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In vitro Anti-Diabetic Activity and *In-Silico* Studies of α - Amylase (3M07) D on Isolates of Ipomoea Sepiaria

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ABSTRACT

Ipomoea sepiaria is one of the sacred plants from dhashapusham of an ayurvedic system of India. The genus *Ipomoea* is well known for its nutritional, medicinal and folklore values. Molegro Virtual Docker (MVD) studies were performed on isolated moieties of methanolic extract of *Ipomoea sepiaria* and Acarbose for comparison of *in-silico* and *in-vitro* studies. *In silico* studies of α - amylase (3M07) D was carried on the isolated moieties of the methanol extract of *Ipomoea sepiaria* are Dodecyl–p–coumarate, β–sitosterol, Ipobscurine B, and serotonin alkaloid Ipobscurines C. In this study, the results showed that Ipobscurine-B and serotonin alkaloid Ipobscurines C proved to have better H-bonding in α - Amylase (3M7) having a Moldock score -149.326, -148.389 and Re-rank score -92.9283, -86.95 than -93.1984 and Re-rank score -75.7669 of standard Acarbose. Moldock Scores of Dodecyl–p–coumarate, β–sitosterol, ipobscurine B, and a serotonin alkaloid Ipobscurines C were -88.874, -78.866, -149.326 and -148.389, respectively. The Re-rank score of above moieties were -68.2373, -61.0402, -92.9283 and -86.95, respectively. The hydrogen bonds of ipobscurine B, serotonin alkaloid Ipobscurines C and Acarbose with an amino acid of α - Amylase (3M07) are Arginine, Alanine, Threonine and Aspartic acid. All the main content of *Ipomoea sepiaria* studied for the *in-vitro* anti-diabetic activity by alpha-amylase inhibition method. The compounds showed mild to moderate anti-diabetic activity. Ipobscurine B found to be most active among the series of isolates in comparison with Acarbose. Therefore, this class of compound could be a good starting point to develop new lead compound in the treatment of anti-diabetic activity.

KEYWORDS

Ipomoea sepiaria, Molegro virtual Docker, Ipobscurines, Moldock score

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INTRODUCTION

Diabetes is one of the most common serious and chronic diseases in the world[1] and approximately of about 422 million are affected and the count is doubled and nearly 1.5 million deaths are reported in 2012. It is predominant in India mainly due to food, stress and living standards when comparing to other countries.

The genus *Ipomoea* since many years continuously has been in use for its medicinal values. *Ipomoea* species is to treat constipation, diabetes, aphrodisiac, astringent, Acquired Immunodeficiency Syndrome (AIDS) and hypertension[2,3,4]. The review of folk-lore literature sufficiently revealed the hypoglycaemic activity of *Ipomoea sepiaria* plant.

In the present study, we isolated biomarkers from *Ipomoea sepiaria extract* and screening of efficiency in the biological model for *in-vitro* antidiabetic activity and compared with *in-silco* activities.

Fig 1 *Ipomoea sepiaria* plant with flowering parts

MATERIALS AND METHODS

In our study, *Ipomoea sepiaria* plant was collected from rural parts of Guntur and authenticated by Dr Khasim, Acharya Nagarjuna University, Guntur, A.P, India. The leaves were separated, cleaned, shade dried and powdered. The powder was passed sieve no:120# and finally stored in a labelled container.

METHODS:

Preparation of methanolic extract- From the labelled container about 25gms was weighed and soaked in methanol(acetone free) for a period of 3 days. Then the extract was filtered and evaporated at 40^{o}C for solvent elimination by using reduce pressure method, the extract was stored in a desiccator for the few days and stored in the refrigerator for experimental purposes.

Solvents like methanol were distilled prior to use. TLC 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 I_2, 10%H_2SO_4, UV and different reagent for visualization. A mixture of toluene and ethyl acetate in ratio 7:3 is used as eluent. Column chromatography was performed using Silica gel G (MERCK 60-120mesh). IR spectra obtained from JASCO FT/IR-5300 spectrophotometer. ^{1}HNMR and ^{13}C

NMR spectra were recorded with an NMR spectrometer.

LC-MS data was collected from LCMS-2010A SHIMADZU. Elemental analysis was performed from FLASH EA1112 SERIES THERMO FINNIGAN. Our present study used biological databases like drug bank, PDB(protein data bank), Molegro Virtual Docker (MVD), Chem3D Ultra 8.0.

DOCKING STUDY:

Preparation of Ligand

Docking studies were carried out by using MolegroVirtual Docker (MVD) in order to identify the interaction and affinity of molecules between the ligands and receptors, Ligand structures were drawn and saved in .mol format by using ChemDraw 8.0 and optimized by using MM2 force field. finally, the ligand molecules were compared with the standard drug (Acarbose) and docking score was reported by importing the standard and ligand molecules into the workspace.

Preparation of Protein target

The α-Amylase enzyme was used docking studies and it was explained in detail in our previous[6,7] work. Docking analysis is carried out by selecting the target for the antidiabetic disease and followed by downloading the 3D structure of alpha-Amylase (3M07) D from protein data bank in the .pdb format.

***IN-VITRO* ANTI-DIABETIC ACTIVITY:**

In our research activity, we aimed to investigate the effect of all the isolated moieties by the following alpha-enzyme inhibition assay.

Alpha-amylase inhibition assay[8,9]

The alpha-amylase enzyme is mainly useful for carbohydrate digestion in humans. Starch digestion takes place in several steps and at the initial stage, salivary amylase in the mouth helps in conversion of polysaccharide into oligosaccharides. On a later stage, α-Amylases in pancreatic juice into maltose, malt-triose and small malto-oligosaccharides. The α-Amylase used for hydrolysing dietary starch maltose into simple glucose prior to absorption.

Preparation of working solutions:

A solution of NaH_2PO_4 (6.24 g in1L) and Na2HPO4 (7.12 g of in 1L) by adding one litre each to prepare 40 mM Phosphate Buffer with pH 7 at 25^0C.

An α-amylase enzyme of 3.246 mg was dissolved in 100 ml of 40 mM Phosphate Buffer, Acarbose (50 mg) was dissolved in a volume of 50 ml of 40 mM Phosphate buffer as Positive control.

Working stock solution: 25 µL from the stock transfer into a volumetric flask (10 ml) and make up the 10 ml (2.5 µg/ml) by using 40 mM Phosphate buffer as a solvent.

Alpha-amylase inhibition assay procedure:

The selected compounds were serially diluted to get a required concentration to perform both alpha-amylase inhibition assay. The alpha-amylase activity was carried out and the values are tabulated in the Table-3. At 565nm the absorbance of sample, substrate and alpha-Amylase blank determinations were carried by UV-VIS spectrophotometer.

Inhibition of enzyme activity was calculated by the formula:

(%) = (A-C) *100/ (B-C),

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

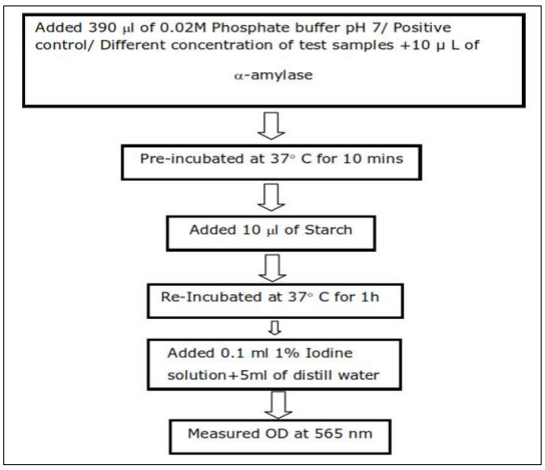

Fig 2 Schematic flow chart of α-amylase enzyme inhibition assay procedure

RESULTS AND DISCUSSION

In Table-1, The molecular docking, re-rank and hydrogen bonding interactions score of isolated molecules like Ipobscurine B and Ipobscurines C was better than the standard Acarbose.

Table 1 *In-silico* docking analysis of designed molecules on α - Amylase (3M07) D ranking based on Molecular Docking score and Hydrogen-Bond Interaction

Ligand	Molecular docking score (Kcal/mol)	Re-rank Score	Hydrogen-Bond Interaction
Dodecyl–p–coumarate	-88.874	-68.2373	-0.21689
β–sitosterol	-78.866	-61.0402	-0.25691
ipobscurine B	-149.326	-92.9283	-0.36515
serotonin alkaloid Ipobscurines C	-148.389	-86.95	-6.30012
ACARBOSE	-93.1984	-75.7669	-12.9581

In Table-2, interactions between different amino acids of active sites with isolated molecules showed that Ipobscurine B and Acarbose both contain ASP-264. Ipobscurine B interacted with 3 amino acids, Ipobscurines C interacted 6 amino acids and the standard interacts with 5 amino acids. The binding pocket and pose

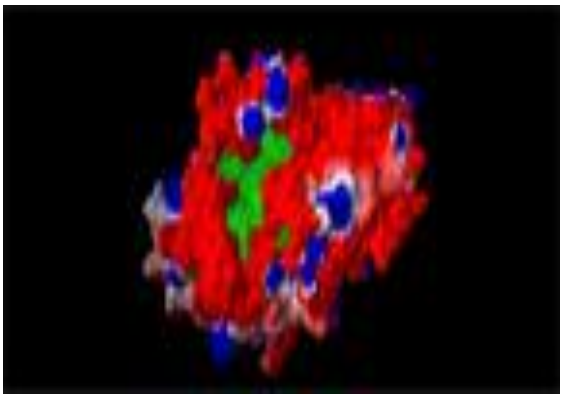

Fig 3 Binding pockets interactions of Acarbose in α-Amylase (3M07)

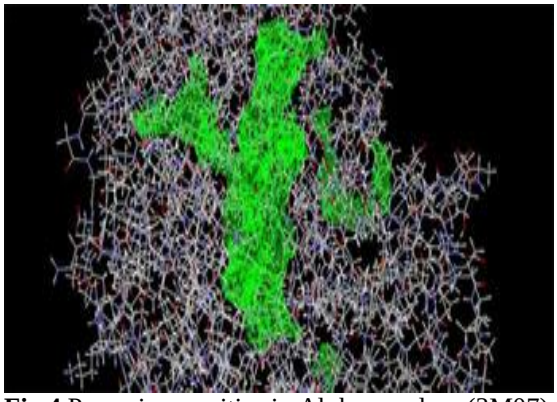

Fig 4 Pose view cavities in Alpha-amylase (3M07)

of the cavity of alpha-Amylase (3M07) were showed in figure 3 and 4. Interactions of Ipobscurine B with ASP-264, ARG-373, TRP-227 and Ipobscurines C with ARG-283,289, ALA-313,318 and ILE-313,318amino acids of active site were shown in figure 5 and 6. On over all molecular docking score of Ipobscurine B

Table 2 Anti-diabetic docking studies score and binding interactions derivatives

Compounds	Docking scores (Kcal/mol)	H-Binding interactions
Dodecyl–p–coumarate	-88.874	ASP-264
β–Sitosterol	-78.866	ASP-264
Ipobscurine B	-149.326	ASP-264, ARG-373, TRP-227
Serotonin alkaloid Ipobscurines C	-148.389	ARG-283,289; ALA-313,318 ILE-313,318
Acarbose	-93.1984	THR-225, ASP-264,TYR151, GLY306, ALA307

and Ipobscurines C is more negative than standard Acarbose. The more is the negative score more is the binding capacity so its clearly indicating that Ipobscurine B and Ipobscurines C are more potent when compared to standard and it was proven from the *in-vitro* antidiabetic α-amylase activity also, where the IC_{50} value is low for Ipobscurine B.

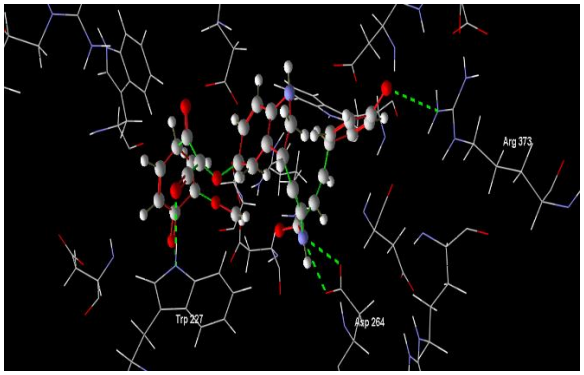

Fig 5 3M07 shows the bonding with the Ipobscurine B

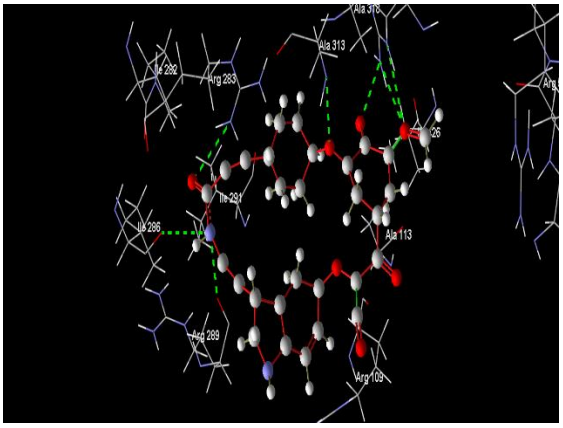

Fig 6 3M07 shows the bonding with the serotonin alkaloid Ipobscurines C

CONCLUSION

The *In-silico* docking studies of 4 compounds with α - Amylase (3M07) (Type-II diabetic targets) proves that they are having more affinity towards the specific receptors. The Dock score and energy minimization confirm that the active

compounds Ipobscurine B, serotonin alkaloid Ipobscurines C have potent antidiabetic activity than the standard and the same was proven by the *in-vitro* antidiabetic of compounds, all of them showed mild to significant activity. Among them ipobscurine B showed the anti-diabetic activity with least Inhibitory Concentration ($IC_{50)}$ value of 5.83 compared with Acarbose with 6.38 µg/mL hopefully, this study could discover a new specific lead to target the α -Amylase (3M07), this research work concludes that among all ipobscurine B showed the anti-diabetic activity, therefore further studies to be carried out in order to confirm as potent biomarker.

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