistency of results are combined to recognize best solution.

3. RESULTS AND DISCUSSION

3.1. GC-MS analysis

GC-MS methodology used for the analysis of the obtained extract may be a stimulating tool for testing the number of some active principles in herbs utilized in cosmetic, drugs, pharmaceutical or food industry. The acidic fractions were silylated and subjected to GC-MS investigation. It is evident from the table one that each one fractions have convoluted chemical composition.

In the GC-MS analysis of aqueous extract of seed of *Phaseolus vulgaris L.* revealed molecular peaks, with typical retention time of analyzed total ions chromatogram which are shown in **Tables (1)** and **figures (3**). MS molecular (ionic) spectrum fragments were referred from Wiley Library sources.

Table 1: Separation of bioactive compound of aqueous extract of seed of *Phaseolus vulgaris* L. using GC-MS

RT	Compounds	Molecular Formula	Molecular Weight	Peak area %
12.98	4-isopropylbenzyl alcohol	$C_{10}H_{14}O$	150.22	2.315
14.16	Beta – Estradiol	$C_{18}H_{24}O_2$	272.38	2.609
19.08	1,4 – Dimethoxybenzene	$C_8H_{10}O_2$	138.16	2.478
20.25	**Decanoic acid**	**$C_{10}H_{20}O_2$**	**172.26**	**49.407**
22.02	**5,12 – Naphthacenequinone**	**$C_{18}H_{10}O_2$**	**258.27**	**49.70**
22.52	**1,4 – Benzenedicarboxylic acid**	**$C_{16}H_{22}O_4$**	**278.34**	**3.928**
26.32	Hentriacontane	$C_{31}H_{64}$	436.84	2.369
27.87	2 - Methyltriacontane	$C_{31}H_{64}$	436.85	2.369

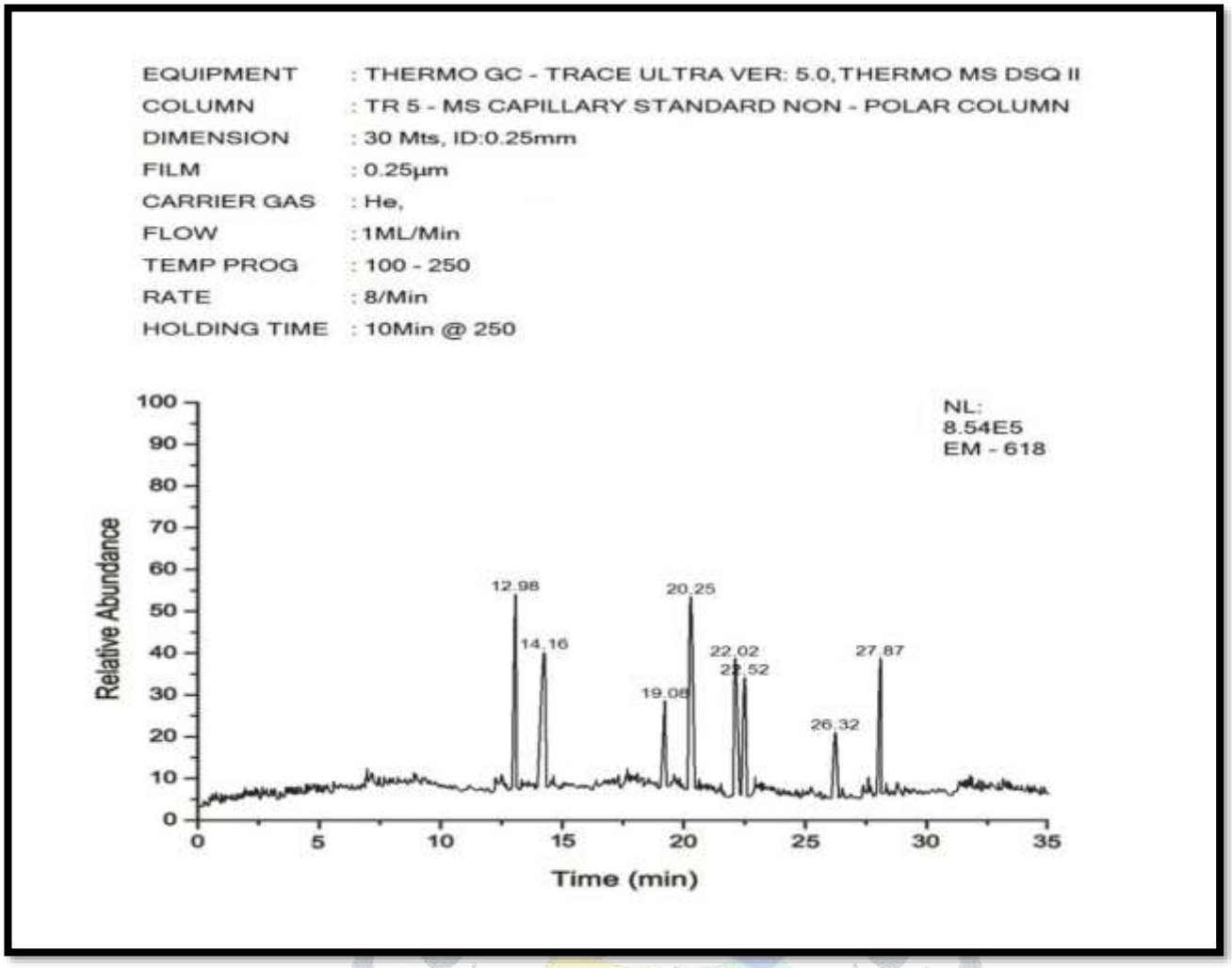


Figure 3: GC spectrum of aqueous extract of seed of *Phaseolus vulgaris* L.

GC-MS analysis of aqueous extract of seed of *Phaseolus vulgaris L.* revealed the presence of 8 major compounds include 4-isopropylbenzyl alcohol, Beta – Estradiol, 1,4 – Dimethoxybenzene, Decanoic acid, 5,12–Naphthacenequinone, 1,4 – Benzenedicarboxylic acid, Hentriacontane and 2 - Methyltriacontane

The prevailing compounds were Decanoic acid, 5,12–Naphthacenequinone and 1,4 – Benzenedicarboxylic acid (49.407, 49.70 and 3.928) respectively.

The biological activities of prevailing compounds are summarized in **Table 2**. The investigation finished that the stronger extraction capability of methyl alcohol and ethyl alcohol might being made range of active constituents accountable for several biological activities. So that those may well be used for the event of ancient medicines and additional investigation has to rinse novel active compounds from the healthful plants which can be created a new way to treat many incurable diseases.

Table 2: Activity of phyto-components identified in the aqueous extract of seed of *Phaseolus vulgaris* L by GC-MS

SI. No	Retention Time	Name of the Compound	Biological activity
1.	12.98	4 – isopropylbenzyl alcohol	• is a flavouring ingredient
2.	14.16	Beta – Estradiol	• is a steroid sex hormone vital to the maintenance of fertility and secondary sexual characteristics in females. • Estradiol exhibits mild anabolic and metabolic properties, and increases blood coagulability.
3.	20.25	Decanoic acid	• used in organic synthesis, perfume manufacturing, medicine, lubricating grease, rubber, and dye
4.	22.52	1,4 – Benzenedicarboxylic acid	• principally as a starting compound for the manufacture of polyester (specifically PET), used in clothing and to make plastic bottles

3.2. Molecular Docking Studies

The compounds 1,4 – Benzenedicarboxylic acid, Decanoic acid, 4-isopropylbenzyl alcohol, 1,4 – Dimethoxybenzene and 5,12 – Naphthacenequinone were selected due to their high pick value. The five compounds were analyzed for the inhibition activity against Aldose Reductase (1US0) receptor of anti-diabetic activity.

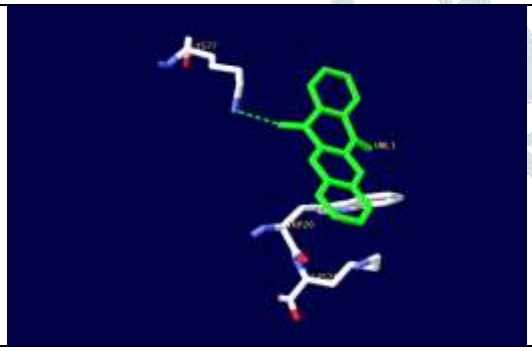
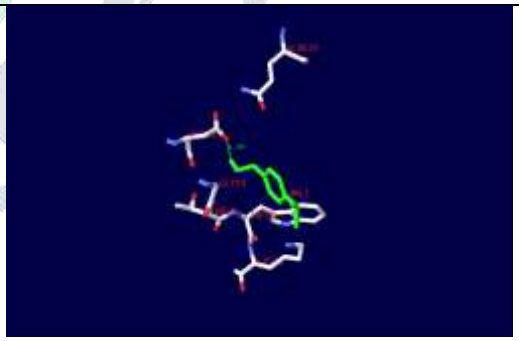
The molecular docking of the two compounds showed good binding mode and interaction energy. The binding energy for each chosen compound with the targets using AutoDock 4.2 is given in **Table 3**.

Table 3: Amino acids residues involved in hydrogen bond interaction with Phytocompounds of *Phaseolus vulgaris* L.

S. No	Phytochemicals	Binding energy kcal/mol	H bonds	Protein Ligand interactions (H bonds)
1.	5,12-Naphathacenequinone	-11.21	1	UNL1:H: - 1US0:A:LYS77:NZ
2.	4 – isopropylbenzyl alcohol	-6.48	1	UNL1:H: - 1US0:A:ASP43:OD2
3.	Decanoic acid	-5.98	1	UNL1:H: -1US0:A:LYS21:NZ

4.	1,4 – Benzenedicarboxylic acid	-5.51	2	UNL1:H: - 1US0:A:SER210:OG & UNL1:H: - 1US0:A:SER159:OG
5.	1,4 – Dimethoxybenzene	-4.99	1	UNL1:H: - 1US0:A:TRP20:N

The five phytochemicals were successfully docked using Auto dock 4 software. **Table 3** lists the interactions and the binding energy of phytochemicals. Great interactions between protein residues and ligand molecules were observed. The binding energy of -11.21, -4.99kcal/mol all values was shown for bioactive compound. The findings were analyzed based on the complex's binding energy. The number of H-bonds was determined using the length of the bond between the protein-ligand-docked complex atoms. Results from the current study showed that 5, 12-naphathacenequinone, and 4-isopropylbenzyl alcohol had the lowest binding energy value of -11.21kcal/mol and -6.48 21kcal/mol respectively (**Figure 4(1, 2)),** which showed high energy activity. Both phytochemical interactions with the aldose reductase receptor showed one hydrogen bond. 5, 12- naphathacenequinone demonstrates interactions with LYS77 predicted amino acid, in active site region with 1US0 receptor. In the active site region with 1US0 receptor, 4-isopropylbenzyl alcohol showed one hydrogen interaction with ASP43 predicted amino acid.

1	Binding mode of 5,12- Naphathacenequinone (green)into the binding site of aldose reductase receptor(PDBID:1US0)	2	Binding mode of 4- Isopropylbenzyl alcohol (green) into the binding site of aldose reductase receptor (PDBID:1US0)
			
3	Binding mode of 1,4- Benzenedicarboxylic acid (green)into the binding site of aldose reductase receptor(PDBID:1US0)	4	Binding mode of 1,4- Dimethoxybenzene (green)into the binding site of aldose reductase receptor(PDBID:1US0)

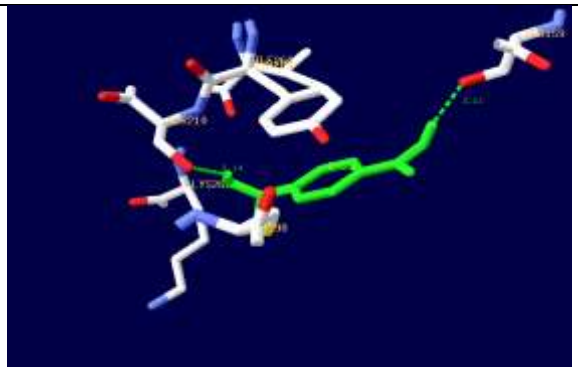
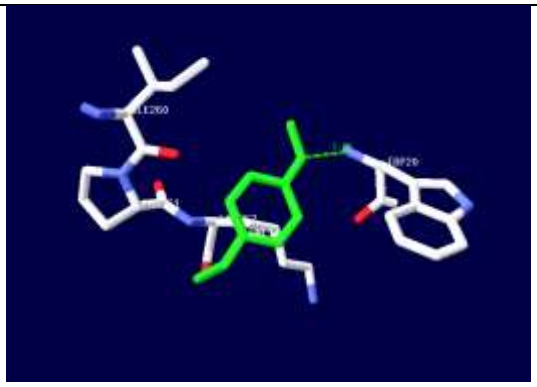
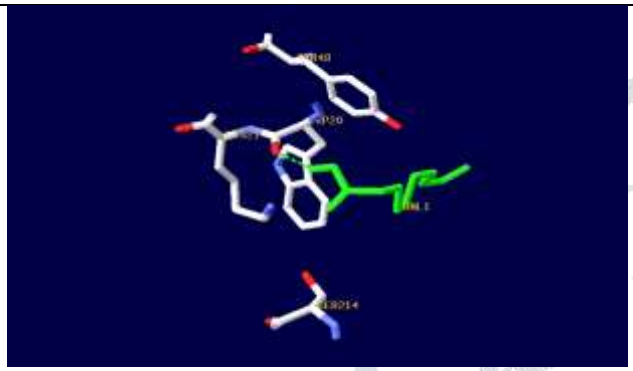
			
5	Binding mode of Decanoic acid acid(green)into the binding site of aldose reductase receptor(PDBID:1US0)		
			

Figure 4: Summary of Docked Pose of the Compounds identified from aqueous extract of seed of *Phaseolus vulgaris* L.

The screening of phytochemicals from aqueous extract of seed of ***Phaseolus vulgaris* L.** against diabetes target aldose reductase receptor was carried out using molecular docking methods. The docked five compounds demonstrated binding affinity towards the aldose reductase receptor. Among the five, 5, 12 – Naphathacenequinone and 4 – isopropylbenzyl alcohol were identified with the least binding energy of -11.21 and -6.48 21kcal/mol respectively which its high activity in a given biological activity.

CONCLUSION

In summary, from the GC-MS analysis of aqueous extract of seed of ***Phaseolus vulgaris* L.** 8 compound were found out namely 4-isopropylbenzyl alcohol, Beta – Estradiol, 1,4 – Dimethoxybenzene, Decanoic acid, 5,12–Naphthacenequinone, 1,4 – Benzenedicarboxylic acid, Hentriacontane and 2 – Methyltriacontane. Out of 8 compounds 5 compounds were selected due to their high peak value for molecular docking. The five phytochemicals were successfully docked using Auto dock 4 software. Results from the current study showed that 5, 12-naphathacenequinone, and 4-isopropylbenzyl alcohol had the lowest binding energy value of -11.21kcal/mol and -6.48 21kcal/mol respectively. The molecular docking of the two compounds indicated great binding mode and collaboration energy. The H-bond pattern was examined and confirmed inhibition of diabetes target and showed that the phytochemicals possessed possible anti-diabetic activity.

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