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Molecular Docking Analysis of Flavonoid Compounds with *C. igneus* for the Identification of Potential Effective α -amylase Inhibitors

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ABSTRACT

Introduction: Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from fault in insulin secretion, insulin action or both. most effective treatment for type 2 diabetes is the control of postprandial hyperglycemia after a meal. stabilization of blood glucose is necessary for diabetic patients, because it prevents hyperglycemia and the complexity associated with diabetes.

Objective: The objective of this study is to evaluate the *in silico* activities of *Costus igneus* flavonoids on α -amylase.

Methods: Human Pancreatic Alpha-Amylase (PDB ID-4GQR) was retrieved. The chemical structures of 1-Ethoxy-1-propene, 2-Pentadecanone, Epicatechin, Pentatriacontane, Squalene, and Undecanal flavonoids were retrieved from PubChem and ChemSpider database for molecular docking. The docking analysis of ligand with Human Pancreatic Alpha-Amylase was carried by autodock software using default parameters. The values were obtained in terms of energy (e-value) Kcal/mol.

Results: Squalene, a flavonoid compound present in *Costus igneus* has good docking activity with the least e-value of -7.22 kcal/mol and the residues ASN:5, 481; PRO:4, 223, 332; ASP:402, ARG:10, 252, 398, 421; VAL:401, GLY:403, 9, 334; THR: 6, 11; GLN:8; PHE:222, 335 SER:3, 226; LEU:217 were might play important roles in binding with this compound. Lesser the E-value greater the acceptability of compound as a drug and so it is considered as an effective ligand in inhibiting Human Pancreatic Alpha-Amylase.

Conclusion: The results are expected to be useful in conducting *in vitro* and *in vivo* screenings on animal model which may lead to the development of more effective and potent new chemical entities with anti-diabetic property.

Keywords: Docking, α -Amylase, *Costus igneus*, Diabetes mellitus

Introduction

Diabetes mellitus is a metabolic disease characterized by hyperglycemia resulting the insulin secretion, insulin action or both. Stabilization of blood glucose is necessary for diabetic patients, because it prevents hyperglycemia and associated with diabetes (Kotowaroo 2006). The natural products or medicinal plants reduce the absorption of glucose by reduce the carbohydrate hydrolyzing enzymes, such as pancreatic α -amylase. The inhibition of this enzyme slow down the carbohydrate digestion and extend the overall carbohydrate digestion time, ensuing in the decrease in glucose absorption rate and as a result reduce the postprandial plasma glucose rise. Several indigenous medicinal plants have a great potential in the α -amylase inhibition (Zinjarde SSet al. 2011).

Costus pictus (syn. *Costus igneus* Nak, *Costus mexicanus* or *Costus congenitus*), a perennial herbs belonging to Costaceae (Zingiberaceae) family, grows up to height of two to three feet. Popularly known as Insulin plant where as its botanical name is *Costus pictus* D. Don (Bakrudeen and Arun, et al. 2009). The flowers appear on branches tip and orange in colour, ultimately develops into cone like fruits (Gilman, 2012). It originally belong South and Central America (Mexico) and was introduced to India (Beena and Joji, 2012) . In southern India, it usually grows as an ornamental plant and its leave are used as a dietary supplement in the treatment of diabetes mellitus (Vishalakshi et al, 2006). Leaves of *Costus igneus* is known to be effectively used for treating diabetes by the tribal people of Kolli Hills of Namakkal district, Tamil Nadu (Elavarasi and Saravanan, 2012).

In this present study the flavonoids of *Costus igneus* were subjected to extensive computational study to predict binding interactions with target enzyme α -amylase using computer aided active site prediction. Docking allows virtually screening of compounds and predicts the strongest binders based on various scoring functions. several researches of flavonoids gives the details about their valuable biological activities such as anti-allergenic, anti-cancer, anti-inflammatory, anti-oxident, anti-viral, properties (annath P et al, 2011). However, the *in silico* evaluation of α -Amylase the current study is to predict the *in silico* evaluation of α -Amylase inhibitory activity and the stereochemistry binding of the flavonoids on α -Amylase has been carried out, which may helpful in the development of potent α -Amylase inhibitors.

Materials and Methods

Software's required

Open Babel software used to convert chemical file formats from .sdf to .pdb. AutoDock4.2 is used for docking. Discovery studio visualizer 2.5.5 is for post docking analysis. ChemSketch is a comprehensive structure editor with a variety of tools and functionality that ease the communication of scientific and chemical information.

Preparation of protein receptor

The pancreatic α -amylase structure was downloaded from protein database (PDB ID: 4GQR). It was a wild type human pancreatic α -amylase at true atomic resolution (1.07 Å). All water molecules were removed, hydrogen atoms were added, non-polar hydrogen atoms were merged, and Gasteiger charge was assigned to the receptor molecule.



Figure-1. Crystal structure of α -amylase(PDB ID: 4GQR)

Preparation of ligand

The major flavonoid compounds of *Costus igneus* such as 1-Ethoxy-1-propene (CID-536509), 2-Pentadecanone (CID-61303), Epicatechin (CID-72276), Pentatriacontane (CID-12413), Squalene (CID-638072), and Undecanal (CID-8186) were used as ligands. The 3D structure of each ligand was obtained from Pubchem (<http://pubchem.ncbi.nlm.nih.gov/>) and finally converted into PDF files using Open Babel.

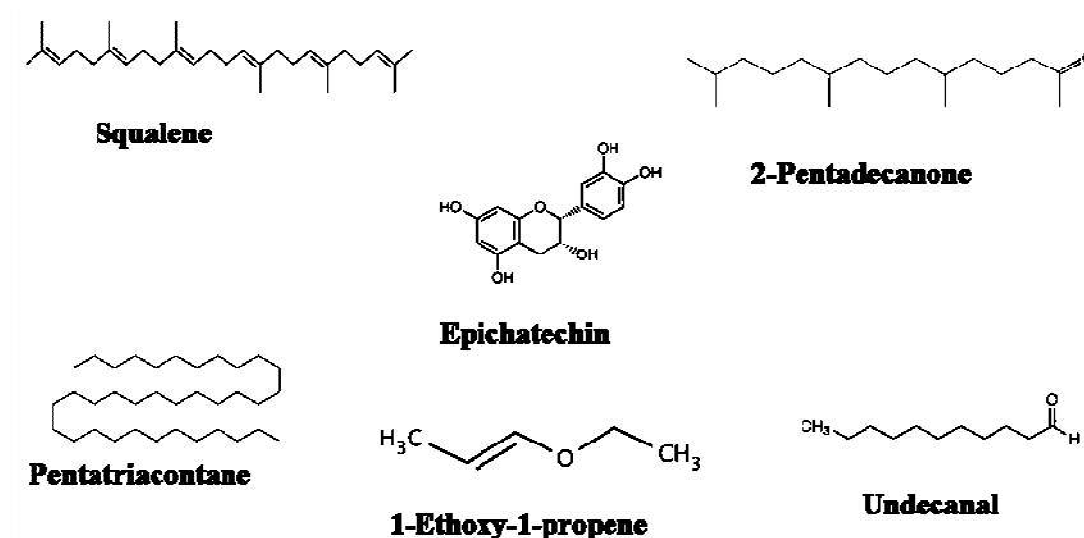


Figure-2. General chemical structures of different types of flavonoids.

Docking methodology

We employed the Lamarckian genetic algorithm (LGA) for ligand conformational searching, which is a hybrid of a genetic algorithm and a local search algorithm. This algorithm first builds a population of individuals (genes), each being a different random conformation of the docked molecule. Each individual is then mutated to acquire a slightly different translation

and rotation and the local search algorithm then performs energy minimizations on a userspecified proportion of the population of individuals. The individuals with the low resulting energy are transferred to the next generation and the process is then repeated. The algorithm is called Lamarckian because every new generation of individuals is allowed to inherit the local search adaptations of their parents. An extended PDB format, termed as PDBQT file was used for coordinate files which includes atomic partial charges. AutoDock Tools was used for creating PDBQT files from traditional PDB files (Umamaheswari et al. 2011). Crystal structure of α -Amylase enzyme was downloaded from the even protein data bank. The flavonoid ligands like optimized using “Prepare Ligands” in the AutoDock 4.2 for docking studies (Madeswaran et al. 2011).

Molecular docking analysis

A computational ligand-target docking approach was used to analyze structural complexes of the (target) with α -Amylase (ligand) in order to understand the structural basis of this proteintarget specificity. Finally, docking was carried out by, AutoDock 4.2 Vina option based on scoring functions. The energy of interaction of Squalenewith the is assigned “grid point.” At each step of the simulation, the energy ofinteraction of ligand and protein was evaluated using atomic affinity potentials computed on a grid. The remaining parameters were set as default.

RESULTS AND DISCUSSION

Docking orientations of the flavonoids

Among the reported flavonoids, epicatechinhas the best docking score of -7.05 kcal/mol. The compound was found to involved carbon hydrogen bond with SER:3, LEU:211 amino acid residues at a distance of 2.13 Å to 2.14Å respectivelyin the active site of α -amylase receptor. The compound was observed to involvedvandar waals interaction withbASN:5, PHE:229, LEU:214,LYS:208, ASP:212 residues, pi-cation interaction with TYR:2, LYS:227 amino acid residues, pi-Donar hydrogen bond with ASN:250, pi-sigma bond interaction with PRO:228 and pi-alkyl interaction with ILE:230 amino acid residues in the active site of the receptors.

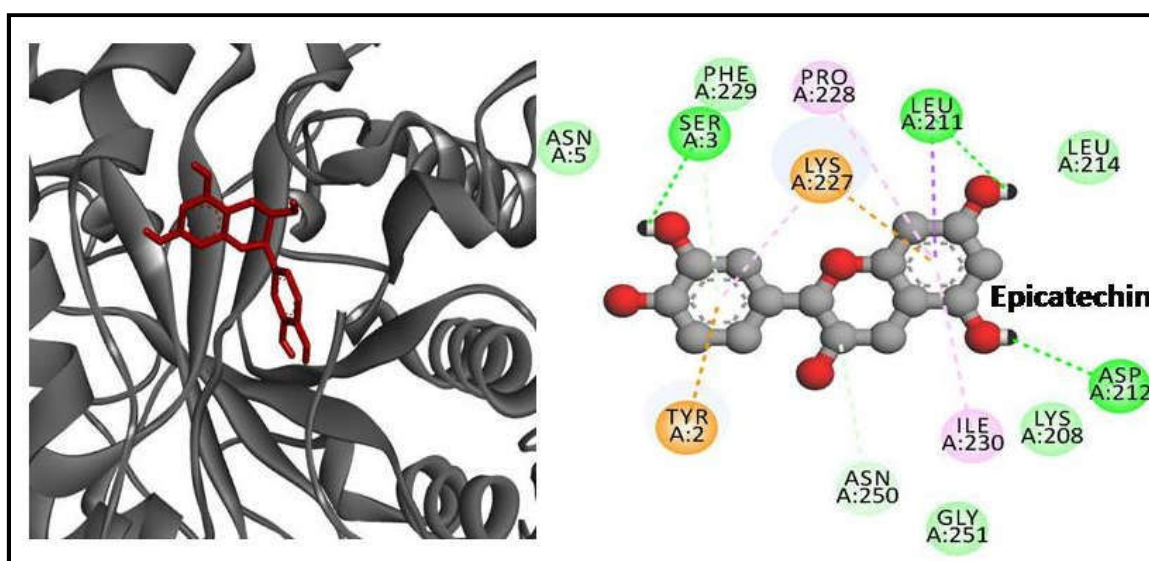


Figure 3. 3D and 2D visualizations of epicatechin in the active site of α -amylase(4GQR)

The second best among the docking score of -7.22kcal/mol among the reported flavonoid compounds was squalene and it involves van der Waals interaction with GLY:334,403,ARG:10,252,421,PRO:332,THR:6,11,GLN:8,SER:3,226, PHE:335, ASN:5 residues, alkyl interactions with ARG:398, pi-alkyl interactions with PRO:4,223 PHE:222 amino acids residues in the active site of the receptors.

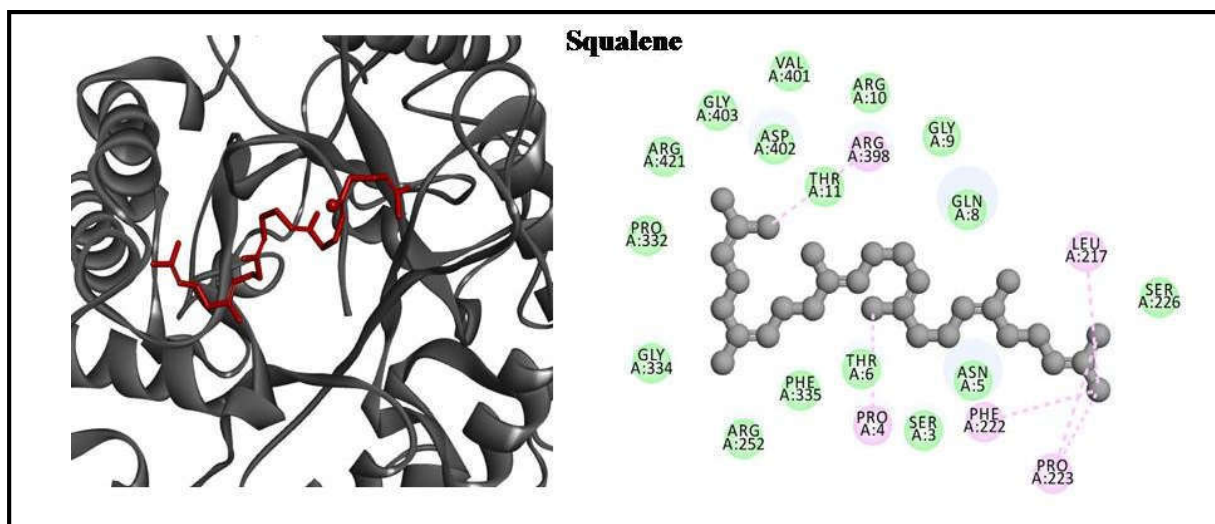


Figure 4. 3D and 2D visualization of compound squalene in the active site of α -amylase (4GQR)

Another compound 2-Pentadecanone with a docking score of -4.82 kcal/mol. It involved Alkyl interaction with VAL:49, 107 residues, Van der Waals interactions with PRO:54, ASN:53, ILE:51, TYR:52, ALA:50, SER:108,112, GLY:104, TRP:59, GLN:63, THR:163, LEU:162,165, HIS:10 and GLU:233 amino acids residues in the active site of the receptors.

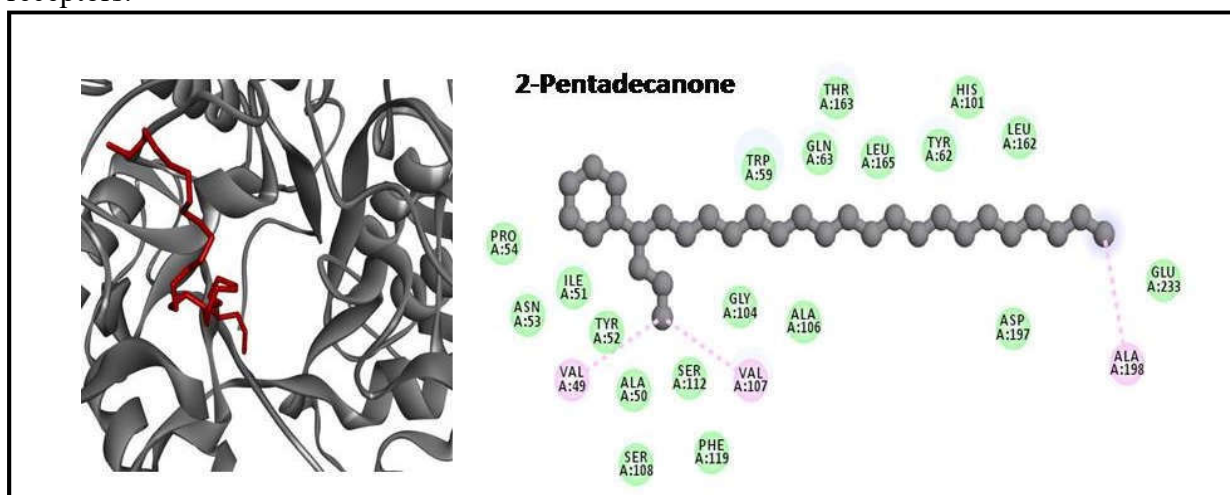


Figure 5. 3D and 2D visualization of flavonoid, 2-Pentadecanone in the active site α -amylase (4GQR)

Flavonoid, 1-Ethoxy-1-propene also showed docking score of -4.79 kcal/mol and was observed to form carbon hydrogen bond with GLN:63, THR:163 and with bond of distance 2.08 Å and 2.12 Å. Carbon hydrogen bonds interaction with TRP:59, TYR:62, GLU:233, ASP:30, LEU:165 ASN:105 amino acids residues in the active site of the receptors. Vander-Waals interaction with ALA:106 GLY:104,164 ARG:195 HIS:299 ASP:197 ILE:235 amino acid residues.

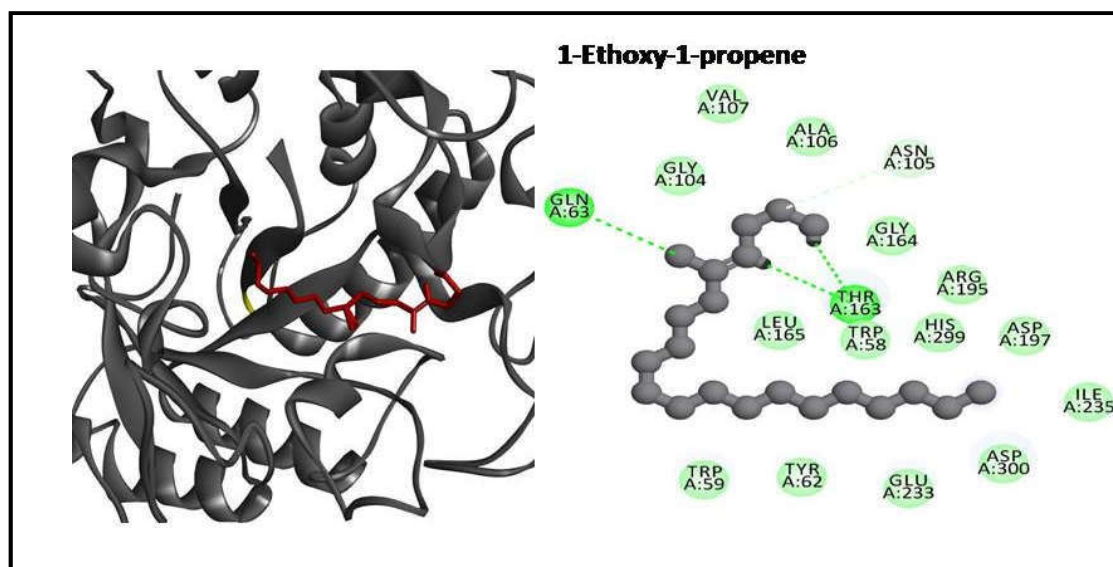


Figure 6. 3D and 2D visualization of flavonoid, 1-Ethoxy-1-propene in the active site of α -amylase (4GQR)

The Compound Undecanal with a docking score of -3.87 kcal/mol which formed vander waals interactions in the active site of the target receptor with ARG:421, ASP:402, THR:11, PHE:335, GLY:334, GLN:7,8 and formed carbon hydrogen bonds with THR:6 residue and alkyl interactions with ARG:398, PRO:332 amino acids residues in the active site of the receptors.

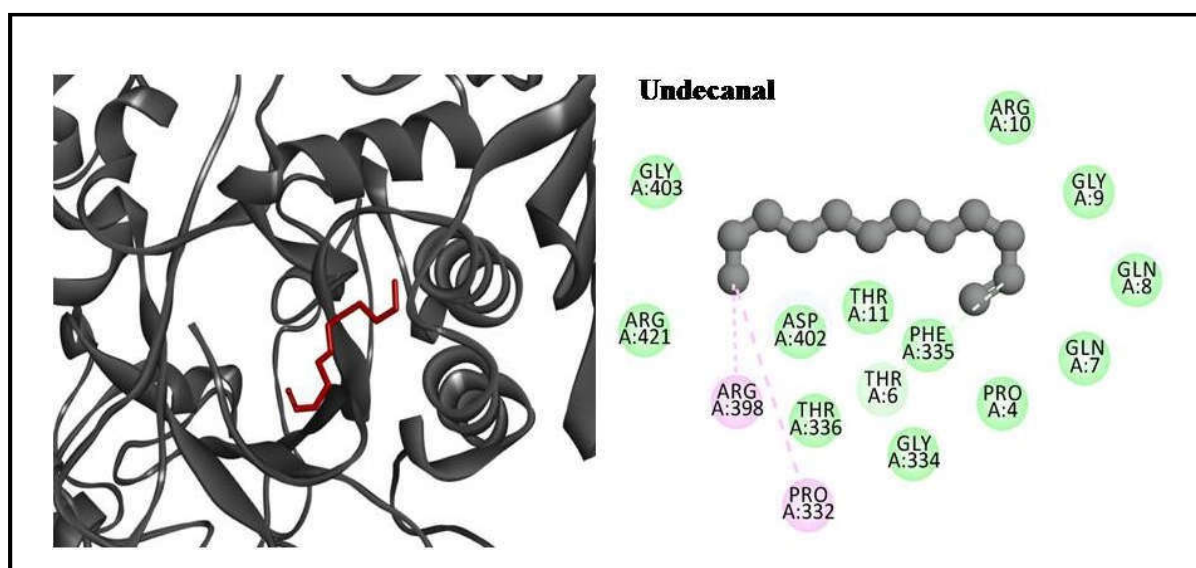


Figure 7. 3D and 2D visualization of compound Undecanal in the active site of α -amylase (4GQR)

The other flavonoid pentatriacontane with docking score of -3.18 kcal/mol was found to have Vander-Waals interaction with the TYR:62,151, ILE:235, HIS:101,201, GLU:233, ALA:198, ASP:197,300, LEU:165, TRP:58, THR:163 and GLN:63 amino acid residues and pi-alkyl interaction with TRP:59 residue in the active site of the receptors.

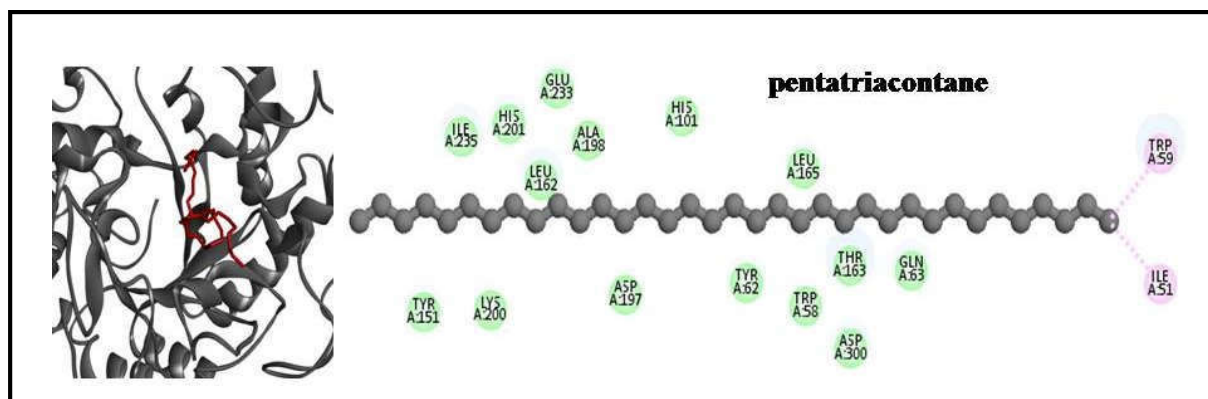


Figure 8. 3D and 2D visualization of flavonoid pentatriacontane in the active site of α -amylase (4GQR)

Molecular interaction of phytochemical constituents of α -amylase enzyme has been investigated in this study using Autodock software, and the results are given in Table-1. Epichatechin (07.05 kcal/mol), squalene (-7.22 cal/mol) 2-pentadecanone (-4.82 kcal/mol), 1-ethoxy-1-propane (-4.79 kcal/mol), undecanal (-3.87 kcal/mol), pentatriacontane (-3.18 kcal/mol) were exhibited remarkable interaction with α -amylase at lower energy level. This indicates, the phytoconstituents of *costus igneus* could inhibit α -amylase in a more efficient way even with low binding energy than the synthetic compound. The results obtained from the present investigation given significant information that the phytochemicals of *Costus igneus* plant could work better with the diabetic targets by binding with α -amylase and slows down the starch digestion and leads to slow release of glucose in the blood stream. Due to this mechanism, the *Costus igneus* plant could control the blood sugar level more effectively. Hence, we could take some leads from phytochemicals of *Costus igneus* towards the development/synthesis of α -amylase inhibitors for the management of diabetic patients.

As shown in table-1, flavonoids showed binding energy ranging between -7.22 kcal/mol to -3.18 kcal/mol. Squalene showed better binding energy (-7.22 kcal/mol) than all the selected flavonoids. This proves that flavonoids consist of potential α -Amylase inhibitory binding sites.

In addition, two other parameters like inhibition constant (K_i) and intermolecular energy were also determined. The inhibitory constant is a measure of the effectiveness of a compound in inhibiting biological or biochemical function which act between neighboring particles such as, atoms, molecules or ions. Inhibition constant is directly proportional to intermolecular energy of a compound.

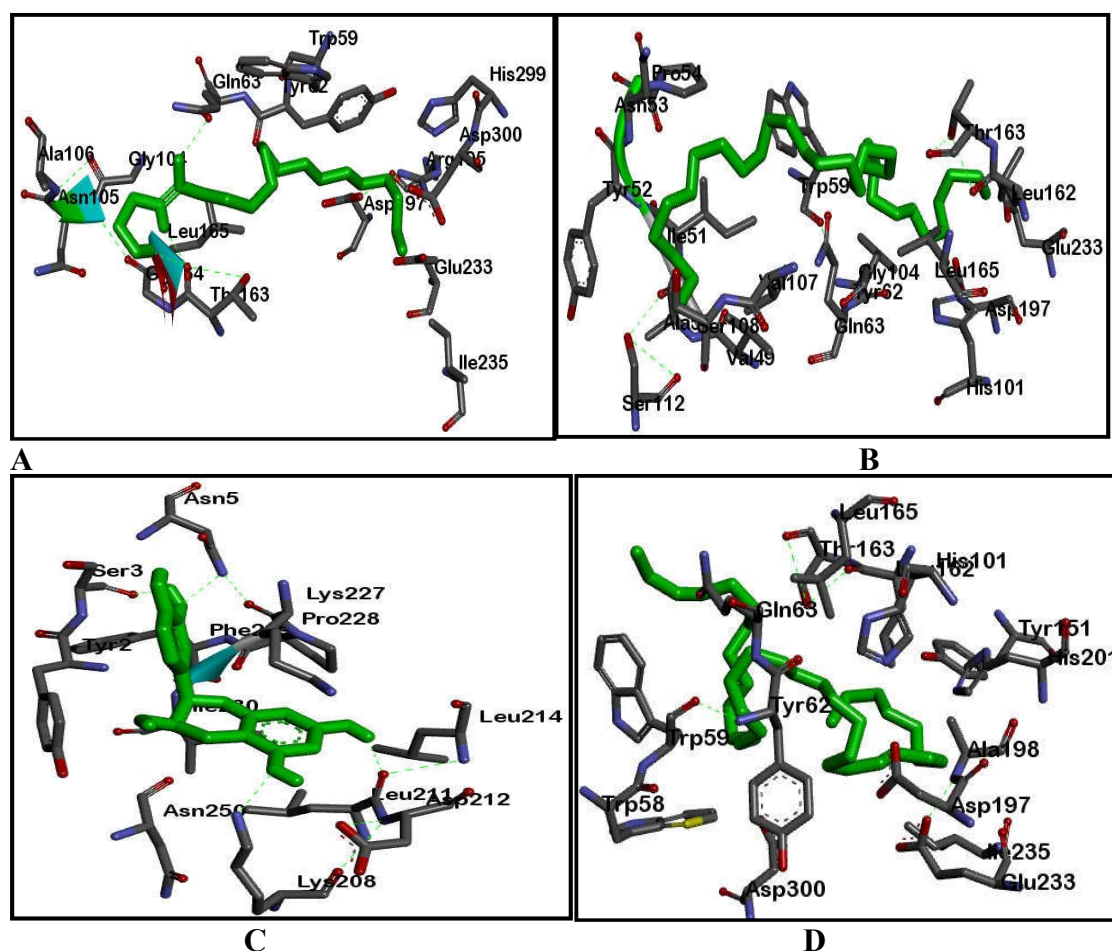
Table 1:- *C. igneus* flavonoids docking results against target enzyme α -amylase

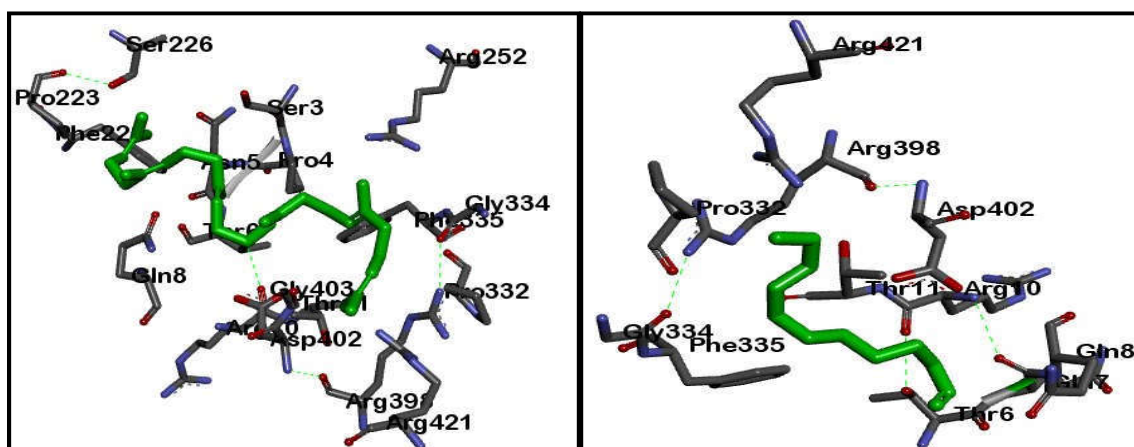
S. N	Compound Name	Binding energy	Inhibition constant	Intermolecular energy (kcal/mol)	Residue involving interaction	No. of H bonds	Interaction of residues forming H ₂ bonds
1	1-Ethoxy-1-propene	-4.79 kcal/mol	308.08 uM	-9.86 kcal/mol	ASP:197,300,THR:163,GLU:233,ARG:195,TYR:62,GLY:164,TRP:58,59,ASN:105,ILE:235,ALA:106,HIS:299,VAL:107,LEU:165,GLN:63	2	GLN:63 , THR:163
2	2-Pentadecanone	-4.82 kcal/mol	292.83 uM	-13.47 kcal/mol	THR:163,ILE:51,HIS:101,ASN:53,TRP:59,TYR:52,GLN:63,VAL:49,107,LEU:162,165,ALA:50,106,198,TYR:62,PRO:54,SER:108,112,GLY:104,PHI:119,ASP:197,GLU:233	0	-
3	Epicatechin	-7.05 kcal/mol	6.76 uM	-8.84 kcal/mol	ASN:5,25,ILE:230,PHI:229,GLY:251,SER:3,LYS:208,PRO:228,ASP:212,LEU:211,214,LYS:227,TYR:2	3	SER:3 LEU:211 ASP:212
4	Pentatriacontane	-3.18 kcal/mol	4.66 uM	-12.73 kcal/mol	GLU:233,LYS:200,HIS:101,201,ASP:197,300,ILE:51,235,THR:163,LEU:162,165,GLN:63,ALA:198,TRP:58,59,TYR:151	-	-
5	Squalene	-7.22 kcal/mol	5.13 uM	-11.63 kcal/mol	VAL:401,ARG:10,252,421,398,GLY:9,334,403,ASP:402,THR:11,PRO:4,223,332,GLN:8,PHE:222,335,THR:6,SER:3,226,ASN:5,LEU:217	-	-
6	Undecanal	-3.87 kcal/mol	1.46uM	-6.55 kcal/mol	GLY:9,334,403,ARG:10,398,421,ASP:402,THR:6,11,336,PHE:335,PRO:4,335,GLN:7,8	-	-

Inhibition constant is directly proportional to binding energy. As shown in Table 1, flavonoids showed inhibition constant ranging from 1.46 μ M to 308.08 μ M. 1-ethoxy-1-propene showed excellent inhibition constant (308.08 μ M) than the 2-pentadecanone (292.83 μ M). All the selected compounds had lesser inhibition constant when compared to the standard. Thus, the potential α -Amylase inhibitory activity of the flavonoids

Intermolecular energy is also directly proportional to binding energy. As shown in Table-1, flavonoids showed intermolecular energy ranging between -13.47 kcal/mol to -6.55 kcal/mol. We found a decrease in intermolecular energy of all the selected compounds with a simultaneous decrease in the binding energy. This result further proved the α -Amylase inhibitory activity of all the selected flavonoids.

Based on the docking studies, the α -Amylase inhibitory activity of the selected compounds was found to be decreased in the order of Squalene, Epicatechin, 2-Pentadecanone, 1-Ethoxy-1-propene, Pentatriacontane and Undecanal possess potential α -amylase inhibitory binding sites. This may be attributed due to the differences in the position of the functional groups in the compounds.





EF

Figure-9.Docking images showing the interactions of ligandA. 1-Ethoxy-1- propene,B. 2-pentadecanone,C. epicatechin, D. pentatricontane,E. squalene, F. undecanal

CONCLUSION

In conclusion, the results of the present study clearly demonstrated the *in silico* molecular docking studies of selected flavonoids with α -amylase enzyme exhibited binding interactions and warrants further studies needed for the development of potent α -amylaseinhibitors for the treatment of DM. This *in silico* studies is actually an added advantage to screen the α -amylaseinhibition. Further investigations on the above compounds and *in vivo* studies are necessary to develop potential chemical entities for the prevention and treatment of metabolic disorders.

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