

**Original Research Article**

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**IN SILICO SCREENING OF PHYTOCHEMICALS IDENTIFIED FROM *AEGICERAS CORNICULATUM* (L.) BLANCO FOR ITS ANTI-DIABETIC ACTIVITY****P. G. Geegi<sup>1\*</sup>, N. Manoharan<sup>2</sup>**

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**ABSTRACT:** In present study the phytochemical analysis of the leaves of *Aegiceras corniculatum* (L.) Blanco was done using GCMS method and five compounds were identified as 4,4'-(Tetramethylenebisoxo)bis(2-nitro-5-methoxybenzoic acid methyl) ester, Ethyl 2,2-diethoxyacetate, Pentadecan-4-yl cyclopentanecarboxylate, 2-O-(2-methylpropyl) 1-O-pentyl oxalate and, N-(4-nitrobenzilidene)-tert-butylamine. The anti-diabetic activity of these five compounds was analyzed with molecular docking study against the dipeptidyl peptidase IV (DPP-IV) which is found elevated in person with diabetes. Among the five phytochemicals identified, 2-O-(2-methylpropyl) 1-O-pentyl oxalate was observed to have maximum score with DPP-IV in molecular docking study and taking into consideration of all the scoring parameters, 2-O-(2-methylpropyl) 1-O-pentyl oxalate is considered as the best fit. Extended studies are required for the isolation of bioactive phytochemical and subject it to additional cell line and animal testing for anti-diabetic studies and can be treated to be an effectual and inexpensive drug because of higher biomass obtainability of *Aegiceras corniculatum* (L.) Blanco.

**KEYWORDS:** *Aegiceras corniculatum* (L.) Blanco; Anti-diabetic Study; Autodock; In silico Studies; Molecular Docking.

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**1. INTRODUCTION**

Diabetes mellitus (DM) is recognized as a metabolic disorder which results from the injured in insulin secretion and action [1]. According to World Health Organization, an assessed 422 million grownups are existing with DM (WHO 2016) [2]. In 2013, 381 million persons have been shown to

have diabetes rendering to the International Diabetes Federation (Simple treatment to curb diabetes) [3] and the number is projected to double by 2030[4,5]. Glucagon-like peptide-1 (GLP-1) [6] is an incretin hormone released from the L cells of the small intestine in reaction to food consumption. This hormone performs numerous biological functions including the stimulus of insulin secretion, inhibition of glucagon release, obstruction of gastric clearing, generation and encouraging the regeneration and distinction of islet  $\beta$ -cells[7-11]. However, GLP-1 (GLP-1amide) is swiftly destroyed in vivo (lifetime: about 1 min) through the performance of dipeptidyl peptidase IV (DPP-IV), which conjoins a dipeptide from the N-terminus to offer the inactive GLP amide [12,13]. DPP-IV is a serine protease slashing the N-terminal dipeptide with an inclination for L-proline or L-alanine at the last but one position [14]. This protease is expressed in many tissues and body fluids, and arises as either a membrane-bound or a soluble enzyme[15]. Retardation of DPP-IV intensifies the level of flowing GLP-1 and thus upsurges insulin release, which can upgrading hyperglycaemia in type 2 diabetes[16]. Recently, the therapeutic value of mangroves and related plants persevere to deliver precious medicinal agents, both in modern medicines and in traditional systems[17]. The traditional applications of black mangrove, *Aegiceras corniculatum* (L.)Blanco has been chosen for the present study, which belongs to the myrsinaceae family scattered in coastal and estuarine regions of India[18]. Also, the ethno pharmacological significance aimed out the study plant conventionally operated for the healed of painful arthritis, inflammation, free radical scavenging, asthma antioxidant, rheumatism, antinociceptive, anti-inflammatory, hepatoprotective and diabetic actions [19]. The purpose of this study is to examine the anti-diabetic bioactive compounds present in the leaves of *Aegiceras corniculatum* (L.) Blanco using GC-MS analysis and molecular docking studies to ascertain the effective active compounds which can be used in the therapy of DM.

## 2. MATERIALS AND METHODS

### Collection and Authentication of the Plant Material

The plant named *Aegiceras corniculatum* (L.) Blanco. was collected from Muthupettai region. mangrove forest situated in Thiruvarur district, Tamil nadu, South india (Lat' 10°20' long. 79°35' N) is covering an area of about 20,000 ha.[20] The mangrove are always an association of halophyte trees, shrubs, and other plants growing in brackish to saline tidal water of tropical and subtropical coastlines[21]. The healthy plant material of *Aegiceras corniculatum*, were collected from the muthupettai and used for study. The material were identified and authenticated by taxonomist Rev. Fr. Dr. John Britto MSc., M Phil., PhD

### Dry Powder Preparation

The plant leaf sample was dried in hot air oven at 40°C for 24 hours and ground into powder.

### Sample Preparation for GCMS Analysis

*A. corniculatum* leaves were collected and shade dried. The leaves were powdered to moderately coarse grade. Ethanolic extract of leaves was obtained by using Soxhlet apparatus. The extraction is

continued for 12 cycles or until the solvent in the thimble was clear. After evaporating the solvent, the greenish semisolid extract was kept in an air tight container at 37°C for further use. Suspensions of extract were freshly prepared using Dimethyl sulfoxide (DMSO), for experimental use. The cleaned extract was then concentrated to about 1 ml using nitrogen concentration before being introduced into the GC-MS analyser.

### **Gas Chromatography-Mass Spectrometry (GC-MS) Analysis**

The GCMS analysis was conducted at the TanBio R&D Solution, Vallam, Thanjavur. 2µL aliquot was injected into a Fisons GC8000 series GC coupled to a MD800 MS with quadrupole mass analyzer. The chromatography was performed by using the DB5-MS column. Injection temperature was 230°C. Helium flow was 1 mL/min. After a 5 min solvent delay time at 70°C; the oven temperature was increased at 5°C/min to 310°C, 1 min isocratic, and cooled to 70°C, followed by the additional 5 min delay. The ion trace integration was done using the mass lab find target method for the characteristic fragment of assigned peaks[22,23].

### **Identification of Components**

Interpretation of mass spectrum GCMS was conducted using data base of National Institute Standard and Technology (NIST) and Wiley spectra libraries. Spectrum of the unknown component was compared with the spectrum of known components stored in the NIST library[24]. The molecular weight, molecular formula, and the number of hits used to identify the name of the compound from NIST and Wiley spectra libraries were recorded[25,26].

### **Docking Analysis**

All computational studies were carried out using AutoDock 4.0.121 with MGL tools 1.5.6 installed in a 8 CPU machine running on a 2.0 GHz Intel core i5 processor with 2GB RAM and 2 TB hard disk with LINUX (RED HAT 6) operating system[27].

### **Ligand Preparation**

Ligand 2D structures were drawn using Chem Draw Ultra 7.0. Chem3D Ultra 7.0 was used to convert 2D structure into 3D and the energy minimized using semi empirical AM1 method. Minimize energy to minimum RMS gradient of 0.100 was set in each iteration[28]. All structures were saved as .pdb file format for input to Auto Dock Tools (ADT) version 1.5.6.22 All the ligand structures were then saved in PDBQT file format, for input into AutoDock version 1.5.6.22[29]

### **Protein Structure Preparation**

For the molecular docking study, protein structure was obtained from the Brookhaven protein data bank; the DPP-IV structure PDB ID was 2P8S[30] The co-crystallized ligand (PF2) in the DPP-IV structure was removed. For the protein structure, all hydrogen atoms were added, lower occupancy residue structures were deleted, and any incomplete side chains were replaced using the ADT version 1.5.6. Further ADT was used to remove crystal water, added Gasteiger charges to each atom, and merged the non-polar hydrogen atoms to the protein structure[31]. The distance between donor

and acceptor atoms that form a hydrogen bond was defined as 1.9 Å with a tolerance of 0.5 Å, and the acceptor–hydrogen–donor angle was not less than 120°. The structures were then saved in PDBQT file format, for input into AutoDock version 1.5.6.

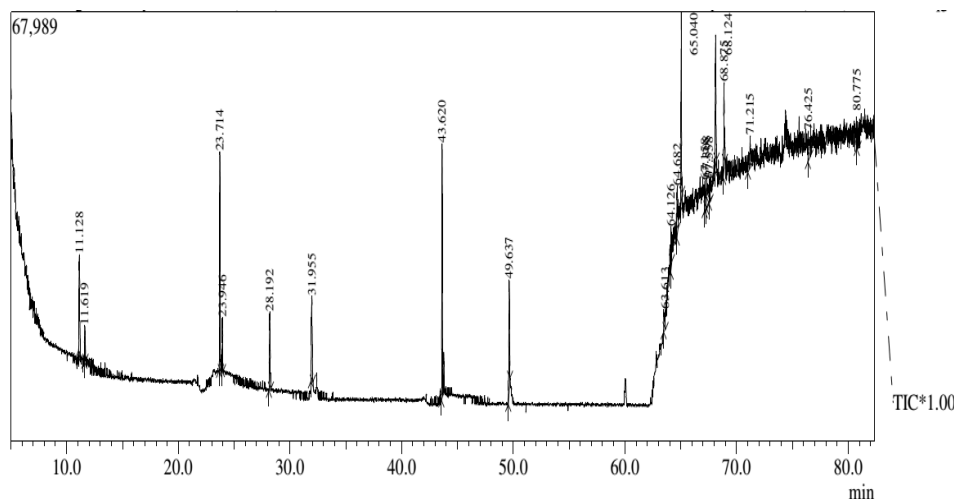
### Docking Protocol and their validation

A grid box with dimension of  $40 \times 40 \times 40$  Å<sup>3</sup> and was centred on 34.840, 6.101, 61.781 was created around the binding site of PF2 on DPP-IV protein using autodock tools. The centre of the box was set at PF2 and grid energy calculations were carried out. For the Autodock docking calculation, default parameters were used and 50 docked conformations were generated for each compound. In order to verify reproducibility of the docking calculations, the bound ligand (PF2) was extracted from the complexes and submitted for one-ligand run calculation. This reproduced top scoring conformations of 4 falling within root-mean-square deviation (RMSD) values of 0.71 to 0.74 Å from bound X-ray conformation for DPP-IV, suggesting this method is valid enough to be used for docking studies of other compounds

## 3. RESULTS AND DISCUSSION

### GC-MS analysis

The GC-MS results showed presence of bioactive compounds in ethanol leaf extract of *A. corniculatum*. The recognition of the active compounds was verified based on the retention time (RT), peak area, molecular weight and molecular formula[32]. The active principle with their RT, peak area in percentage, molecular formula and MW are presented in Figure 1 and Table 1.



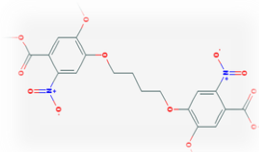
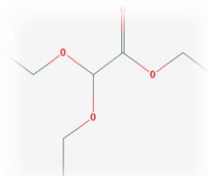
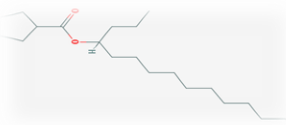
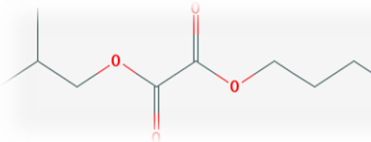
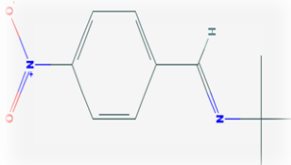
**Figure 1: GC-MS spectra of isolated compound from *A. corniculatum***

### Docking Results

The greatest methods is to appropriate ligand molecules (DPP IV inhibitors), into DPP IV 3D structure, using Autodock software ensued in docking files that comprised thorough data of docking (Table 2). The achieved log files were read in Auto Dock Tool (ADT) to investigate the outcomes of docking. The resemblance of docked structures was precised by calculating the root mean square deviation (RMSD) between the coordinates of the atoms and generating collecting of the

conformations depends on the RMSD scores[33]. Binding energies between ligand-receptor are computed with a free energy-based representation. The lowest energy of binding conformation in whole clusters was measured as the most promising docking position. Binding energies that are described signify the quantity of the whole total internal energy, intermolecular energy, and torsional free energy minus the energy of the unbound system[34]. The docking values were created by Autodock component with IC<sub>50</sub> were displayed in Table 2. From the results of this study, the lowermost docked energy structure was studied in detail in an attempt to recognize the usual pharmacophore for DPP IV inhibitors. The lowest energy docked structures of five DPP IV inhibitors are displayed in Figure 2. The highest low energy interactions of five DPP IV inhibitors had docking energies fluctuating from – 9.06 to –6.84 kcal/mol. Only one way of interactions were detected for all five inhibitors with the docked energy within a scale of 2.2 kcal/mol of the lowest docked energy structure. The main amino acid of DPP IV implicated in the hydrogen bonding with lowest energy docked structures of different ligands performed were displayed in Table 2.

**Table 1: GC-MS profile of ethanol leaf extract of *A. corniculatum***

S. No.	Retention Time	Name of the Compound	PUBCH EM ID	Molecular Weight (g/mol)	Molecular Formulae	Molecular Structure
1.	71.215	4,4'-(Tetramethylenebis(oxy)bis(2-nitro-5-methoxybenzoic	15329165	508.436	C <sub>22</sub> H <sub>24</sub> N <sub>2</sub> O <sub>12</sub>	
2.	80.775	Ethyl 2,2-diethoxyacetate	80169	176.212	C <sub>8</sub> H <sub>16</sub> O <sub>4</sub>	
3.	76.425	Pentadecan-4-yl cyclopentanecarboxylate	549695	324.549	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	
4.	68.875	2-O-(2-methylpropyl) 1-O-pentyl oxalate	6420703	216.277	C <sub>11</sub> H <sub>20</sub> O <sub>4</sub>	
5.	67.558	N-(4-nitrobenzilidene)-tert-butylamine	136558	206.245	C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>2</sub>	

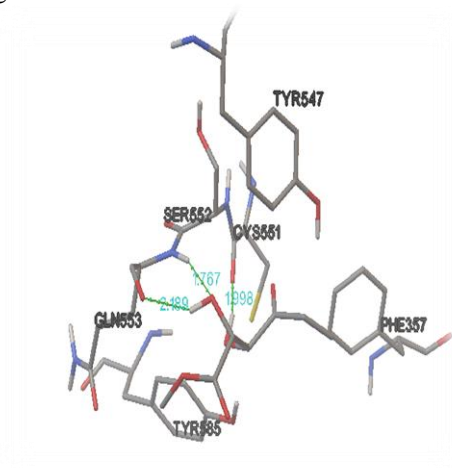
**4,4'-(Tetramethylenebisoxo)bis(2-nitro-5-methoxybenzoic acid methyl) ester:** Figure 2(a) shows the lowest energy docked structures of 4,4'-(Tetramethylenebisoxo)bis(2-nitro-5-methoxybenzoic acid methyl) ester with DPP IV showed the binding energy -3.81 kcal/mol.  $IC_{50}$  was calculated as 1.6. The lowest energy docked structure bounded with DPP IV with three hydrogen bonds, the hydrogen bond interactions from GLN553, SER552 and CYS551 with H bond distances 2.189, 1.767 and 1.998 respectively (Table 2).

**Ethyl 2,2-diethoxyacetate:** Figure 2(b) shows the low energy docked structures of Ethyl 2,2-diethoxyacetate with DPP IV protein domain showed the binding energy -3.11 kcal/mol.  $IC_{50}$  was calculated as 5.24. The lowest energy docked structure interacted with DPP IV with two hydrogen bonds, the hydrogen bond interactions from LYS554 and GLY63O with H bond distances 1.740 and 2.124 respectively (Table 2).

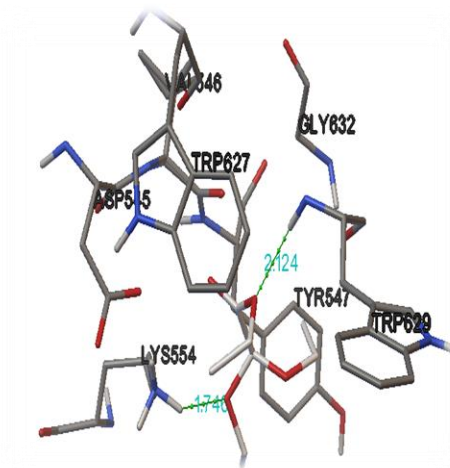
**Pentadecan-4-yl cyclopentanecarboxylate:** Figure 2(c) shows the low energy docked structures of Pentadecan-4-yl cyclopentanecarboxylate with DPP IV protein domain showed the binding energy -3.69 kcal/mol.  $IC_{50}$  was calculated as 1.96. The lowest energy docked structure interacted with DPP IV with a hydrogen bonds, the hydrogen bond interaction from TYR585 with H bond distance 1.961 (Table 2).

**2-O-(2-methylpropyl) 1-O-pentyl oxalate:** Figure 2(d) shows the low energy docked structures of 2-O-(2-methylpropyl) 1-O-pentyl oxalate with DPP IV protein domain showed the binding energy -4.34 kcal/mol.  $IC_{50}$  was calculated as 1.23. The lowest energy docked structure interacted with DPP IV with a hydrogen bonds, the hydrogen bond interaction from GLN553 with H bond distance 1.182 (Table 2).

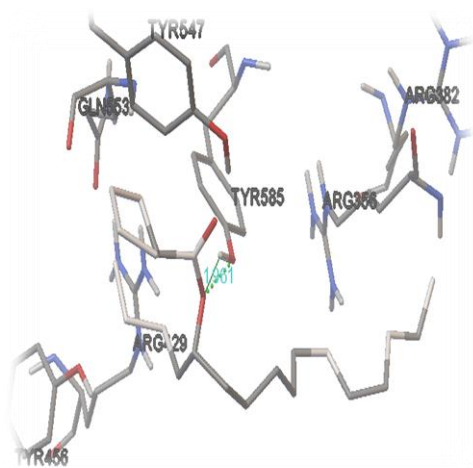
**N-(4-nitrobenzilidene)-tert-butylamine:** Figure 2(e) shows the low energy docked structures of N-(4-nitrobenzilidene)-tert-butylamine with DPP IV protein domain showed the binding energy -3.45 kcal/mol.  $IC_{50}$  was calculated as 1.67. The lowest energy docked structure interacted with DPP IV with a hydrogen bonds, the hydrogen bond interaction from ARG356 with H bond distance 2.219 (Table 2).



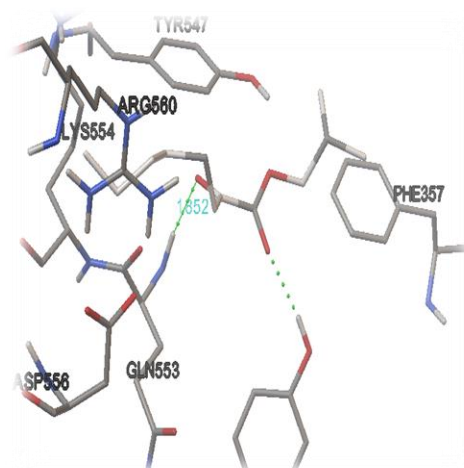
(a) 4,4'-(Tetramethylenebisoxo)bis(2-nitro-5-methoxybenzoic acid methyl) ester



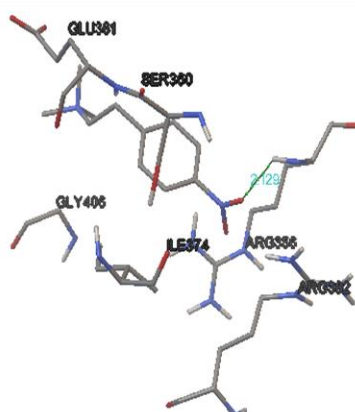
(b) Ethyl 2,2-diethoxyacetate



(c) Pentadecan-4-yl cyclopentanecarboxylate



(d) 2-O-(2-methylpropyl) 1-O-pentyl oxalate



(e) N-(4-nitrobenzylidene)-tert-butylamine

**Figure 2: Interaction of dipeptidyl peptidase IV (DPP-IV) with ligands (3D View).**

**Table 2: The docking results of identified bioactive compounds of *Aegiceras corniculatum* (L.) Blanco against DPP IV.**

Compound Name	4,4'-(Tetramethylenebis(2-nitro-5-methoxybenzoic acid methyl) ester	Ethyl 2,2-diethoxy acetate	Pentadecan-4-yl cyclopentanecarboxylate	2-O-(2-methylpropyl) 1-O-pentyl oxalate	N-(4-nitrobenzylidene)-tert-butylamine
Binding energy (kcal mol <sup>-1</sup> )	-3.81	-3.11	-3.69	-4.34	-3.45
IC <sub>50</sub>	1.6	5.24	1.96	1.23	1.67
Number of bonds involved	3	2	1	1	1
H-bond interaction	GLN553 O-H...O SER552 N-H...O CYS551 O-H...O	LYS554 N-H...O GLY63O N-HO...O	TYR585 O-H...O	GLN553 N-H...O	ARG356 N-H...O
Distance of H-Bond interaction	2.189 1.767 1.998	1.740 2.124	1.961	1.182	2.219

#### 4. CONCLUSION

The receptor (Protein) and ligand plays an important role in structural based drug design. In the present work, phytochemicals were obtained from the leaves of *Aegiceras corniculatum* (L.) Blanco (Black Mangrove) by Gas Chromatography Mass spectrometry (GC- MS) analysis. The presence of various bioactive compounds delivers indication for the effective use of medicinal plant parts for different illnesses by conventional healers. In this study, we interacted the receptor of DPP-IV with the bioactive compounds and from this, 2-O-(2-methylpropyl) 1-O-pentyl oxalate from the leaves of *Aegiceras corniculatum* (L.) Blanco clinches a guaranteeing lead target molecule development against diabetes based on molecular docking analysis. *In-vivo* and *in-vitro* methods are suggested to explicate the molecular mechanism of this phytochemical to perform as effective drug for type 2 diabetes.

#### CONFLICT OF INTEREST

Authors have no conflict of interest.



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