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SCREENING AND *IN SILICO* PROFILING OF A - AMYLASE (3M07) D ANTI-DIABETIC ACTIVITY IN THE ELUCIDATED COMPOUNDS OF *TECOMARIA CAPENSIS*

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ABSTRACT

Diabetes mellitus is a common problem in most of the countries, especially in India where 90-95 % people have diabetes. It occurs due to inadequate insulin levels. Insulin is essential for maintaining blood glucose level which is essential for the body. When inadequate amount of insulin is produced, it has to be compensated by administration of insulin injections which is a painful procedure. So now a days, as there is a renewed interest in the traditionally used herbal drugs, the present study aims to screen the identified constituents of *Tecomaria capensis* for determining the potent constituent for antidiabetic activity using *in-silico* approach. The elucidated compounds were evaluated for the antidiabetic activity using *in-silico* Docking method. The receptor was analyzed for the active site and pocket finder tools. The amino acids such as Arg-307, Ile-291, Thr-225 and Asp-264 were predicted as active site binding residues. Docking studies were done through MVD (Molegro Virtual Docker) software. Out of six compounds, two compounds from leaves of *Tecomaria capensis* showed good docking profiles with Alpha - Amylase (3M07) D. Finally, the result from the study demonstrates that the 3, 7-dimethyloct-6-en-1-ol possess potent anti-diabetic activity against Type-II diabetes mellitus through drug action on Alpha - Amylase inhibition system.

KEYWORDS: ***Alpha – Amylase inhibition, Diabetes mellitus, Molegro virtual docker Mol-dock software, Tecomaria capensis, In silico approach.***

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INTRODUCTION

Diabetes mellitus[1] is one of the common metabolic disorders with micro and macrovascular complications that result in significant morbidity and mortality. Hence it is one of the life -threatening diseases in the current society. Nowadays, no ideal therapy is available to cure diabetes mellitus. As the side effects associated with the use of insulin and other oral hypoglycemic agents are more, there is an escalating demand by patients to use natural products with antidiabetic activity. There are numerous traditional medicinal plants reported to have hypoglycemic properties. *Tecomaria capensis Lindl* is commonly known as honey suckles. Previously ferulic, rutin, luteolin-7-O-β-D- glucuronopyranoside, apigenin-7-O-β-D-24glucuronopyranoside and luteloin-7-O-(6-O-E-p. coumaroyl) β-D-glucopyranoside were isolated from methanolic extract[2]. Various pharmacological activities such as antibacterial and antifungal[3], cytotoxic, antinociceptive, antiulcer, anti-tumor, anti-inflammatory and wound healing [4] etc., have been reported. The main objective of the present research work is to separate biomarkers from *Tecomaria capensis* and assess its efficiency in *in vitro* anti diabetic activity.

Figure 1
Whole plant of Tecomaria capensis

MATERIALS AND METHODS

Materials and tools used

In the present study the leaves of *Tecomaria capensis* (Thunb.) was collected from Guntur district, A.P and it was authenticated by professor Dr.B.Sandhya, SIMS College of life sciences, Mangaldas nagar, Guntur. All solvents like n-hexane, ethyl acetate, petroleum ether, chloroform and ethanol were distilled prior to use. TLC (standard grade 2-25mm average particle size) was performed on silica gel in different solvents like toluene, n-hexane, pet. ether, ethyl acetate, chloroform, methanol, ethanol and glacial acetic acid. Spots were identified with the help of Iodine to detect double bond & triple bonds, 10% H_2SO_4 to detect involatile organic compounds, UV light to detect compounds having absorption of UV light (220-380nm)and others like molisch, Dragondroff's reagents for visualization. A mixture of toluene and ethyl acetate in the ratio 7:3 is used as eluent. Column chromatography was performed by using Silica gel G (MERCK 60-120 mesh). IR V_{max} (KBr) cm^{-1} spectra was measured on a JASCO FT/IR-5300 spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded with a BRUKER AVANCE III 400.1328 MHz NMR spectrometer. LC-MS data was collected from LCMS-2010A SHIMADZU. Elemental analysis was performed using FLASH EA1112 SERIES THERMO FINNIGAN. The present study used biological databases like drug bank, PDB (protein data bank), Molegro Virtual Docker (MVD) and Chem3D Ultra 8.0, melting points and boiling points of compounds are determined by using melting point apparatus (model:$\mu Thermocal_{10}$) and capillary method respectively.

Methodology

Preparation of Extracts [5,6]

The extract was prepared by Soxhlet apparatus using individual solvents like petroleum ether, n-hexane, ethyl acetate, chloroform, ethanol and water. About 150 gm of dried flower powder was taken in a muslin cloth bag. The purified solvent was passed through the tube where the powder bag was kept. The solvent will pass through siphon tube and reach the round bottom flask in which porcelain chips are provided. The vapours containing the constituents pass through the condenser and reach the tube containing powder bag and the process is repeated. This is continued for 24hrs. Then the round bottom flask containing the extract is transferred to a beaker and is allowed to evaporate in a water bath. This semisolid concentrated extract is used for further studies.

Determination of Total Ash

About 2 or 3 gms of the grounded drug is taken and incinerate in a tarred platinum or silica dish by maintaining 450℃, until free from carbon, the product is cooled and weighed. If we could not able to collect carbon-free ash in the above method, incinerate the residue and filter paper along with filtrate, allow it to dry, ignite at a temperature not more than 450℃, finally calculate the percentage of ash with the air-dried drug as a reference.

TLC Analysis for Selected Crude Extract [7,8]

Toluene, ethyl acetate and glacial acetic acid combination gave good results in TLC (thin layer chromatography) analysis among the different phases. The mobile phase ratios of toluene and ethyl acetate were in 5:5 and 7:3, toluene, ethyl acetate and glacial

acetic acid in 5:5:3 ratios were used as mobile phases for better elution

Preparation of Ligand structure

To investigate the detailed intermolecular interactions between the analogues we carried out docking of 4 molecules using Molegro Virtual Docker (MVD), Ligand structures were drawn and optimized using MM2 force field by using Chem3D Ultra 8.0 and saved in mol format. The prepared ligands were uploaded to the working space. The docking scores of the active constituents are compared against the standard drugs (Acarbose [9]) obtained from the drug bank in .mol format.

Preparation of Enzyme structure

The target for docking studies is selected as α - Amylase. Docking analysis is done by initially selecting the target for the disease and followed by obtaining the 3D structure of α - Amylase (3M07) from protein data bank in .pdb format. Proper bonding, bond orders, hybridization and charges were assigned using the MVD. The possible attaching locus of both the objects were calculated using built in cavity detection algorithm enabled in MVD[9]. The search space of the duplication maneuver in the docking was studied as a subset region of 25^0 A circumfered the effective side left. The water molecules are also taken into consideration and the replaceable water molecules were given a score of 0.50.

Molegro Virtual Docker's docking search algorithms and scoring functions

Mol-Dock software is a search algorithm which mainly works on combining differential evolution with cavity prediction. Evolutionary algorithm is an interactive optimization technique inspired from Darwin's evolution theory of individuals of population is exposed to selection of competitive that weeds out poor solution and they are solved by mutation and recombination[9]. Molecular docking function of scoring which is based on PLP (piecewise linear potential), parameter of simplified potential are fit to protein ligand structure and scoring function of binding data is further extended by new hydrogen bonding term and charge scheme in GEMDOCK(generic evolutionary method for molecular dock).Recombination and mutation are used to generate new solutions[9].

Mol-Dock Optimizer[9]

In MVD, selected parameters were used for the guided differential evolution algorithm, number of runs=5 by checking constrain poses to cavity option. Population size=50, maximum interactions =2000, cross over rate=0.9, and scaling factor=0.5.A^o variance-based termination scheme was selected rather than root mean square deviation (RMSD). To ensure the most suitable binding mode in the binding cavity, Pose clustering was employed, which lead to multiple binding modes.

Parameters for scoring functions

Mol-Dock score

Atoms which are far away from binding site are ignored by distant atoms option and hydrogen bonding between potential donors and acceptors are checked by hydrogen bonding directionality. A selected cavity with a radius of 25 A^0 in X, Y, Z directions for extending was defined as binding site of protein.

In-Silico Toxicity Risk Assessment

While drawing a structure the toxicity risk predictor will start looking for potential toxicity risks as long as the currently drawn structure is a valid chemical entity. Toxicity risk alert is an evidence for drawn structure on specified risk point of view however risk alert is not meant to be a fully reliable toxicity predictions. It is a conclusion for the absence of risk alerts that selective compound is absolutely free of any toxic effects.

In vitro *screening method*

In the present study, we propose to investigate the effect of all the selected compounds in the following enzyme inhibition assay.

Alpha-amylase inhibition assay[10]

Importance of Alpha-amylase enzyme in human body: The digestion of starch involves several stages. Initially, partial digestion by the salivary amylase results in the degradation of polymeric substrates into shorter oligomers are further hydrolyzed by pancreatic α-amylases into maltose, malt-triose and small malto-oligosaccharides. The digestive enzyme (α-amylase) is responsible for hydrolyzing dietary starch (maltose), which breaks down into glucose prior to absorption. Inhibition of α-amylase can lead to reduction in post-prandial hyperglycemia in diabetic condition. Alpha-amylase activity can be measured *in-vitro* by hydrolysis of starch in presence of α-amylase enzyme. This process was quantified by using iodine which gives blue colour with starch. The intensity of blue colour in test sample is directly proportional to α-amylase inhibitory activity. Enzyme: (Type VI B: From porcine pancreas, 5, 00,000 U) [15.8 U/mg solid at pH 6.9]- Stored at 2-8^0C- Sigma, USA (A3176). Substrate: Starch 1%, Positive Control: Acarbose- Stored at RT-Glucobay (Bayer pharma, India), Sodium dihydrogen orthophosphate (NaHat RT, Disodium hydrogen phosphate (Na Indicator: Iodine solution 1% Instrument- UV- Visible Spectrometer(220-380).

Preparation of working solution

Phosphate Buffer (40 mM, pH 7, 25^0C): Solution A: 6.24 g of NaH_2PO_4 -1L, Solution B: 7.12 g of Na_2HPO_4 -1L, Enzyme (0.5128 U/ml)- 3.246 mg α-amylase in 100 ml of 40 mM Phosphate Buffer, NaCl solution (0.006 M), Positive control:Stock- 50 mg of Acarbose in 50 ml of 40 mM Phosphate buffer, Working stock: Take 25 µl of stock, made upto 10 ml (2.5 µg/ml) with 40 mM Phosphate buffer

Method [11,12]

Starch iodine method is used to accomplish α -amylase activity. 10µL of α -amylase solution was added to 390 µL of phosphate buffer with pH 7.0, containing different concentration of extracts, incubate them for 10 min at 37 °C , add 100 µL of starch solution (1%), re-incubated for 1 hrs at 37 °C, add 0.1 mL of 1% iodine solution and 5 mL distilled water. Finally take the absorbance at 565 nm with α-amylase substrate blank. Enzyme activity inhibition was determined by using the following formula.

$$(\%) = (A-C)\ X100/(B-C)$$

where,

A= sample absorbance,
B= blank absorbance (without α-amylase) and
C= control absorbance (without starch).

RESULTS AND DISCUSSION

Physicochemical constituents are determined according to Ayurvedic pharmacopoeia [10] standard protocols which are tabulated in Table 1 and Table 2. 2% of foreign material was adulterated in one gram of powder.

Table 1
Different Extractive Values of *Tecomaria capensis* flowers

Extractive value	Values
Alcohol soluble extraction	0.25 gm
Water soluble extraction	0.36 gm
Ether soluble extraction	0.04 gm

Table 2
Different Ash Values of Tecomaria Capensis flowers

ASH VALUES	In grams
Total ash value	0.93
Acid insoluble ash value(dil.Hcl)	0.01
Sulphated ash value (H_2SO_4)	0.06
Water soluble ash value (H_2O)	0.05

Table 3
Percentage yield of Tecomaria capensis Extracts

S. No.	Solvents	Nature of extract	Color	% yield
1	Pet-ether	Semisolid	Dark yellow	0.35
2	N-Hexane	Semisolid	Dark yellow	0.13
3	Chloroform	Semisolid	Dark Green	0.20
4	Ethyl acetate	Semisolid	Dark Green	0.70
5	Ethanol	Semisolid	Dark Green	0.52
6	Aqueous	Semisolid	Dark brown	0.25

Preliminary Phytochemical Screening

Phytochemical studies of *Tecomaria Capensis* leaves revealed the presence of cardiac glycosides, saponin glycosides and volatile oils in pet ether extracts. In n-hexane extracts cardiac glycosides, steroids and triterpinoids and volatile oils are present. In chloroform extract flavonoids, cardiac glycosides, saponin glycosides, steroids, triterpenoids and volatile oils are present. In ethyl acetate extract flavonoids, cardiac glycosides, saponin glycosides, steroids, terpenoids, carbohydrates, proteins and volatile oils are present. In ethanolic extract flavanoids, cardiac glycosides, coumarin glycosides, tannins, steroids, terpinoids, proteins, inulin, volatile oil and mucilage are present. In water extract flavanoid, inulin and mucilage are present. In ethanol and ethyl acetate extracts, the maximum phytochemical constituents which are present are tabulated below (Table 4):

Table 4
Preliminary Phytochemical Screening* of *Tecomaria Capensis Extracts

Phyto constituents	Pet. Ether	n-hexane	Chloroform	Ethyl acetate	Ethanol	water
Alkaloids	--	--	--	--	++	--
Flavinoids	--	--	++	++	++	++
Cardiac Glycosides	++	++	++	++	++	--
Saponin Glycosides	++	++	++	++	--	--
Coumarin Glycosides	--	--	--	++	++	--
Tannins	--	--	--	--	++	--
Steroids and terpinoids	--	++	++	++	++	--
Carbohydrates	--	--	--	++	--	--
Protein	--	--	--	++	++	--
Inulin	--	--	--	--	++	++
Volatile oil	++	++	++	++	++	--
Waxes	--	--	--	--	--	--
Mucilage	--	--	--	--	++	++

++ = Present, -- = Absent

TLC Analysis for Selected Crude Extract

Toluene, ethyl acetate and glacial acetic acid combination gave good results in TLC analysis among the different phases. The mobile phase ratios of toluene and ethyl acetate were in 5:5 and 7:3, toluene, ethyl acetate and glacial acetic acid in 5:5:3 ratios showed an effective response. Toluene and ethyl acetate in 7:3 ratio was more effective among the above three ratios.

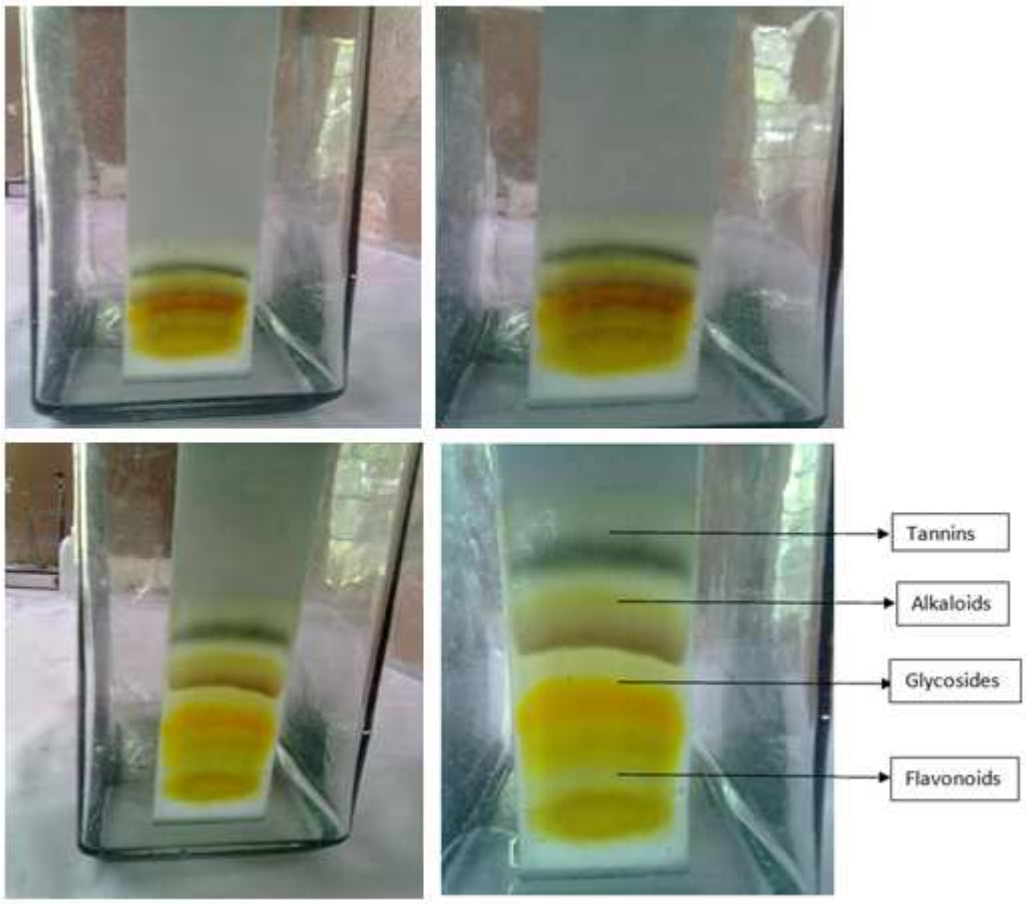


Figure 2
TLC shows separation of compounds in toluene & ethyl acetate (5:5&7:3)

Isolation of Biomarker By Column Chromatography
Six different biomarkers were isolated from the liquid column chromatographic technique. The above Six different biomarkers are characterized and structurally elucidated by the IR, ^{1}HNMR, ^{13}C NMR, LC-MS and elemental analysis.

Compound 1
Orange solid compound with melting point was 150-153^{0}C; IR V_{max} (KBr) cm$^{-1:}$groups1731 (C=O), 1650 (C=C), 1395 (CH_3), 1228.7 (C-O). ^{1}H NMR (ð ppm) : 7.3280-7.8891 (4H, m), 2.5070 (2H, s), 1.9955 (2H, s), 1.399 (6H, s),referred for two methyl groups. ^{13}C NMR(ð ppm) : 137.93, 137.40, 136.71, 136.04, 133.89, 127.49, 164.33, 112.21, 181.11, 177.20, 78.80, 35.14, 15.94, 27.74, 27.74.(shows 15 carbons, 137.92, 137.40, 136.71, 136.04, 133.89, 127.49 to benzene ring, 164.33, 112.21 to ethylene group, 181.11, 177.20 referred to carbonyl group, 78.80 to fully substituted C, 35.14, 15.94 referred to CH_2, 27.74, 27.74 referred to CH_3), LC-MS m/z: 242.09 (calculated for $C_{15}H_{14}O$, 242.26). Elemental analysis:C-74.38, H-5.82, O-19.81 were obtained, from above the structures elucidated as 3,4-dihydro-2,2-dimethyl-2H-benzo chromene-5,6-dione.

Compound 2
Grey solid compound with melting point was 371-374^{0}C; IR V_{max} (KBr) cm$^{-1:}$ 3477.90 (O-H), 2948.16 (C-H), 1647.43 (C=O), 1105.38 (C-O). ^{1}H NMR (ðppm) : 7.21-7.19(2H,d), 6.17(1H,s), 5.52(1H,s), 4.26-4.22(2H,d), 2.56-2.47(2H,d), 3.7(1H,s), 1.49(3H,s), 1.83(1H,s) 1.52-1.49(2H,s), 1.12-1.11(2H,d),1.10(1H,s), 2.18-2.0(2H,d), 4.06(1H,s),1.83(1H,s), (referred to furan ring group, CH, CH_2,CH_2, 1 H , 3H, CH_3 group, OH group,Valero-Lactone ring CH_2, CH_2, H, cyclohexane ring, CH_2, H, OH group, hydrogen's on cyclohexanone ring, hydrogen's on three CH_3 groups, hydrogen's on CH_3 group respectively) ^{13} C NMR (ð ppm) : 215.11, 171.19, 170.79, 124.88, 76.66, 69.19, 60.51, 57.49,47.34,38.59,141.99,139.29,127.48, 78.42, 77.78, 39.54, 32.15, 113.41, 67.14, 37.14, 30.54, 29.13, 19.30, 28.73, 14.19, 12.11 .(^{13}C NMR shows 27 carbons, 215.11 referred to carbonyl group, 171.19, 170.79 referred to carboxyl group, 124.88, 76.66, 69.19, 60.51, 57.49, 47.34, 38.59 referred to fully substituted C, 141.99, 139.29, 127.48, 78.42, 77.78, 39.54, 32.15 referred to CH, 113.41, 67.14, 37.14,30.54, 29.13, 19.30 referred to CH_2, 28.73, 14.19, 12.11 referred to CH_3 and from elemental analysis C-64.53, H-6.82, O-28.65) LC-MS m/z: 502.21 calculated for C_{27} H_{34} O_9, 502.55).Elemental analysis: unknown nitro phenanthrene carboxylic acid with conjugated double bond.

Compound 3
Marigold liquid compound with boiling point was 465-467^{0}C; IR V_{max} (KBr) cm^{-1}: 3522.34 (OH), 2859.95 (C-H), 2359.15 (C=N), 1633.85 (C=C), 1263.22 (C-N). ^{1}H NMR (ð ppm) : 7.845-7.362 (3H, t, 6.342 (1H, s), 3.79 (1H, s), 3.195 (1H, s), 2.612-2.502 (2H, d, 1.512 (2H, s), 1.145 (1H, s), (^{1}H NMR displayed a signal of 3H m 7.845-7.362 refer to aromatic ring, 1H singlet with 6.342 referred to aromatic OH group, 2H d 2.612-2.502 referred to aromatic CH_2 group, 2H singlet with 1.512 referred to CH_2 group, 2H singlet with 3.79 referred to CH_2 group and two 1H singlet's 3.195 referred to CH

group, 2.21 referred to OH group).[13] C NMR (ð ppm) : 155.51, 115.63, 123.20, 138.78, 120.66, 114.99, 167.11, 70.32, 29.24, 47.66, 52.06, ([13]C NMR shows 11 carbons, 155.51, 115.63, 123.20, 138.78, 120.66, 114.99 referred to benzene ring, 167.11 referred to carbon in imine ring, 70.32 referred to CH, 29.24, 47.66, 52.06 referred to CH_2) LC-MS m/z: 204.08 (calculated for $C_{11}H_{12}N_2O_2$, 204.22). Elemental analysis: Elucidated as 1,2,3,9-tetrahydropyrrolo quinazoline-3,7-diol, the structure was represented in Figure 4.

Compound 4

Brown compound with melting point was 275-277^{0}C; IR V_{max} (KBr) cm^{-1}:3515.15(O-H), 3321.11(N-H), 1633.85(C=0), 1191.45(C-O) (1H with d 4.79-4.62 referred to CH_2 group,four 1H singlet with 7.91 referred to secondary amine group, 5.23 referred to CH group, 3.81 referred to amine group and 2.27 referred to OH group). ^{1}H NMR (ð ppm): 4.79-4.62(2H, d, 7.91 (1H, s), 5.23 (1H, s), 3.81 (1H,s), 2.27 (1H,s). ^{13} C NMR (ð ppm): 170.15, 115.83,75.75.(170.15 C, 115.83 referred to CH, 75.75 referred to CH). LC-MS m/z: 119.04 (calculated for $C_3H_6N_2O_3$, 119.05). Elemental analysis:Structure elucidated as 6-hydroxy-1,3,4-oxadiazinan-5-one.

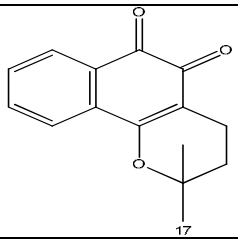
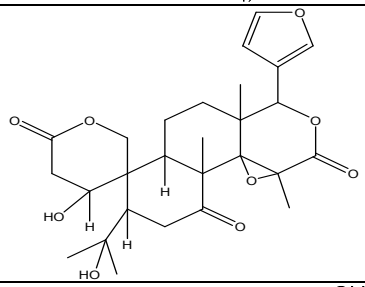
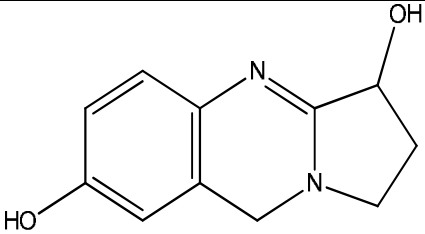
Compound 5

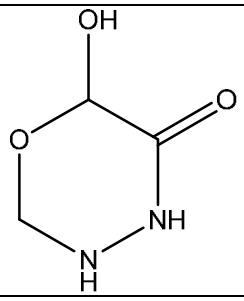
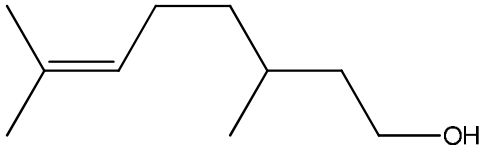
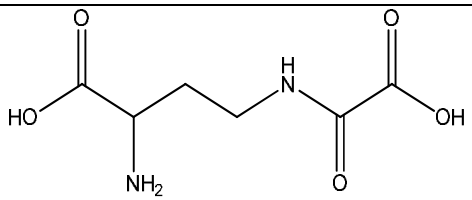
Colourless liquid compound with boiling point was 225-227^{0}C; IR V_{max} (KBr) cm^{-1} : 3607.21(O-H), 2915.92(C-H), 1622.28(C=C), 1453.54(CH_2), 1370.28(CH_3), 1153.54(C-O). ^{1}H NMR (ð ppm) : 1.777(6H,s), 1.06(3H,s), 1.29(2H,s), 1.4(2H,s), 1.96(2H,s), 3.11(2H,s), (1H,s), 6.927(1H,s), 5.267(1H,s), 1.65(1H,s) (referred to OH group, CH group,H directly attached to carbon group respectively), ^{13} C NMR (ð ppm) : 131.41, 126.82, 29.35,60.54, 40.17, 38.02, 24.44, 19.63, 21.17, 25.61, (131.41 referred to fully substituted C, 126.82, 29.35 referred to CH,60.54, 40.17, 38.02, 24.44 referred to CH_2, 19.63, 21.17, 25.61 referred to CH_3), LC-MS m/z: 156.15 (calculated for $C_{10}H_{20}O$, 157.15). Elemental analysis: Structures elucidated as 3,7-dimethyloct-6-en-1-ol, the structure was represented in Figure 5.

Compound 6

Yellow liquid compound with boiling point was 467-469^{0}C; IR V_{max} (KBr) cm^{-1} :3607.21(O-H), 3299.21(N-H), 2912.98(C-H), 1613.11(C=0), 1405.67(CH_2), 1212.10(C-N). ^{1}H NMR (ð ppm) : 10.117-10.17(2H, d, 2.986-2.976(2H, d, 2.112-2.0768 (2H, d, 8.617(1H, s, 3.470(1H, s), 2.00(1H, s) (refered to carboxylic acid group, CH_2 group, secondary amine group, CH group, amine group), ^{13} C NMR (ð ppm) : 174.91, 157.03, 159.95, 53.06,30.55, 35.09, (6 carbons, 174.91, 157.03, 159.95 referred to fully substituted C, 53.06 referred to CH, 30.55, 35.09 referred to CH_2 and from elemental analysis C-37.90, H-5.30, N-14.73, O-42.07), LC-MS m/z: 190.05 (calculated for $C_6H_{10}N_2O_5$, 191.06). Elemental analysis: Structures elucidated as 2-amino-4-[(carboxy carbonyl) amino] butanoic acid. The six isolated compounds are tabulated below (Table 5):

Table 5
Structures and IUPAC names of isolated compounds

S.NO	COMPOUND	IUPAC	STRUCTURE
1	COMPOUND 1	(3,4-dihydro-2,2-dimethyl-2H-benzo[h]chromene-5,6-dione)	
2	COMPOUND 2	nitrophenanthrene carboxylic acid with conjugated double bond	
3	COMPOUND 3	1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-3,7-diol	

4	COMPOUND 4	6-hydroxy-1,3,4-oxadiazinan-5-one	
5	COMPOUND 5	3,7-dimethyloct-6-en-1-ol	
6	COMPOUND 6	2-amino-4-[(carboxycarbonyl)amino]butanoic acid	

Docking results

In silico docking analysis of designed molecules on α - Amylase (3M07) D ranking based on MolDock Score and H-Bond Interaction Among 6 isolated compounds 4 compounds were docked.

Table 6

MolDock Score and H-Bond Interaction of isolated compounds

Ligand	Mol-Dock Score (Kcal/mol)	Re-rank Score	H-Bond Interaction
3,7-dimethyloct-6-en-1-ol	-91.5379	-70.7999	0
1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-3,7-diol	-82.4848	-70.8979	-4.17936
3,4-dihydro-2,2-dimethyl-2H-benzo[H]chromene-5,6-dione	-81.2034	-72.2155	-7.30612
6-hydroxy-1,3,4-oxadiazinan-5-one	-56.7609	-52.5031	-2.04071
ACARBOSE	-93.1984	-75.7669	-12.9581

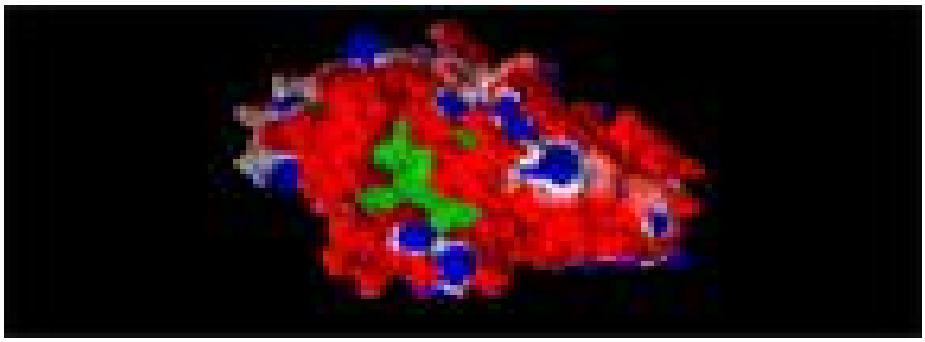


Figure 3

Binding pockets interactions of Acarbose in α - Amylase (3M07)

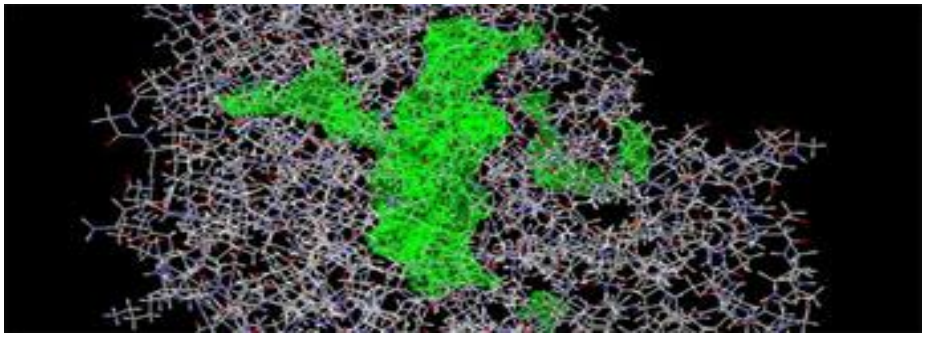


Figure 4
Pose view cavities in Alpha- amylase (3M07)

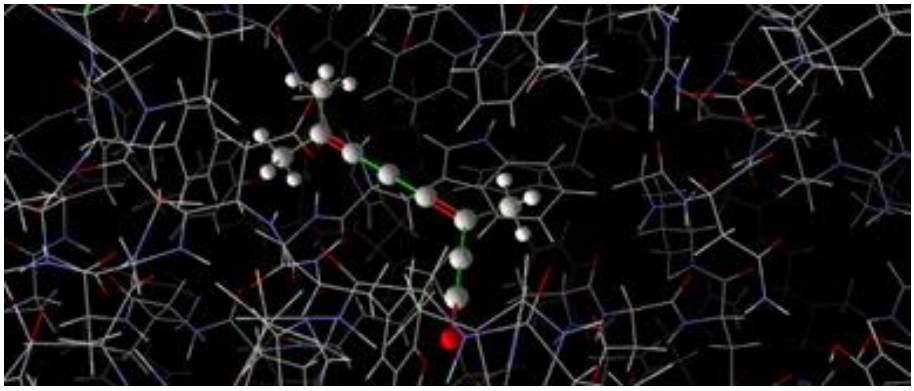


Figure 5
Bonding with the 3, 7-dimethyloct-6-en-1-ol

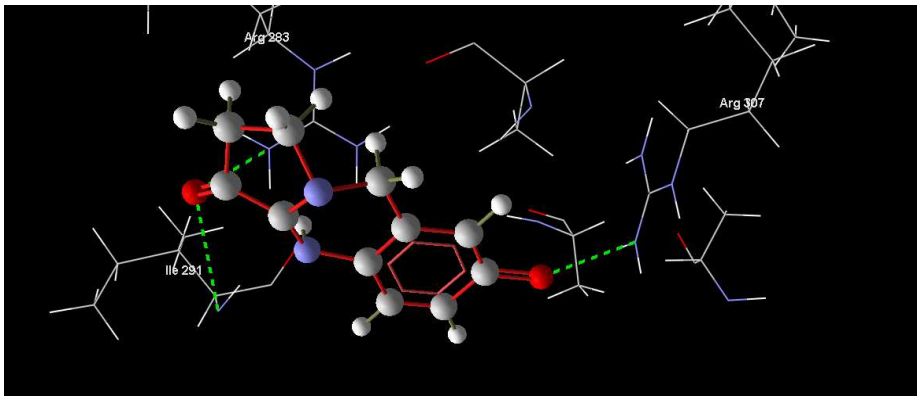


Figure 6
Bonding with the 1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-3,7-diol

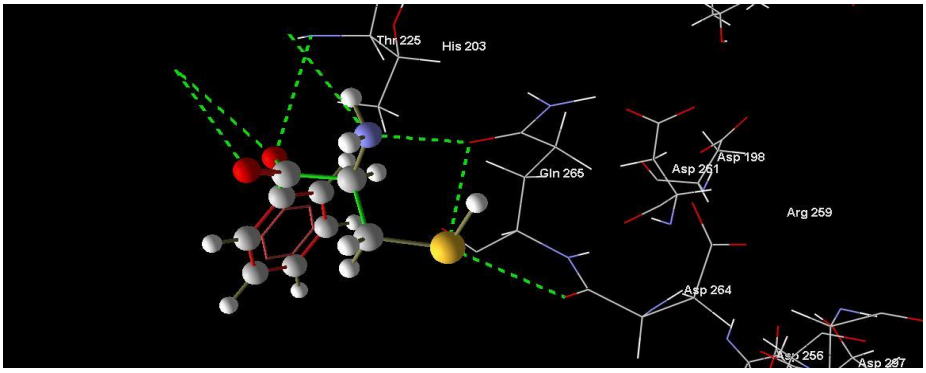


Figure 7
3M07 shows the bonding with the ligand Acarbose

Table 7
Anti-diabetic activity docking studies score and binding interaction derivatives

Compounds	Docking scores (Kcal/mol)	H-Binding interactions
3,7-dimethyloct-6-en-1-ol	-91.5379	ARG-307 LLE-291
1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-3,7-diol	-82.4848	NA
3,4-dihydro-2,2-dimethyl-2H-benzo[H]chromene-5,6-dione	-81.2034	NA
6-hydroxy-1,3,4-oxadiazinan-5-one	-56.7609	NA
ACARBOSE	-93.1984	THR-225, ASP-264, TYR151, GLY306, ALA307

NA =Not applicable

Table 8
Comparison of compounds with Acarbose IC_{50} in µg/ml against α-amylase activity

COMPOUND	IC_{50} µg/ml
3,7-dimethyloct-6-en-1-ol	7.76
1,2,3,9-tetrahydropyrrolo[2,1-b]quinazoline-3,7-diol	11.12
3,4-dihydro-2,2-dimethyl-2H-benzo[H]chromene-5,6-dione	16.17
6-hydroxy-1,3,4-oxadiazinan-5-one	22.09
ACARBOSE	6.38

The *In-silico* docking studies of 4 compounds with α - Amylase (3M07) (Type-II diabetic targeting) demonstrates, all the compounds were docked. The Dock score and energy minimization reveals that the active compounds 3,7-dimethyloct-6-en-1-ol have potent antidiabetic activity. In the *in-vitro* antidiabetic evaluation of compounds, all of them showed moderate to superior *in-vitro* antidiabetic Alpha-amylase inhibition. Among them 3,7-dimethyloct-6-en-1-ol showed the anti-diabetic activity with least IC_{50} value of 7.76 µg/mL compared with Acarbose, IC50 value of 6.38 µg/mL. Hopefully, this study could discover a new specific lead to target the α - Amylase (3M07) D. This finally confirms that the compound activates the IRS-receptors in carbohydrate metabolism by inducing secretion of necessary proteins and maintains glucose homeostasis in Diabetes mellitus.

CONCLUSION

The study concludes the compound 3,7-dimethyloct-6-en-1-ol is further profiled for pre clinical studies which are derived from methanolic extract of *Tecomaria capensis* for the therapeutic treatment of Diabetes mellitus.

AUTHORS CONTRIBUTION STATEMENT

Madhuri latha thadanki- Conceived of the presented idea, developed the theory and performed computations. Pavan kumar chadalawada- Carried out the experiment and performed analytical caluculations and wrote the manuscript with support from author Pooja boayapati- Helped and supervised the findings of project work and analysed the data.

CONFLICT OF INTEREST

Conflict of interest declared none.

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