

GC-MS Characterization of the Anti-Diabetic Compounds From the Flowers of *Cassia Auriculata* (AVARAM): A structure Based Molecular Docking Studies

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ABSTRACT: Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alterations in carbohydrate, and classified as Type I and Type II. The incidence of type II diabetes has a major health risk and significant increases in the incidence of metabolic diseases, efficient strategies for preventing and treating of these common disorders are urgently needed. This includes the development of phytopharmaceutical products or functional foods to prevent or cure metabolic diseases. Plants have been an important source of medicine with qualities for thousands of years. Mainly on traditional remedies such as herbs for their history it has been used as a popular folk medicine. *Cassia Auriculata* has medicinal values; ethanol flower extract of this plant was analyzed using Gas chromatography Mass Spectrometry. While the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. Gas Chromatography Mass Spectrometry (GC-MS) analysis revealed the presence of 8 compounds. In GC-MS analysis some of the phyto-compounds Screened were Preganane-3,11,12,14,20-pentol, 3,12,20-triacetate 11-(hydroxyacetate), 2-propenoic acid, 3-(4-methoxyphenyl), ethyl ester, (E)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)undec-1-ene-3,6-dione. These compounds were analyzed for anti-diabetic activities with their protein targets using Molecular docking studies. Docking results showed best Glide energy, Docking score, H-bonding interactions compared with molecular targets and has the potential to prevent or treat type 2 diabetes and related disorders.

KEYWORDS: *Cassia Auriculata*, GC-MS, Anti-diabetic, Propenoic acid, Molecular docking, Glide energy, H-bonding interaction, docking score.

I. INTRODUCTION

According to the World Health Organization (WHO), in the world there are over 347 million people suffering from diabetes mellitus (DM), becoming a global epidemic. Projections indicate that by 2030 diabetes mellitus will become the seventh leading cause of death worldwide, and type 2 DM (T2DM) will be the responsible for 90% of cases.^[1] The medicinal plants as sources of bioactive compounds continue to play a dominant role in the maintenance of human health. Reports available on green plants represent a reservoir of effective chemotherapeutants; these are non-phototoxic, more systemic and easily biodegradable.^[2-4] Hence a thorough validation of the herbal drugs has emerged as a new branch of science emphasizing and prioritizing the standardization of the natural drugs and products because several of the phytochemicals have complementary and overlapping mechanism of action. Mass spectrometry, coupled with chromatographic separations such as Gas chromatography (GC/MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants. In recent years GC-MS studies have been

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increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of non polar components, volatile essential oil, fatty acids and lipids. ^[5, 6]

T2DM, a metabolic disease characterized by a persistent increase in blood glucose above normal values (hyperglycemia) due to a progressive insulin secretory defect on the background of insulin resistance ^[7] is the most common form of the disease. In recent years, many reviews focusing on natural products and medicinal plants with anti-diabetic potential have been published. ^[8] In other types of reviews, the authors were focused their efforts on compiling other antidiabetic medicinal plant from different parts was analyzed by GC-MS. The Identification of Compounds was structure based molecular docking studies were carried out by Sivasubramanian Suhitha and Seenivasan Karthiga Devi et al. ^[9] The aim of the present study is to identify the phytochemicals of this plant and subjecting the ethanol extract of the plant flowers to Gas chromatography – Mass Spectrum analysis. A structure based activity was carried out using molecular docking studies was reported for the first time. This work will help to identify the newer compounds, which may be used in anti-diabetic drug design products and for its therapeutic values.

II. MATERIALS AND METHODS

2.1. Collection and processing of plant material

The plant *Cassia Auriculata*, (family-Fabaceae, Subfamily: *casalpinioideae*) the flowers were collected from the natural habitats of Cheyyar, Tamil Nadu and India. Flowers were cleaned with tap water later cleaned twice with distilled water. Then they were allowed to shade dry and pulverized to powder using a mechanical grinder. The powder was stored in an air-dried packet.

2.2. Plant sample extraction- Soxhlet extraction method

The plant material 50 g was extracted successively with 500 ml of ethanol by using a Soxhlet extractor until a complete extract were effected (4-6 h) at a temperature not exceeding the boiling point. The ethanol extract was collected after filtration and evaporated to dryness under reduced pressure using a Rota vapor and the resulting pasty form extracts analysis the phytochemical screening and GC-MS analysis was carried out for this ethanol extract.

2.3. Phytochemical analysis

The extracts were subjected to preliminary Phytochemical testing to detect for the presence of different chemical groups of compounds. The plant extracts was carried out qualitatively for the presence of Alkaloids, carbohydrates, fixed oils, tannins, flavonoids, saponins, terpenoids, lignin and sterols by using the standard method given by (Harborne, 1998). ^[10]

2.4. GC – MS ANALYSIS

Gas Chromatography–Mass Spectrometry using JEOL GCmate™ II GC/MS Double-Focusing Mass Spectrometer. A HP-5 MS capillary column (28 m × 0.25 mm × 0.25 μm) with helium as carrier gas (1.0 ml/min) was used for the gas chromatographic separation. The injection mode was split (split ratio: 20:1), injection volume was 2 μL, and the temperature of vaporization chamber ranged from 50°-250°C. The column was eluted with ethanol at a flow rate of 0.3ml/min with an increase of 10°C every min. Mass detector was EI-Detection (70 eV). The mass spectra of the compounds from compared with the NIST08 GC-MS database library with 346,757 Kovats retention index values for 70,835 compounds (38,648 in the EI library), covering both polar data.

2.5. The Protein targets

Binding DataBase is public, web-accessible database of measured binding affinities, focusing chiefly on the interactions of proteins considered to be the drug-target molecules ^[11,12] This page allows to enter one or more compounds and quickly see a list of Targets that compound(s) might bind, based on the principle that similar these compounds tend to bind the same proteins. One has to draw one compound, or upload a file of compounds, and click the GO button to see a list of protein Targets bound by similar compounds. Human monoamine oxidase B (MAO B), ^[13] Aldo-keto reductase family 1 member B10 (AKR 1 B10), ^[14] Liver fatty acid-binding protein (LFABP), ^[15] Human cytochrome P450 2E1 (CYP450-2E1) ^[16] and Glucocorticoid Receptor (GR) ^[17] have been chosen from the PDB IDs (4A79), (1ZUA), (3STM), (3E6I), (3BQD), respectively.

2.6. Molecular Docking Studies

Molecular docking is a method to predict the exact orientation of one molecule to another molecule when bound to each other to form stable complexes. This study has been carried out with the commercial Schrodinger software.

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2.6.1. Ligand Preparation

The structures of the compounds were drawn using the software Chemsketch. The ligand preparation is to prepare the three dimensional structure of drug like molecules in Maestro format. The impact module performs conversions, apply corrections to the structures, generate variations on the structures and optimize the structures. The structures were minimized using impact energy minimization with 1000 cycles of Steepest Descent and 5000 cycles of Conjugate gradient.

2.6.2. Protein Preparation:

The X-ray crystal structures retrieved from PDB database as raw could not be suitable for molecular docking studies. A typical PDB structure consists only of heavy atoms, waters, cofactors, metal ions and can be of multimeric. These structures do not have the information about bond orders, topologies or formal atomic charges. The terminal amide groups may be misaligned because the X-ray structure analysis cannot distinguish between O and NH₂. Ionization and tautomeric states are also unassigned. So, the raw PDB structure retrieved from PDB should be prepared in a suitable manner for docking. Protein Preparation Wizard of GLIDE software was used to process and prepare the protein. This Wizard allows one to properly prepare a protein for docking studies. This also follows the Optimized Potential for Liquid Simulations-All Atoms (OPLS-AA) force fields for energy minimization. The protein was prepared by removing all the water molecules and sulphate ions present in the structure. Since the raw data do not contain any hydrogen in it, the implicit hydrogen atoms were added to the atoms to satisfy their appropriate valencies. Then the structure was optimized by assigning the bond orders, bond angles and topology. The formal atomic charges were fixed for the amino acid residues and the optimized structure was then energy minimized to remove the steric hindrance between the atoms.

2.6.3. Induced Fit Docking

In the docking studies, for the sake of faster calculations the receptor site is considered as rigid and ligand is considered as flexible and this is known as rigid docking. But when limited number of ligands is used, both the receptor and ligand are considered to be flexible and this is known as Induced Fit Docking (IFD). In IFD, conformational changes occur in the side chain and backbone of the active site during the ligand binding. These changes allow the receptor to alter its binding site, so that it more closely conforms to the shape and binding mode of the ligand. This is often referred as "induced fit" and it is one of the important complicating factors in structure based drug design. This induced fit docking has two main applications. i) Generation of an exact complex structure for a ligand, which is known to be active but that cannot be docked in an existing structure of the receptor. ii) Release of false negatives during virtual screening, where instead of screening against a single conformation of the receptor, additional conformations are obtained with the induced fit protocol used. In this study IFD is one of the main complicating factors which predicts accurate ligand binding modes and concomitant structural movements in the receptor using the modules of Glide and Prime. The energy minimized complex was loaded in the workspace and the ligand was selected to specify the active site. All the screened compounds were docked with respective protein targets. In IFD results, the best conformation was chosen for further calculations based on the docking score, Glide energy and non-bonded interactions. The glide score is the sum of the energy terms such as lipophilic, hydrogen bonding (neutral-neutral, neutral-charged and charged-charged), metal interaction, rotatable bond and solvation terms. The glide energy is the ligand binding energy calculated based on the OPLS-AA force field. The best compound is selected based on the least glide score or the least glide energy^[9, 18].

III. RESULTS AND DISCUSSION

3.1. GC-MS

The peaks are marked with retention time in the GC-MS chromatogram of the ethanol extract of the flowers of Cassia Auriculata. Their retention time (RT) and amount of their presence as indicated by listed in (Table II). The qualitative analysis of the extract, showed the presence of eight compounds in ethanolic extract. Pentadecane with retention time (10.4), Pregnanane-3,11,12, 14, 20-pentol, 3, 12, 20-triacetate 11-(hydroxyacetate) with retention time (12.33), 2-propenoic acid, 3-(4-methoxyphenyl), ethyl ester with retention time (14.12), Dodecanoic acid with retention time (15.3), Hexadecanoic acid, ethyl ester with retention time (15.95), 8-Octadecanoic acid, methyl ester with retention time (17.03), 9-Octadecanoic acid (Z) with retention time (17.68) and E)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)undec-1-ene-3,6-dione with retention time (19.37). The biological activities based on Dr. Duke's Phytochemical and Ethnobotanical Databases and Passonline Database, Binding Database were tabulated in (Table I)^[19]

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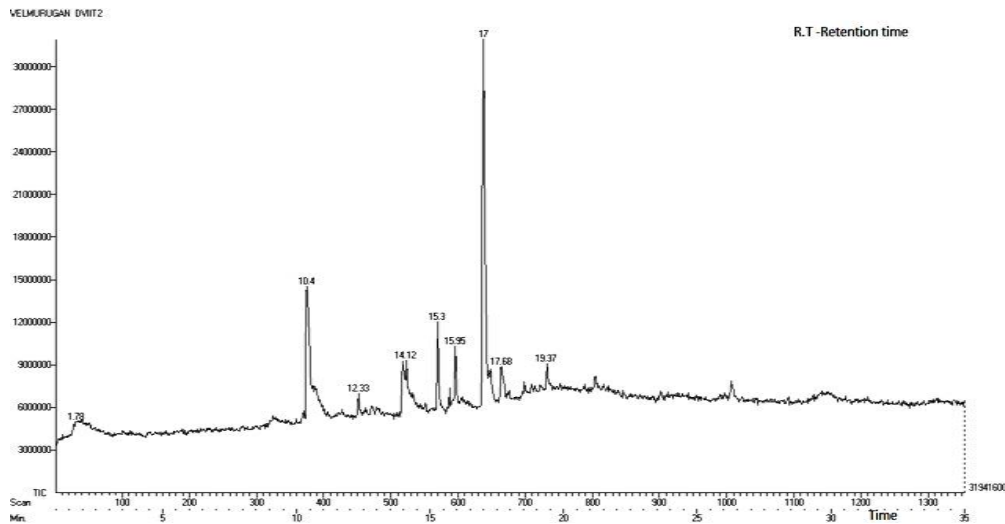


Fig 1: GC MS of flowers of *Cassisaauriculata*Linn

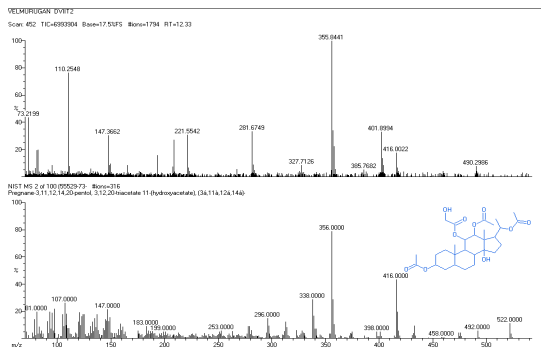


Fig.2.Preganane-3, 11, 12, 14, 20-pentol,3, 12, 20-triacetate 11-(hydroxyacetate) - $C_{29}H_{44}O_{10}$

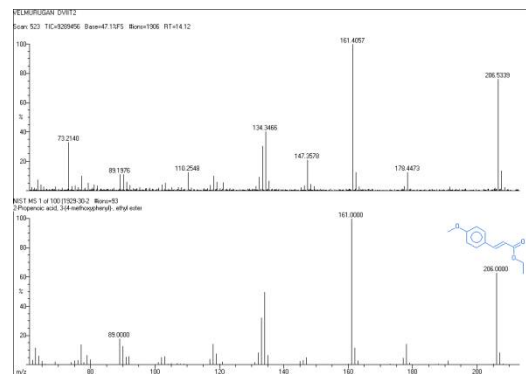


Fig 3. 2-propenoic acid,3-(4-methoxyphenyl), ethyl ester - $C_{12}H_{14}O_3$

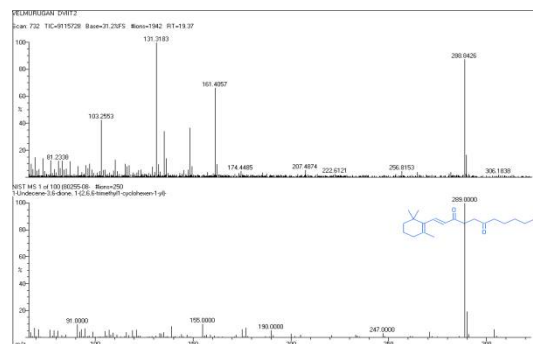


Fig.4. (E)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)undec-1-ene-3,6-dione- $C_{20}H_{32}O_2$

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Table 1. The Chemical Composition of flowers of *Cassisaauriculata* Linn

No	RT	Name of the compound	Molecular formula	MW	Compound Nature	Activity
1.	10.4	Pentadecane	C ₁₅ H ₃₂	212.41	alkane hydrocarbon	Sugar-phosphatase inhibitor, Acrocyllindropepsin inhibitor, Chymosin inhibitor
2	12.33	Preganane-3,11,12, 14, 20-pentol,3, 12, 20-triacetate 11-(hydroxyacetate)	C ₂₉ H ₄₄ O ₁₀	552.25	Steroids	UDP-glucuronosyltransferase substrate, Alkenylglycerophosphocholine hydrolase inhibitor
3.	14.12	2-propenoic acid,3-(4methoxyphenyl), ethyl ester	C ₁₂ H ₁₄ O ₃	206.04	Alkene compound	Anticancerous activity, Antidiabetes,
4.	15.3	Dodecanoic acid	C ₁₄ H ₂₈ O ₂	228.37	Lauric acid	Antioxidant, Antibacterial, COX-1&COX-2 inhibitor, Antiviral, Hypocholesterolemic, candidicide.
5.	15.95	Hexadecanoic acid, ethyl ester	C ₁₈ H ₃₆ O ₂	284.48	Fatty acid ester	Antioxidant, Flavor, Hypocholesterolemic Nematicide, Pesticide, Lubricant,Antiandrogenic, Hemolytic, 5-Alpha reductase inhibitor
6.	17.03	8-Octadecanoic acid, methyl ester	C ₁₉ H ₃₆ O ₂	296.49	Fatty acid ester	Antioxidant, Antimicrobial
7.	17.68	9-Octadenoic acid (Z)	C ₂₁ H ₄₀ O ₄	356.54	Linoleic acid	Hypocholesterolemic, 5-Alpha reductaseinhibitor,Antihistaminic,Insectifuge, Antieczemic, Antiacne
8.	19.37	(E)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)undec-1-ene-3,6-dione	C ₂₀ H ₃₂ O ₂	304.47	Norterpenoide	Antioxidant, anticancer,

3.2. Molecular Docking Studies

3.2.1. Glucocorticoid Receptor(GRLBP) Target

The co-crystal ligand Deaccylcortivazol was redocked with the target Glucocorticoid receptor (GR) (PDB ID:3BQD) at the active site. The docked complex had the docking score of -12.11 and glide energy of -60.952 kcal/mol. The docked complex had three hydrogen bond interactions with the active site residues ASN 574, ASN 564, GLN 642 at distance of 3.39,2.76,3.14 Å, respectively Preganane-3,11,12,14,20-pentol,3,12,20-triacetate 11-(hydroxyacetate) showed docking with residues GLN 642, MET 601, LEU 563 at a distance of 3.09Å, 2.62 Å, 3.10Å with glide score of -14.01 and glide energy -65.33kcal/mol (Table-II) the list the IFD results and (fig.5) shows the hydrogen bond interactions at active site.

Table 2: Molecular Docking Studies of the Compound and native crystal

COMPOUND NAME	DOCKING SCORE	GLIDE ENERGY Kcal/mol	HYDROGEN BOND INTERACTION (D-H...A)	DISTANCE (Å)
Preganane-3,11,12,14,20-pentol,3,12,20-triacetate 11-(hydroxyacetate),	-14.011	-65.332	GLN 642 (N-H...O)	3.09
			MET 601(O-H...O)	2.62
			LEU 563 (O-H...O)	3.10
<u>Native crystal</u> Deaccylcortivazol	-12.112	-60.952	ASN 574 (N-H...O) ASN 564 (O-H...O) GLN 642 (O-H...O)	3.39 2.76 3.14

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COMPOUND NAME	DOCKING SCORE	GLIDE ENERGY Kcal/mol	HYDROGEN BOND INTERACTION (D-H...A)	DISTANCE (Å)
<u>Co-crystal-(PDB ID: 4A79)</u> Pioglitazone	-5.98	-26.84	TYR 435 (O-H...O)	3.22
<u>Co-crystal-(PDB ID :1ZUA)</u> Tolrestat	-10.53	-45.84	TYR 112 (N-H...O) HIS 111 (N-H...O) HIS 111 (N-H...O)	2.97 3.01 3.34
<u>(E)-1-(2,6,6-trimethylcyclohex-1-en-1-yl)undec-1-ene-3,6-dione</u> (PDB ID: 4A79)	-12.09	-45.38	TYR 435 (O-H...O) GLN 206 (N-H...O)	2.89 2.80
<u>(PDB ID :1ZUA)</u>	-8.72	-45.94	TYR 112 (N-H...O) HIS 111 (N-H...O)	3.08 2.76

Table 4: Molecular Docking Studies of the Compounds and native crystals

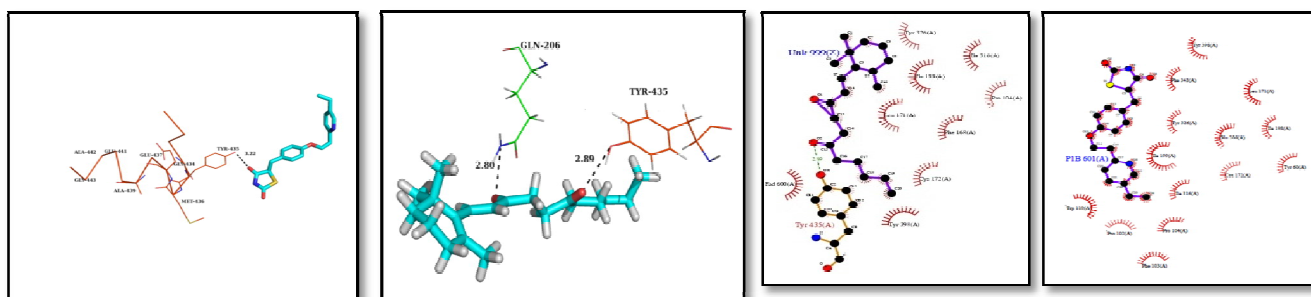


Fig.8.Pymol and ligplot view of compound and co-crystal Human monoamine oxidase B (MAO B)

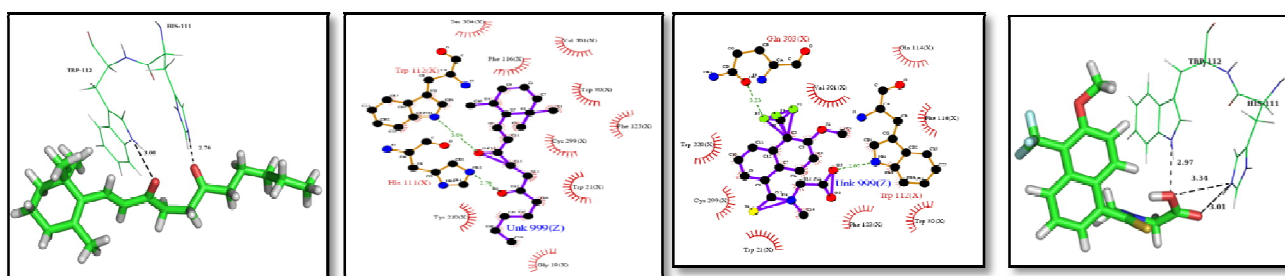


Fig.9.Pymol and ligplot view of compound and co-crystal Aldo-keto reductase family 1 member B10

IV. CONCLUSION

Molecular docking results clearly substantiate the binding of selected compounds and active site of the respective target suggesting the possibility for action of the compounds in curing the type 2 Diabetes. The GC-MS study reveals that *Cassia auriculata* contains a number of phytoconstituents which are to be used for various therapeutic purposes. In this study, identified compounds from GCMS data of *Cassia auriculata* flower, have been characterised as antidiabetic drugs as several targets like Human monoamine oxidase B (MAO B), Aldo-keto reductase family 1 member B10(AKR 1 B10), Liver fatty acid-binding protein(LFABP), Human cytochrome P450 2E1(CYP450-2E1) and Glucocorticoid Receptor(GRLBP) have been implicated in diabetic disease. An in Silico docking studies were carried for molecular understanding of inhibition mechanism of identified compounds against diabetic drug target (MAO B, AKR 1 B10, CYP450 2E1, LFABP, GRLBP). Further, the docking results suggest that GC-MS Com-2, GC-MS Com-3

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and GC-MS Com-8 can be used as better drugs against MAO B, CYP450 2E, LFABP, GRLBP compared to available drugs.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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