Identification of Novel Protein Targets for Fenugreek to Treat Diabetes: A Molecular Docking Study

Haasini Nandyala¹,², Ariel Pham¹,³, Anushka Wagle¹,⁴, Hansika Daggolu¹,⁵, Amrita Guha¹,⁶, Reya Sankar¹,⁷, Chloe Chan¹,⁸, Dipti Venkatesh¹,⁹, Gayathri Renganathan¹#

¹Department of Chemistry, Biochemistry, & Physical Science, Aspiring Scholars Directed Research Program (ASDRP) Fremont, CA, USA
²BASIS Independent Silicon Valley, San Jose, CA, 95126.
³Piedmont Hills High School, San Jose, CA, 95132.
⁴Fremont High School, Sunnyvale, CA, 94087.
⁵Mission San Jose High School, Fremont, CA, 94539.
⁶Alsion Montessori Middle/High School, Fremont, CA, 94539.
⁷Dougherty Valley High School, San Ramon, CA, 94582.
⁸Lowell High School, San Francisco, CA, 94132.
⁹Evergreen Valley High School, San Jose, CA, 95148.
#Advisor

ABSTRACT

Trigonella foenum-graecum has been shown to have anti-diabetic potential through a wide variety of in-vivo assays as well as by inhibiting enzymes such as alpha-glucosidase and alpha-amylase. Studies have indicated the therapeutic potential of different phytoconstituents found in Trigonella foenum-graecum including diosgenin trigonelline, 4-hydroxyisoleucine, leucine, and L-lysine. This study aims to find novel protein targets that these specific phytoconstituents from fenugreek can bind to, thereby helping to treat diabetes mellitus. Through multiple stages of molecular docking and analyzing the binding sites in comparison to previously reported inhibitors, a suitable and novel target protein for four of the compounds was found and the relevance to diabetes was discussed, setting up these compounds as novel inhibitors for the target proteins.

Introduction

Trigonella foenum-graecum, also known as fenugreek, is a short, annual plant from the Fabaceae family. It is found in various parts of the globe, and fenugreek leaves and seeds have been often used as a spice, condiment, and medication. In Africa, fenugreek is used as a supplement during bread preparation and the seed components of fenugreek are known to enhance the nutritional quality of the bread. In India, the leaves and seeds are utilized as flavoring and seasoning agents. In China, it is used to cure edema, and the ancient Egyptians employed fenugreek to incense the mummies¹. Additionally, fenugreek has been recently studied for its therapeutic potential, including its antidiabetic², antibacterial³, antihyperlipidemic⁴, anti-angiogenic⁵, and anticoagulant factors⁶. Fenugreek seeds have been the most widely studied part of the plant and many potent active compounds have been identified. These major phytoconstituents include diosgenin, a sapogenin, which are lipophilic triterpene derivatives which protect plants against microbes, fungi, and other hostile organisms; trigonelline, an alkaloid⁷, which is a class of basic, naturally occurring organic compounds that contain at least one nitrogen atom; and 4-hydroxyisoleucine⁸, leucine, and L-lysine⁹, which are all amino acids. Amino acids are organic compounds that contain amino and carboxyl functional groups, along with a side chain specific to each amino acid, which are polymerized to form proteins.
Diabetes mellitus is a chronic metabolic disease that is prevalent around the world; the IDF estimated that in 2019, the global estimate of adults with diabetes was 463 million\textsuperscript{11}. Diabetes mellitus is characterized by hyperglycemia, which is when there is too much sugar in the blood. This results in defective insulin secretion, resistance to insulin action, or both\textsuperscript{12}. In type 1 diabetes mellitus, autoimmune destruction of pancreatic β-cells leads to a deficiency of insulin secretion, which results in metabolic derangements such as increased waist circumference, increased serum triglyceride levels, hypertension, and insulin resistance\textsuperscript{13}. In addition, the function of pancreatic α-cells is also abnormal, so there is excessive secretion of glucagons, which would normally be suppressed by hyperglycemia. On the other hand, in type 2 diabetes mellitus, individuals have detectable levels of insulin circulating in the body. However, they often are resistant to the action of insulin, due to impaired insulin secretion by the pancreas. This then causes increased glucose production in the liver, which leads to receptor and post-receptor defects, causing insulin resistance\textsuperscript{14}. Diabetes has been thoroughly studied, yet in spite of new medical devices and technology, it is projected that diabetes in the United States will increase by 54% to more than 54.9 million Americans between 2015 and 2030, annual deaths attributed to diabetes will climb by 38% to 385,800, and total annual medical and societal costs related to diabetes will increase 53% to more than $622 billion by 2030\textsuperscript{15}.

With these projections, finding anti-diabetic therapeutics is crucial. Fenugreek has shown to have quite a lot of anti-diabetic potential. In terms of its hypoglycemic effect, many studies have tested fenugreek generally through animal and \textit{in vivo} studies where animals were fed with either the seeds powders or extracts for a period of time from 5 days to several weeks or with a single dose treatment regimen\textsuperscript{16}. The results generally showed that pretreatment with fenugreek resulted in lowered blood glucose and lipid levels in the mice or rats, indicating antidiabetic potential. Additionally, current research has focused on finding effective alpha-glucosidase and alpha-amylose inhibitors. Both of these enzymes are involved in the digestion of carbohydrates. Alpha-glucosidase is a membrane-bound enzyme present in the epithelium of the small intestine, which works to facilitate the absorption of glucose by the small intestine by catalyzing the hydrolytic cleavage of oligosaccharides into absorbable monosaccharides. α-glucosidase inhibitors can retard the liberation of D-glucose from dietary complex carbohydrates and delay glucose absorption, resulting in reduced postprandial, or occurring after a meal, plasma glucose levels and suppression of postprandial hyperglycemia\textsuperscript{17}. Alpha-amylose enzymes, most importantly pancreatic alpha-amylose, act as catalysts in the reaction which involves the hydrolysis of the alpha-1,4 glycosidic linkages of the starch, amylopectin, amylose, glycogen, and numerous maltodextrins and is responsible for starch digestion. Similar to alpha-glucosidase, inhibiting alpha-amylose has been shown to decrease postprandial blood glucose levels\textsuperscript{18}. In terms of fenugreek’s ability to inhibit target proteins, diosgenin was shown to inhibit both alpha-glucosidase and alpha-amylose quite significantly through \textit{in-vitro} studies\textsuperscript{19}.

Molecular docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred pose is important to predict the strength of association or binding energy between two molecules. Characterization of the binding behavior to biomolecules plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes. For example, finding an inhibitor, which binds to that particular protein could prevent the action of some harmful proteins. Due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target-binding site, molecular docking is one of the most frequently used methods in structure based drug design\textsuperscript{20}. Traditionally, multiple ligands are screened against a certain protein target to find the ligand with the best binding energy score. However, when there is not much information about the molecular mechanism of the ligands, they undergo reverse docking, a process in which multiple protein targets are screened against a certain ligand and their docking complexes are reviewed.

**Methods**

Reverse Docking
Our group obtained SDF files of the five phytoconstituents from fenugreek: trigonelline\textsuperscript{21}, diosgenin\textsuperscript{22}, lysine\textsuperscript{23}, leucine\textsuperscript{24}, and 4-hydroxyisoleucine\textsuperscript{25} were from PubChem. We then individually submitted these files to ACID, a novel web server that conducts inverse docking by using AutoDock Vina, LEDOCK, PLANTS, and PSOVina for binding pose search and docks the compounds against a i9 target database containing 831 protein structures from PDB\textsuperscript{26} covering 30 therapeutic areas\textsuperscript{27}. After obtaining the reverse docking results for each compound, the top 30 target proteins (ranked in terms of binding energy) for each compound were selected and filtered first based on their enzyme class. The enzyme classes that our group selected were transferases, hydrolases, and oxidoreductases. The proteins from these classes were then screened for relevance to diabetes and the benefits of the protein being inhibited in relation to diabetes.

Validation with AutoDock Vina

In order to confirm the docking scores received from ACID, our group manually blind docked the target proteins that passed the screening against their respective compound using AutoDock Vina\textsuperscript{28}. The docking score received from AutoDock Vina (ADV) was then compared with the score received from ACID.

Analysis of Binding Sites

Since blind docking was conducted, we used AutoDock Vina and Discovery Studio Visualizer\textsuperscript{29} (DS) to identify the amino acid residues for each protein-ligand complex and we also used Discovery Studio Visualizer to identify the specific interactions for each amino acid residue. In cases where an error occurred with AutoDock Vina, PyMol\textsuperscript{30} was used to determine the amino acid residues. In order to determine the amino acid residues through AutoDock Vina and PyMol, the output files created by manually docking and the target protein file were opened and analyzed by looking at the interactions. Similarly, the output file and protein file were opened on Discovery Studio Visualizer and the 2D interactions map was opened to identify the amino acid binding residues and the specific types of interactions. Additionally, in order to identify which proteins the ligands were actually inhibiting, we blind docked previously reported inhibitors for each target protein using AutoDock Vina and the amino acid residues from these dockings were compared to binding residues of the respective fenugreek compound. When comparing the phytoconstituents’ docking to the inhibitors’ docking, only conformations with the lowest RMSD values were studied.

Results

For each of the five compounds, there are three tables displaying the different results we obtained. The first table shows the top 30 target proteins based on binding energy we received from ACID. The second table depicts the ACID docking score, AutoDock Vina docking score, AutoDock Vina binding residues and the Discovery Studio binding residues for the screened and selected target proteins. The discrepancies between the ACID docking score and the AutoDock Vina score has been attributed to the fact that ACID used multiple docking softwares to come up with a docking score, and these other softwares are not accounted into the score received from AutoDock Vina. Additionally, the binding residues received from each software are very similar, but Discovery Studio was more specific in that for each amino acid residue, it also included what type of interaction was occurring between the residue and the ligand. Lastly the third table depicts the AutoDock Vina binding residues and docking score of the fenugreek compound and the reported inhibitor for each of the selected target proteins.

Table 1.1. Top 30 target proteins scored for diosgenin from ACID

| Protein Code | Protein Name | Free Binding Energy (kcal/mol) | Docking Score (kcal/mol) |
|--------------|--------------|--------------------------------|--------------------------|

ISSN: 2167-1907 www.JSR.org
| PDB Code | Name                                      | Classification                               | AutoDock Docking | AutoDock Vina Docking | AutoDock Vina Amino Acid Residues and Interactions |
|---------|-------------------------------------------|----------------------------------------------|-------------------|-----------------------|---------------------------------------------------|
| 1NRL    | Human PXR-LBD                              |                                               | -38.12            | -11.1                 |                                                   |
| 5HBS    | Human cellular retinol binding protein 1   |                                               | -37.74            | -10.95                |                                                   |
| 1FCY    | Human Retinoic Acid Nuclear Receptor Hrar  |                                               | -37.64            | -11.13                |                                                   |
| 1SQN    | Progesterone Receptor Ligand Binding Domain|                                               | -37.07            | -11.6                 |                                                   |
| 1R20    | Heterodimer EcR/USP                        |                                               | -36.82            | -11.21                |                                                   |
| 4UDD    | Glucocorticoid Receptor                    |                                               | -36.66            | -11.5                 |                                                   |
| 5HJP    | LXRbeta Selective Agonists                 |                                               | -36.55            | -11.25                |                                                   |
| 4DM6    | RARb LBD homodimer                         |                                               | -35.98            | -11.05                |                                                   |
| 2BZG    | Thiopurine S-methyltransferase             |                                               | -35.94            | -11.04                |                                                   |
| 3KMR    | RAR alpha ligand binding domain             |                                               | -35.81            | -10.89                |                                                   |
| 1SQB    | Bovine Bc1                                 |                                               | -35.66            | -10.22                |                                                   |
| 3MY0    | ACVRL1 (ALK1) kinase                       |                                               | -35.27            | -10.35                |                                                   |
| 4QE6    | Human FXR                                  |                                               | -35.26            | -11.27                |                                                   |
| 2GL8    | Retinoic acid receptor RXR-gamma           |                                               | -34.78            | -9.07                 |                                                   |
| 2P1T    | Retinoid X receptor alpha                  |                                               | -34.71            | -11.48                |                                                   |
| 1CQX    | Flavohemoglobin                            |                                               | -34.37            | -10.04                |                                                   |
| 3B0T    | Human VDR ligand binding domain            |                                               | -33.67            | -10.72                |                                                   |
| 2E2R    | Human estrogen-related receptor gamma      |                                               | -33.59            | -11.07                |                                                   |
| 4L8U    | Human serum albumin                        |                                               | -33.18            | -10.41                |                                                   |
| 3C6K    | Human spermine synthase                    |                                               | -33.1             | -9.55                 |                                                   |
| 3BRT    | NEMO/IKK                                   |                                               | -33.09            | -10.21                |                                                   |
| 1W78    | E.coli FolC                                |                                               | -32.44            | -9.42                 |                                                   |
| 4QT8    | Human ERK1                                 |                                               | -32.43            | -10.99                |                                                   |
| 2HI4    | Human Microsomal P450 1A2                  |                                               | -32.41            | -10.71                |                                                   |
| 4BVM    | Peripheral membrane protein P2             |                                               | -32.13            | -10.96                |                                                   |
| 4GQS    | Human Microsomal Cytochrome P450(CYP) 2C19|                                               | -31.78            | -10.19                |                                                   |
| 1D2S    | N-Terminal Laminin G-Like Domain of SHBG   |                                               | -31.57            | -10.19                |                                                   |
| 1T46    | Autoinhibition and STI-571 Inhibition of C-KIT Tyrosine Kinase | | -31.56 | -9.84 |                                                   |
| 2Q80    | Human geranylgeranyl pyrophosphate synthase bound to GGPP | | -31.28 | -10 |                                                   |
| 4UYB    | SEC14-like protein 3                       |                                               | -31.28            | -11.15                |                                                   |

Table 2.1. Validation of docking of selected target proteins for diosgenin
Table 3.1 Diosgenin vs. the reported inhibitors for each target protein

| PDB Code | Protein Name          | Diosgenin ADV Docking Score (kcal/mol) | Diosgenin ADV Amino Acid Residues | Reported Inhibitor Name   | Reported Inhibitor ADV Docking Score (kcal/mol) | Reported Inhibitor ADV Amino Acid Residues |
|----------|-----------------------|---------------------------------------|-----------------------------------|---------------------------|-----------------------------------------------|------------------------------------------|
| 4QT B    | Human ERK1 Transferase/Transf erase Inhibitor | -10.99                                | THR288, LYS289, PHE313, THR312, ASN314, LYS317 | Ulixertinib               | -9.7                                         | TYR53, LYS71, VAL56, ASP184, GLU88, GLY186, ARG84 |
| 2HI4     | Human Microsomal P450 1A2 Oxidoreductase          | -10.71                                | GLN304, ASN309, GLU305, VAL308, PHE288, TYR272, VAL268, GLN265 | Asenapine                 | -9.0                                         | LEU382, THR385, THR124, ALA317, LEU497 |

After comparing diosgenin’s binding site to many reported inhibitors of each of the two target proteins 4QTB and 2HI4, human extracellular signal-regulated kinase (ERK1) and Cytochrome P450 1A2 (CYP1A2), our group found that diosgenin did not act as an inhibitor for either of the proteins. The reported inhibitor we used for ERK1 was ulixertinib31. For CYP1A2, we used asenapine32. For ERK1, diosgenin was compared to Figures 1 and 2, displaying the PyMol visualization of diosgenin’s binding site and the reported inhibitors’ binding sites for ERK1 and CYP1A2, respectively.
Table 1.2. Top 30 target proteins scored for trigonelline from ACID

| PDB Code | Protein Name                                                                 | Free Binding Energy (kcal/mol) | Docking score (kcal/mol) |
|----------|------------------------------------------------------------------------------|-------------------------------|--------------------------|
| 3L6B     | Human serine racemase                                                        | -55.07                        | -6.56                    |
| 4E1O     | Human histidine decarboxylase                                                 | -53.4                         | -6.68                    |
| 3I10     | Human Glutamate oxaloacetate transaminase 1                                  | -52.42                        | -6.85                    |
| 3KQJ     | MurA binary complex with UDP-N-acetylgalacosamine                             | -45.79                        | -6.4                     |
| 5HHY     | Human Alanine-Glyoxylate Aminotransferase major allele                        | -45.22                        | -6.86                    |
| 2O0B     | Mycobacterium tuberculosis epsp synthase                                      | -44.56                        | -6.24                    |
| 3C8Y     | Fe-only hydrogenase                                                           | -43.35                        | -6.59                    |
| 1D4D     | Flavocytochrome e fumarate reductase of shewanella putrefaciens strain mr-1  | -42.7                         | -6.42                    |
| 4USA     | Aldehyde Oxidoreductase                                                       | -41.27                        | -6.79                    |
| 2E1Q     | Human Xanthine Oxidoreductase mutant                                          | -40.83                        | -6.64                    |
| 1OTH     | Human ornithine transcarbamoylase complexed with n-phosphonacetyl-1-ornithine| -39.46                        | -6.82                    |
| 1FW1     | Glutathione transferase zeta/maleylacetoacetate isomerase                     | -38.78                        | -6.75                    |
| 4OQV     | Human dihydroorotate dehydrogenase bound with DSM338                         | -38.49                        | -6.87                    |
| 1SHA     | Phosphotyrosine recognition domain sh2 of v-src complexed with tyrosine-phosphorylated peptides | -33.81 | -6.58 |
| 2F71     | Protein tyrosine phosphatase 1B with sulfamic acid inhibitors                 | -33.26                        | -6.87                    |
| 2OO0     | Ornithine decarboxylase                                                       | -33.15                        | -6.61                    |
| 5KQL     | LMW-PTP in complex with 2-oxo-1-phenyl-2-(phenylamino)ethanesulfonic acid      | -33                            | -6.44                    |
| 4ZZ1     | Human gar transformylase                                                      | -31.89                        | -6.25                    |
| 2Z98     | AzoR (azoreductase)                                                           | -31.39                        | -6.68                    |
| 2GF3     | Monomeric sarcosine oxidase                                                   | -31.2                         | -6.75                    |
| 3DDS     | Glycogen phosphorylase                                                       | -30.06                        | -6.53                    |
| 1P5J     | Human Serine Dehydratase                                                     | -28.5                         | -6.83                    |
| 3N9Z     | Human CYP1A1                                                                  | -27.19                        | -6.46                    |
| 1FDR     | Flavodoxin reductase                                                          | -26.57                        | -6.33                    |
| 1PBE     | P-hydroxybenzoate hydroxylase                                                 | -26.37                        | -6.87                    |
| 5AX8     | Human mitochondrial aspartate aminotransferase                               | -26.27                        | -6.74                    |
| 2ZB4     | Human 15-ketoprostaglandin delta-13-reductase                                 | -25.95                        | -6.46                    |
| 1Z6T     | Apoptotic protease-activating factor 1                                        | -25.86                        | -6.58                    |
| 4XNH     | Yeast N-terminal acetyltransferase NatE                                      | -25.86                        | -6.58                    |
| 1QO8     | Flavocytochrome c3 fumarate reductase                                         | -25.18                        | -6.43                    |
Table 2.2 Validation of docking of selected target proteins for trigonelline

| PDB Code | Protein Name                        | Classification  | ACID Docking Score (kcal/mol) | AutoDockVina Score (kcal/mol) | AutoDockVina Amino Acid Residues | DS Amino Acid Residues and Interactions |
|----------|------------------------------------|-----------------|-------------------------------|------------------------------|---------------------------------|----------------------------------------|
| 2E1Q     | Xanthine Oxidoreductase            | Oxidoreductase  | -6.64                         | -5.8                         | THR262, SER347, GLY350*         | LEU257: Carbon Hydrogen Bond GLY350: Van der Waals SER347: Conventional Hydrogen Bond GLU263: Conventional Hydrogen Bond THR262: Conventional Hydrogen Bond VAL259: Alkyl |
| 2F71     | Protein tyrosine phosphatase 1B    | Hydrolase       | -6.87                         | -5.4                         | ARG199, ARG79, LEU204           | LEU204: Alkyl ARG199: Conventional Hydrogen Bond, Salt Bridge ARG79: Attractive Charge |
| 5KQ      | Low molecular weight Protein tyrosine phosphatase | Hydrolase/hydrolase inhibitor | -6.44                         | -5.5                         | LEU13, GLY14, ARG18, ILE16, TYR131 | TYR131: Alkyl ILE16: Pi-Alkyl ARG18: Salt Bridge, Conventional Hydrogen Bond LEU13: Conventional Hydrogen Bond GLY14: Conventional Hydrogen Bond GLU16: Conventional Hydrogen Bond ASP129: Carbon Hydrogen Bond |
| 3DD      | Glycogen phosphorylase             | Transferase     | -6.53                         | -4.9                         | ASN631*                         | ASN631: Conventional Hydrogen Bond ASP628: Unfavorable Negative-Negative ILE170: Alkyl |

* PyMol was used to identify amino acid residues
Table 3.2 Trigonelline vs. the reported inhibitors for each target protein

| PDB Code | Protein Name                                    | Trigonelline ADV Docking Score (kcal/mol) | Trigonelline ADV Amino Acid Residues | Reported Inhibitor Name | Reported Inhibitor ADV Docking Score (kcal/mol) | Reported Inhibitor ADV Amino Acid Residues |
|----------|-----------------------------------------------|------------------------------------------|-------------------------------------|-------------------------|-----------------------------------------------|------------------------------------------|
| 2E1Q     | Xanthine Oxidoreductase                       | -5.8                                     | THR262, SER347, GLY350*             | Allopurinol             | -6.0                                          | GLN1195, ARG913, SER1081, ALA1080*        |
| 2F71     | Protein tyrosine phosphatase 1B               | -5.4                                     | ARG199, ARG79, LEU204                | Ertiprotafib            | -7.8                                          | VAL49, ASP48, ALA217, ILE219, TYR46, PHE182 |
| 5KQL     | Low molecular weight Protein tyrosine phosphatase | -5.5                                   | LEU13, GLY14, ARG18, ILE16, TYR131  |                         | -7.8                                          | ASP129, ARG18, TYR131, GLY14, GLU50, ILE16  |
| 3DD      | Glycogen phosphorylase                        | -4.9                                     | ASN631*                              | CP-316819               | -7.8                                          | GLU123, GLU124, LYS655*                    |

* PyMol was used to identify amino acid residues

With trigonelline as the ligand, the target protein it inhibits is 5KQL, Low Molecular Weight Protein Tyrosine Phosphatase (LMWPTP). When trigonelline’s binding site is compared to the reported inhibitor 2-(4-([3-(1-Piperidinyl)propyl]amino)-2-quinolyl)benzonitrile’s binding site, trigonelline binds to 5 out of the 6 same amino acid residues that the reported inhibitor binds to. These residues are ASP129, ARG18, TYR131, GLY14, and ILE16. Figure 3 and 4 shows the PyMol visualization of both ligands docked on to 5KQL, displaying how they have binded to the same site of the protein. Some of the interactions between the residues and trigonelline include alkyl, pi-alkyl, salt bridge, conventional hydrogen bonding and Carbon Hydrogen Bonding, showing a mix of strong polar bonds and noncovalent attractions that make it a stable complex.

![Fig 3](image1.png)  ![Fig 4](image2.png)
Figure 3 Trigonelline and 2-(4-[(3-(1-Piperidinyl)propyl]amino)-2-quinolinyl)benzonitrile bound to Low Molecular Weight Protein Tyrosine Phosphatase (LMWPTP)

Figure 4 Close-up view of binding complexes with Low Molecular Weight Protein Tyrosine Phosphatase (LMWPTP) with common binding residues labeled. The pink and blue ligand is 2-(4-[(3-(1-Piperidinyl)propyl]amino)-2-quinolinyl)benzonitrile and the yellow and red ligand is trigonelline.

Table 1.3 Top 30 target proteins scored for 4-hydroxyisoleucine from ACID

| PDB Code | Name                                                                 | Free Binding Energy (kcal/mol) | Docking Score (kcal/mol) |
|----------|----------------------------------------------------------------------|-------------------------------|--------------------------|
| 5CMM     | GluK2EM LBD Dimer Assembly Complex                                   | -35.12                        | -7.31                    |
| 4MXU     | Asparthoacylase Mutant K213E Complex                                  | -25.87                        | -6.87                    |
| 3T3M     | Integrin Alphallbeta3 Receptor                                       | -23.08                        | -6.37                    |
| 3B6R     | Human Brain-type Creatine Kinase                                     | -22.51                        | -6.33                    |
| 1R76     | Pectate Lyase from Azospirillum Irakense                              | -22.35                        | -6.13                    |
| 2WAD     | Penicillin-Binding Protein 2B (PBP-2B)                               | -21.75                        | -6.6                     |
| 4V11     | Synaptotagmin-1 with SV2A Peptide                                     | -21.44                        | -6.12                    |
| 4D1P     | Nitric Oxide Synthases                                               | -20.27                        | -6.17                    |
| 1U8V     | 4-Hydroxybutyryl-CoA Dehydratase from Clostridium Aminobutyricum     | -20.23                        | -6.52                    |
| 2OZL     | Pyruvate Dehydrogenase                                               | -20.03                        | -6.8                     |
| 1UNQ     | Protein Kinase B                                                      | -20                            | -6                       |
| 3HIJ     | Alanine Aminotransferase 2                                            | -19.58                        | -6.57                    |
| 3B6R     | Human Brain-type Creatine Kinase                                     | -19.52                        | -6.31                    |
| 5FQD     | Lenalidomide-Induced CK1a degradation by the Crl4crbn Ubiquitin Ligase| -19.38                        | -7.32                    |
| 2Y2M     | Penicillin-Binding Protein 1B                                         | -19.33                        | -6.52                    |
| 1CQX     | Flavohemoglobin from Alcaligenes Eu trophus                          | -19.2                         | -6.14                    |
| 5K3S     | Acetohydroxyacid Synthase                                            | -18.93                        | -7.14                    |
| 1BUC     | Butyryl-CoA Dehydrogenase                                            | -18.85                        | -6.84                    |
| 3P0C     | Nischarin PX-Domain                                                   | -18.81                        | -6.14                    |
| 2F2S     | Sialidase Neu2 E111Q                                                 | -18.71                        | -6.5                     |
| 1BUC     | Butyryl-CoA Dehydrogenase                                            | -18.59                        | -6.85                    |
| 4XNH     | N-Terminal Acetyltransferase NatE                                    | -18.5                         | -6.5                     |
| 4ZJB     | C-terminal Domain of PyIRS Mutant Bound with 3-Benzothienyl-l-alanine and ATP | -18.34                        | -6.5                     |
| 4HBU     | CTX-M-15 extended-spectrum Beta-Lactamase                            | -18.17                        | -6.53                    |
| 1Y93     | Matrix Metalloproteinase 12                                           | -18.06                        | -6.37                    |
| 3VE6     | Venezuelan Equine Encephalitis Virus Capsid Protein NLS and Importin Alpha | -17.95                        | -6.17                    |
| PDB Code | Name                                      | Classification | ACID Docking Score (kcal/mol) | AutoDock Vina Docking Score (kcal/mol) | AutoDock Vina Amino Acid Residues | DS Amino Acid Residues and Interactions |
|---------|-------------------------------------------|----------------|-----------------------------|---------------------------------------|-----------------------------------|----------------------------------------|
| 2V40    | Human AdenylosuccinateSynthetaseIsozyme 2 in Complex with GDP | 17.74 | -6.31                       |                                       |                                   |                                        |
| 4MR7    | Extracellular Domain of Human GABA(B) receptor | 17.56 | -6.54                       |                                       |                                   |                                        |
| 2OUR    | Phosphodiesterase 10                      | -17.35        | -6.42                       |                                       |                                   |                                        |
| 3ZOO    | Cytochrome C                              | -17.28        | -6.79                       |                                       |                                   |                                        |

Table 2.3 Validation of docking of selected target proteins for 4-hydroxyisoleucine
Table 3.3 4-hydroxyisoleucine vs. the reported inhibitors for each target protein

| PDB Code | Protein Name                          | 4-Hydroxyisoleucine ADV Docking Score (kcal/mol) | 4-Hydroxyisoleucine ADV Amino Acid Residues | Reported Inhibitor Name | Reported Inhibitor ADV Docking Score (kcal/mol) | Reported Inhibitor ADV Amino Acid Residues |
|----------|--------------------------------------|-------------------------------------------------|---------------------------------------------|--------------------------|-------------------------------------------------|---------------------------------------------|
| 3B6R     | Human Brain-type Creatine Kinase      | -4.8                                            | VAL75, VAL72, CYS283, CYS74, GLY73, THR59, LEU201, LEU202 | BU99006                  | -5.8                                            | THR71, MET70, CYS283, VAL72, LEU201, LEU202, THR59, VAL75 |
| 4D1P     | Nitric Oxide Synthases                | -5.9                                            | THR80, VAL465, GLY440, CYS441, GLN435, LEU431 | L-N-Methylarginine (L-NMA) | -6.3                                            | LYS72, SER78, ASN73, GLN462, THR80, VAL465, ASP82, GLN435, LEU431 |
| 3IHJ     | Alanine Aminotransferase 2            | -4.4                                            | GLU421, ALA417, LEU435                        | L-cycloserine            | -3.9                                            | PRO309, LEU435, LYS418, GLU421               |
| 1YP3     | Matrix Metalloproteinase 12           | -6                                              | THR239, TYR240, LYS241, LEU214, VAL235, PHE237 | RXP470.1                 | -4.8                                            | VAL235, LEU214                               |

With 4-hydroxyisoleucine as the ligand, all the target proteins were inhibited in some capacity but based on docking scores, interactions, and the number of common residues between trigonelline and the reported inhibitor, the most suitable protein for 4-hydroxyisoleucine is 4D1P, Nitric Oxide Synthase. Comparing 4-hydroxyisoleucine’s binding to 4D1P with the reported inhibitor L-N-Methylarginine showed that 4-hydroxyisoleucine binds to 6 out of the 9 same amino acid residues as L-N-Methylarginine, with those being GLN462, THR80, VAL465, ASP82, GLN435, and LEU431. Figure 5 and 6 shows the PyMol visualization of both ligands docked on to 4D1P, displaying how they have binded to the same site of the protein. Interactions in this complex include many conventional hydrogen bonds and a Carbon Hydrogen bond and there are no unfavorable forces. These are very strong polar forces, making the attraction between the ligand and protein very stable.
Figure 5 4-hydroxyisoleucine and L-N-Methylarginine bound to 4D1P

Figure 6 Close-up view of binding complexes with 4D1P with common binding residues labeled. The blue ligand is L-N Methylarginine and the pink ligand is 4-hydroxyisoleucine.

Table 1.4 Top 30 target proteins scored for leucine from ACID

| PDB Code | Name                                                                 | Free Binding Energy (kcal/mol) | Docking Score (kcal/mol) |
|----------|----------------------------------------------------------------------|-------------------------------|--------------------------|
| 5CMM     | GluK2EM LBD dimer                                                   | -32.07                        | -7.13                    |
| 3FV2     | Human glutamate receptor: GluR5                                      | -28.85                        | -7.11                    |
| 1ITU     | Human renal dipeptidase                                             | -28.66                        | -6.51                    |
| 5D6E     | Human methionine aminopeptidase 2                                   | -27.54                        | -6.6                     |
| 4V11     | Synaptotagmin-1                                                     | -26.86                        | -6.05                    |
| 2C46     | Human RNA guanylyltransferase                                       | -25.28                        | -6.42                    |
| 3BWY     | Human 108M catechol O-methyltransferase                             | -24.91                        | -7.01                    |
| 3DYD     | Human tyrosine aminotransferase                                     | -24.67                        | -6.6                     |
| 1U8V     | 4-Hydroxybutyryl-CoA dehydratase from clostridium aminobutyricum   | -24.54                        | -6.55                    |
| 2NZ2     | Human argininosuccinate synthase                                    | -24.3                         | -6.64                    |
| 3T3M     | AlphaHbbeta3 receptor antagonist                                     | -24.13                        | -6.35                    |
| 4U3T     | Neisseria gonorrhoeae penicillin-binding protein 2 derived from penicillin-resistant strain 6140 | -23.72                        | -6.27                    |
| 2BM0     | Ribosomal elongation factor G (EF-G) Fusidic acid resistant mutant T84A | -23.58                        | -6.35                    |
| 1KNR     | L-aspartate oxidase: R386L mutant                                   | -22.56                        | -6.74                    |
| 2CVD     | Human hematopoietic prostaglandin D synthase                         | -21.81                        | -6.28                    |
| 1GYX     | YdeE, a 4-Oxalocrotonate Tautomerase Homologue from Escherichia coli | -21.48                        | -6.49                    |
| 1CQX     | Flavohemoglobin from Alcaligenes eutrophus                          | -20.85                        | -6.33                    |
| PDB Code | Name                                      | Classification       | ACID Docking Score (kcal/mol) | AutoDock Vina Docking Score (kcal/mol) | AutoDock Vina Amino Acid Residues | DS Amino Acid Residues and Interactions |
|----------|-------------------------------------------|----------------------|-------------------------------|----------------------------------------|-----------------------------------|------------------------------------------|
| 3I4A     | Dimethylarginine dimethylaminohydrolase-1 (DDAH-1) | -20.84               | -6.73                         |                                        |                                   |                                          |
| 5K3S     | Arabidopsis thaliana acetoxyacid synthase  | -20.44               | -6.89                         |                                        |                                   |                                          |
| 3QFS     | NADPH-Cytochrome P450 Reductase            | -19.89               | -6.29                         |                                        |                                   |                                          |
| 3B6R     | Human Brain-type Creatine Kinase           | -19.87               | -6.35                         |                                        |                                   |                                          |
| 1UNQ     | Pleckstrin Homology Domain Of Protein Kinase B/Akt | -19.85               | -5.98                         |                                        |                                   |                                          |
| 1FDR     | Flavodoxin reductase from E. Coli         | -19.19               | -6.5                          |                                        |                                   |                                          |
| 4RQR     | Human Glutaredoxin                         | -19.15               | -6.07                         |                                        |                                   |                                          |
| 2OUR     | PDE10A2 mutant D674A                      | -19.14               | -6.33                         |                                        |                                   |                                          |
| 3ODU     | CXCR4 chemokine receptor                   | -19.14               | -6.43                         |                                        |                                   |                                          |
| 5F0X     | Human GRP78 (70kDa heat shock protein 5 / BIP) ATPase domain | -18.77               | -6.71                         |                                        |                                   |                                          |
| 1NP7     | Synechocystis sp. PCC6803 cryptochrome     | -18.55               | -6.5                          |                                        |                                   |                                          |
| 1COY     | Cholesterol oxidase                        | -18.45               | -6.56                         |                                        |                                   |                                          |
| 5TC4     | Human mitochondrial methylenetetrahydrofolate dehydrogenase-cyclohydrolase (MTHFD2) | -18.39               | -6.52                         |                                        |                                   |                                          |

Table 2.4 Validation of docking of selected target proteins for leucine
5D6E | Human methionine aminopeptidase 2 | Hydrolase | -6.6 | -5.7 | ASP262, GLU364, ILE338, ASP251, PHE219, HIS231 | TYR607: Pi-Sigma Bond MET638: Alkyl PRO536: Alkyl VAL608: Alkyl

Table 3.4 Leucine vs. the reported inhibitors for each target protein

| PDB Code | Protein Name | Leucine ADV Docking Score (kcal/mol) | Leucine ADV Amino Acid Residues | Reported Inhibitor Name | Reported Inhibitor ADV Docking Score (kcal/mol) | Reported Inhibitor ADV Amino Acid Residues |
|----------|--------------|-------------------------------------|---------------------------------|--------------------------|-----------------------------------------------|-----------------------------------------------|
| 3QFS     | NADPH-Cytochrome P450 Reductase | -4.8 | SER599, TYR607, PRO536, VAL608 | Fumagillin | -7.2 | ASP262, PHE219, HIS231, LEU328, ASN329, HIS331, HIS339, TYR444, PRO443 |
| 5D6E     | Human methionine aminopeptidase 2 | -5.7 | ASP262, GLU364, ILE338, ASP251, PHE219, HIS231 | 7-Ethoxyresorufin | -8.0 | ARG517, TYR458, SER460, TYR459, VAL477, CYS475, TRP679 |

With leucine as the ligand, the target protein that it inhibits is 5D6E, Human Methionine Aminopeptidase 2 (MetAP2). When leucine’s binding site is compared to the reported inhibitor fumagillin’s binding site, leucine binds to 4 out of the 9 amino acid residues that fumagillin binds to. These are ASP262, PHE219, HIS231, and HIS331. Figure 7 and 8 shows the PyMol visualization of both ligands docked on to 5D6E, displaying how they have bound to very similar sites of the protein. Some of the interactions between the residues and leucine include many Salt Bridges, Pi-Alkyl, Pi-Sigma and Positive-Positive interactions. The leucine-5D6E complex has a higher binding energy and docking score in comparison to 3QFS and it also has many salt bridge interactions at the binding site, which are non-covalent interactions between two ionized sites made up of a hydrogen bond and an electrostatic interaction. Therefore, these interactions make the complex with 5D6E stronger and more stable.

Fig 7       Fig 8
Figure 7 Leucine and fumagillin bound to Human Methionine Aminopeptidase 2 (MetAP2)

Figure 8 Close-up view of binding complexes with Human Methionine Aminopeptidase 2 (MetAP2) with common binding residues labeled. The light pink ligand is fumagillin and the light grey ligand is leucine.

Table 1.5 Top 30 target proteins scored for L-lysine from ACID

| PDB Code | Name                                      | Free Binding Energy (kcal/mol) | Docking Score (kcal/mol) |
|----------|-------------------------------------------|-------------------------------|--------------------------|
| 1S1D     | Apyrase                                   | -56.97                        | -6.11                    |
| 3T3M     | alphaIbbeta3 Receptor Antagonist          | -41.63                        | -6.15                    |
| 1CJY     | Human Cytosolic Phospholipase A2          | -39.18                        | -5.82                    |
| 2KFZ     | Klenow Fragment                           | -30.57                        | -5.82                    |
| 1G1T     | E-selectin Lectin/EFG Domains             | -29.75                        | -5.49                    |
| 3ORH     | guanidinoacetate N-methyltransferase      | -27.97                        | -6.53                    |
| 1IMB     | Inositol Monophosphatase                  | -27.87                        | -6.15                    |
| 1JZ8     | E. Coli Beta-galactosidase                | -27.34                        | -6.03                    |
| 1KQP     | NH3-Dependent NAD+ Snythetase             | -25.57                        | -6                      |
| 4LAR     | 5-HT1B-BRIL                               | -24.87                        | -6.33                    |
| 2F25     | Sialidase Neu2 E111Q Mutant               | -24.02                        | -6.15                    |
| 1QGI     | Chitosanase from Bacillus Circulans       | -22.85                        | -5.88                    |
| 4HZE     | human Arginase-2                          | -22.84                        | -6.02                    |
| 5HIA     | Human hypoxanthine-guanine phosphoribosyltransferase | -22.27                        | -6.01                    |
| 2AEB     | human arginase I at 1.29                  | -21.88                        | -6.16                    |
| 3I4A     | Dimethylarginine dimethylaminohydrolase-1 (DDAH-1) | -21.43                        | -6.43                    |
| 3FE4     | Human Carbonic Anhydrase                  | -21.38                        | -6.15                    |
| 3BIU     | human peptidylarginine deiminase 4        | -20.82                        | -6.01                    |
| 3GR4     | Human Pyruvate Kinase M2                  | -19.66                        | -6.06                    |
| 4V11     | Synaptotagmin-1                           | -18.67                        | -5.96                    |
| 4XCT     | hydroxamate based inhibitor ARP101 (EN73) | -17.6                         | -6.62                    |
| 3BRT     | NEMO/IKK association domain structure     | -17.16                        | -6.11                    |
| 2ZC6     | Penicillin-binding protein 1A (PBP 1A) acyl-enzyme complex (tebipenem) from Streptococcus pneumoniae | -17.03                        | -6.13                    |
| 4JNI     | uPA                                       | -16.44                        | -6.36                    |
| 4XIA     | D-Xylose Isomerase                        | -16.35                        | -6.46                    |
| 2QTL     | FAD-containing FNR-like Module of Human Methionine Synthase Reductase | -16.17                        | -6.1                     |
| 2Y2M     | Penicillin-Binding Protein (PBP-1B)       | -16.17                        | -6.03                    |
| 1L14     | Human S-adenosylhomocysteine hydrolyase   | -15.83                        | -6.58                    |
| 2CEV     | Arginase from Bacillus Caldevelox         | -15.79                        | -6.27                    |
| 2Y6D     | MMP7 Inhibitor                            | -15.77                        | -6.08                    |
Table 2.5 Validation of docking of selected target proteins for L-lysine

| PDB Code | Name                          | Classification | ACID Docking Score (kcal/mol) | AutoDock Vina Docking Score (kcal/mol) | AutoDock Vina Amino Acid Residues | DS Amino Acid Residues and Interactions |
|----------|-------------------------------|----------------|------------------------------|----------------------------------------|-----------------------------------|-----------------------------------------|
| 2AE      | Human Aarginase I at 1.29     | Hydrolase/ Hydrolase Inhibitor | -6.16                        | -4.9                                   | PRO167, PHE147, PRO157, ILE156, VAL165, ASP158 | ASP158: Salt Bridge, VAL165: Conventional Hydrogen Bond (2), Alkyl PHE147: Pi-Alkyl ILE156: Alkyl PRO157: Conventional Hydrogen Bond (2) |
| 3BIU     | Human Peptidylarginine Deiminase 4 | Hydrolase/Hydrolase Inhibitor | -6.01                        | -4.3                                   | VAL290, SER288, LEU117*            | ILE121: Alkyl VAL289: Alkyl VAL290: Conventional Hydrogen Bond SER288: Conventional Hydrogen Bond (2), ASP287: Salt Bridge, Charge-Charge LEU117: Conventional Hydrogen Bond |

* PyMol was used to identify amino acid residues

Table 3.5 L-lysine vs. the reported inhibitors for each target protein

| PDB Code | Protein Name                          | L-Lysine ADV Docking Score (kcal/mol) | L-Lysine ADV Amino Acid Residues | Reported Inhibitor Name | Reported Inhibitor ADV Docking Score (kcal/mol) | Reported Inhibitor ADV Amino Acid Residues |
|----------|---------------------------------------|---------------------------------------|---------------------------------|-------------------------|-----------------------------------------------|--------------------------------------------|
| 2AE      | Human Arginase I at 1.29              | -4.9                                   | PRO167, PHE147, PRO157, ILE156, VAL165, ASP158 | L-Lysine               | -4.9                                         | PRO167, PHE147, PRO157, ILE156, VAL165, ASP158 |
| 3BIU | Human Peptidylarginine Deiminase 4 | ASP158 |
|------|----------------------------------|--------|
|      | VAL290, SER288, LEU117*          | GSK484 hydrochloride |
|      | GLN146, SER288, VAL290*          |        |

* PyMol was used to identify amino acid residues

With L-lysine as the ligand, it actually inhibits both the target proteins, 2AEB (Human Arginase I) and 3B1U (Human Peptidylarginine Deiminase 4). However, L-lysine’s inhibition of Human Arginase I has already been reported, so looking at L-lysine’s inhibition of 3B1U will be more novel and significant. When L-lysine’s binding site on 3B1U is compared to the reported inhibitor GSK484 hydrochloride’s binding site, L-lysine binds to 2 out of the 3 residues that GSK484 hydrochloride binds to and these residues are SER288 and VAL290. Figure 9 and 10 shows the PyMol visualization of both ligands docked on to 3B1U, displaying how they have bound to the same binding site of the protein. Some of the interactions between the residues and L-lysine include Alkyl, Conventional Hydrogen Bonds, Salt Bridge, and Charge-Charge and these polar bonds and highly attractive nonpolar interactions provide stability and allow L-lysine to remain bound to 3B1U as an inhibitor.

![Fig 9](image1.png)

Figure 9 L-lysine and GSK484 hydrochloride bound to Human Peptidylarginine Deiminase 4

![Fig 10](image2.png)

Figure 10 Close-up view of binding complexes with Human Peptidylarginine Deiminase 4 with common binding residues labeled. The light green ligand is GSK484 hydrochloride and the orange ligand is L-lysine.

**Discussion**

The goal of this project was to find novel protein targets for fenugreek that can help treat diabetes mellitus. Our results show the different target proteins that each phytochemical constituent of fenugreek, exempting diosgenin, is able to inhibit well, but it is more important to look at the significance of these inhibitions and their relevance to diabetes. Trigonelline inhibits low molecular weight Protein tyrosine phosphatase, a member of the PTP family, but is significantly less researched in its potential therapeutic ability in comparison to PTP1B. LMPTP has been proposed to regulate insulin signaling through IR dephosphorylation. Knockdown of LMPTP expression by antisense oligonucleotides improves the glycemic profile and decreases insulin resistance in diet-induced obese (DIO) C57BL/6 (B6) mice and overexpression of catalytically inactive LMPTP in immortalized mouse fibroblasts increases insulin-induced IR tyrosine phosphorylation, showing that LMPTP regulates insulin signaling through its phosphatase activity. Possible future research could include a deeper study into trigonelline’s inhibition of LMPTP, or synthesis of selective LMPTP inhibitors in order to increase insulin secretion in diabetic patients. 4-Hydroxyisoleucine inhibits Nitric Oxide
Synthase the best, and this protein is a key regulator of endothelial function, which increases skeletal muscle perfusion, and the surface area of capillaries to allow insulin exchange. However, this is the opposite for what is wanted. Several studies have demonstrated that a reduction of nitric oxide synthases diminishes perfusion and glucose uptake in the skeletal muscle in both humans and rodents. Hence, inhibiting nitric oxide synthases is important in improving the condition of patients with type 2 diabetes\textsuperscript{32}. Leucine has been shown to inhibit human methionine aminopeptidase 2, an enzyme that, under normal cellular conditions, methionine aminopeptidase 2 (MetAP2) catalyzes the removal of the N-terminal methionine, the starting amino acid of a polypeptide chain, from novel proteins shortly after translation. In Type II diabetic preclinical studies, MetAP2 inhibitors have shown to produce clinically significant weight loss characteristics and reduced average blood glucose levels\textsuperscript{43}. Leucine’s high binding affinity to MetAP2 and potential as a MetAP2 inhibitor can help extend the catalog of already synthesized MetAP2 inhibitors. Lastly, in this study, it was found that L-lysine inhibited Human peptidylarginine deiminase 4, a nuclear citrullinating enzyme that is critically involved in the release of decondensed chromatin from neutrophils as neutrophil extracellular traps (NETs). NETs, together with fibrin, are involved in defense against pathogens, but the formation of NETs (NETosis) has negative effects related to many diseases, such as diabetes mellitus\textsuperscript{44}. In parallel with increased NETosis, neutrophil PAD4 protein expression is elevated approximately 4-fold in patients with type 1 diabetes mellitus and type 2 diabetes mellitus\textsuperscript{44}. Increased NETs levels also create many complications that are often associated with diabetes patients such as impaired wound healing, especially in relation to diabetic foot ulcers\textsuperscript{45}, and increased incidences of heart disease and thrombosis\textsuperscript{46}. Further research on PAD4 inhibitors like L-lysine would therefore be quite beneficial for diabetic patients.

**Conclusion**

The phytoconstituents from fenugreek have long been studied for their anti-diabetic effects in vivo and in vitro, more specifically for their ability to inhibit enzymes such as alpha glucosidase and alpha amylase. In this study, however, we have shown that the specific compounds trigonelline, 4-hydroxyisoleucine, leucine, and L-lysine are capable of inhibiting novel protein targets that can help treat diabetes mellitus in even more ways. It is important to note that we only picked the highest score and best matching protein, but compounds like 4-hydroxyisoleucine binding to other proteins could also result in the compound’s biological effects. This study only looks at their ideal mechanism of action since it was only a molecular docking study, so future studies should focus on in-vitro studies that look at these compounds’ inhibition of their respective target proteins such as LMPTP. If successful, further research can also be done to create semi-synthetic drugs based on the compounds to target the specific proteins in diabetic patients.

**Acknowledgements**

Gayathri Renganathan conceptualized the project, and Haasini Nandyala, Ariel Pham, and Anushka Wagle developed, mended and conducted the majority of the in-silico work. Amrita Guha and Hansika Daggolu assisted with some in-silico work. In addition to Haasini Nandyala, Amrita Guha, and Hansika Daggolu, Reya Sankar, Chloe Chan, and Dipti Venkatesh assisted in creating and editing the manuscript. We are also grateful for our advisor's support throughout this project by providing us with ideas to improve our work. We would like to thank the Aspiring Scholars Directed Research Program (ASDRP) in Fremont, CA for supporting us with the facilities and funding to conduct this research. Furthermore, we thankfully acknowledge Edward Njoo for providing us with suggestions on our manuscript.

**References**

Rampogu, S., Parameswaran, S., Lemuel, M. R., & Lee, K. W. (2018). Exploring the Therapeutic Ability of Fenugreek against Type 2 Diabetes and Breast Cancer Employing Molecular Docking and Molecular Dynamics
Simulations. Evidence-based complementary and alternative medicine : eCAM, 2018, 1943203.
https://doi.org/10.1155/2018/1943203
Gaddam, A., Galla, C., Thummisetti, S., Marikanty, R., Palanisamy, U., & Rao, P. (2015, October 2). Role of Fenugreek in the prevention of type 2 diabetes mellitus in prediabetes. Retrieved April 28, 2021
Al-Timimi, L. (2019, December 1). Antibacterial and anticancer activities of fenugreek seed extract. Retrieved April 28, 2021
Saxena, B., Saxena, U.. (2009, January). Anti-hyperlipidemic activity of fenugreek (Trigonella foenum-graecum) seeds extract in Triton and high fat diet induced hyperlipidemic model: A potent anti-atherosclerotic agent. Pharmacologyonline. 2. 616-624. Retrieved May 8, 2021
Habib-Martin, Z., Hammad, H., Afifi, F., Zihlif, M., Al-Ameer, H., Saleh, M.,... Nassar, Z. (2017, August 03). In vitro and in vivo evaluation of the antiangiogenic activities of trigonella foenum-graecum extracts. Retrieved April 28, 2021
Taj Eldin, I., Abdalmutalab, M., & Bikir, H. (2013). An in VITRO anticoagulant effect Of fenugreek (Trigonella foenum-graecum) in blood samples of NORMAL Sudanese individuals. Retrieved April 28, 2021
Jesus, M., Martins, A., Gallardo, E., & Silvestre, S. (2016). Diosgenin: Recent highlights on pharmacology and analytical methodology. Retrieved April 28, 2021
Ahmed M.A. Abd-El M., Husam O. (2011). Elicitation of Trigonelline and 4-Hydroxyisoleucine with Hypoglycemic Activity in Cell Suspension Cultures of Trigonella foenum graecum L. The Open Conference Proceedings Journal. Retrieved May 8, 2021
Garg, R. (2016, February 19). Fenugreek: Multiple health benefits. Retrieved April 28, 2021
Mansour, E., & El-Adawy, T. (2002, May 25). Nutritional potential and functional properties of heat-treated and germinated fenugreek seeds. Retrieved April 28, 2021
Worldwide toll of diabetes. (2019). International Diabetes Federation. https://www.diabetesatlas.org/en/sections/worldwide-toll-of-diabetes.html#:text=Today%2C%20we%20calculate%20that%209.3%2C,in%202025%20was%20438%20million.
Baquer, N.Z., Kumar, P., Taha, A. et al. Metabolic and molecular action of Trigonella foenum-graecum (fenugreek) and trace metals in experimental diabetic tissues. J Biosci 36, 383–396 (2011).
JII;, P. M. V. M. (n.d.). The metabolic syndrome: definition, global impact, and pathophysiology. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition The pathogenesis and pathophysiology of type 1 and type 2 diabetes mellitus. Ozougwu, J. C., Obimba, K. C., Belonwu, C. D., and Unakalamba, C. B. JPAP 2017, DOI: 10.5897/JPAP2013.0001 Diabetes 2030: insights from yesterday, today, and future trends. Rowley WR, Bezold C, Arikan Y, Byrne E, Krohe S. Popul Health Manag. 2017;20:6–12
Habtemariam, S. “Chapter 17 - The chemical and pharmacological basis of fenugreek (Trigonella foenum-graecum L.) as potential therapy for type 2 diabetes and associated diseases.” Medicinal Foods as Potential Therapies for Type-2 Diabetes and Associated Diseases- The Chemical and Pharmacological Basis of their Action, 2019, p. 579-637
Kumar, S., Narwal, S., Kumar, V., & Prakash, O. (2011, January). A-Glucosidase inhibitors from plants: A natural approach to treat diabetes. Retrieved April 28, 2021
Agarwal, P., Gupta, R. (September 2016). Alpha-amylase inhibition can treat diabetes mellitus. Journal of Medical Physics. 5(4). Retrieved May 8, 2021
Ghosh, S., More, P., Derle, A., Patil, A., Markad, P., Asok, A.,... Chopade, B. (n.d.). Diosgenin from Dioscorea Bulbifera: NOVEL hit for treatment of Type II diabetes Mellitus with inhibitory activity AGAINST α-Amylase And α-glucosidase. Retrieved April 28, 2021
Jemal, K. (2019, January 01). Molecular docking studies of PHYTOCHEMICALS of allophylus serratus Against cyclooxygenase-2 enzyme. Retrieved April 28, 2021
National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 5570, Trigonelline. Retrieved May 7, 2021
National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 99474, Diosgenin. Retrieved May 7, 2021
National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 5962, Lysine. Retrieved May 7, 2021
National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 6106, Leucine. Retrieved May 7, 2021
National Center for Biotechnology Information (2021). PubChem Compound Summary for CID 2773624, 4-Hydroxyisoleucine. Retrieved May 7, 2021
H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne. (2000) The Protein Data Bank Nucleic Acids Research, 28: 235-242.
Wang, F., Wu, F., Li, C., Jia, C., Su, S., Hao, G., & Yang, G. (2019, November 27). Acid: A free tool for drug repurposing using consensus inverse docking strategy. Retrieved April 28, 2021
O. Trott, A. J. Olson,  AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, Journal of Computational Chemistry 31 (2010) 455-461 DOI 10.1002/jcc.21334
Ref. Dassault Systèmes BIOVIA, Discovery Studio Modeling Environment, Release 2017, San Diego: Dassault Systèmes, 2016.
The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC.
Roskoski R., Jr (2019). Targeting ERK1/2 protein-serine/threonine kinases in human cancers. Pharmacological research, 142, 151–168. https://doi.org/10.1016/j.phrs.2019.01.039
Wójcikowski, J., Danek, P. J., Basińska-Ziobrań, A., Pukło, R., & Daniel, W. A. (2020, March 26). In vitro inhibition of human cytochrome P450 enzymes by the novel atypical antipsychotic drug asenapine: a prediction of possible drug–drug interactions. Pharmacological Reports.
Stanford, S. M., Aleshin, A. E., Zhang, V., Ardeeky, R. J., Hedrick, M. P., Zou, J., … Bottini, N. (2017, March 27). Diabetes reversal by inhibition of the low-molecular-weight tyrosine phosphatase. Nature News.
Viteček, J., Lojek, A., Valacchi, G., & Kubala, L. (2012, September 4). Arginine-Based Inhibitors of Nitric Oxide Synthase: Therapeutic Potential and Challenges. Mediators of Inflammation.
Sin N;Meng L;Wang MQ;Wen JJ;Bornmann WG;Crews CM; (n.d.). The anti-angiogenic agent fumagillin covalently binds and inhibits the methionine aminopeptidase, MetAP-2. Proceedings of the National Academy of Sciences of the United States of America.
Di Costanzo, L., Illies, M., Thorn, K. J., & Christianson, D. W. (2010, April 15). Inhibition of human arginase I by substrate and product analogues. Archives of biochemistry and biophysics.
Du, M., Yang, L., Gu, J., Wu, J., Ma, Y., & Wang, T. (2020, December 30). Inhibition of Peptidyl Arginine Deiminase-4 Prevents Renal Ischemia-Reperfusion-Induced Remote Lung Injury. Mediators of Inflammation.
Stanford, S., Aleshin, A., Zhang, V., Ardeeky, R., Hedrick, M., Zou, J., … Bottini, N. (2017, June). Diabetes reversal by inhibition of the low-molecular-weight tyrosine phosphatase. Retrieved April 28, 2021
LMW-PTP is a negative regulator of insulin-mediated mitotic and metabolic signalling. Chiarugi P, Cirri P, Marra F, Raugei G, Camici G, ManaO G, Ramponi G Biochem Biophys Res Commun. 1997 Sep 18; 238(2):676-82. Reduction of low molecular weight protein-tyrosine phosphatase expression improves hyperglycemia and insulin sensitivity in obese mice. Pandey SK, Yu XX, Watts LM, Michael MD, Sloop KW, Rivard AR, Leedom TA, Manchem VP, Samadzdeh L, McKay RA, Monia BP, Bhanot S J Biol Chem. 2007 May 11; 282(19):14291-9. LMW-PTP is a negative regulator of insulin-mediated mitotic and metabolic signalling. Chiarugi P, Cirri P, Marra F, Raugei G, Camici G, ManaO G, Ramponi G Biochem Biophys Res Commun. 1997 Sep 18; 238(2):676-82. Williams, I., McClatchey, P., Bracy, D., Valenzuela, F., & Wasserman, D. (2018, October 01). Acute nitric oxide synthase inhibition accelerates transendothelial insulin efflux in vivo. Retrieved April 28, 2021
Proietto, J., Malloy, J., Zhuang, D., Arya, M., Cohen, N., Looze, F., . . . Kim, D. (2018, July 11). Efficacy and safety of methionine aminopeptidase 2 inhibition in type 2 DIABETES: A randomised, placebo-controlled clinical trial. Retrieved April 28, 2021
Wong, S. L., & Wagner, D. D. (2018). Peptidylarginine deiminase 4: a nuclear button triggering neutrophil extracellular traps in inflammatory diseases and aging. FASEB journal : official publication of the Federation of American Societies for Experimental Biology, 32(12), fj201800691R. Advance online publication. https://doi.org/10.1096/fj.201800691R
Wong, S. L., Demers, M., Martinod, K., Gallant, M., Wang, Y., Goldfine, A. B., Kahn, C. R., and Wagner, D. D. (2015) Diabetes primes neutrophils to undergo NETosis, which impairs wound healing. Nat. Med. 21, 815–819
Laakso, M., and Kuusisto, J. (2014) Insulin resistance and hyperglycaemia in cardiovascular disease development. Nat. Rev. Endocrinol. 10, 293–302
Morel, O., Jesel, L., Abbas, M., and Morel, N. (2013) Prothrombotic changes in diabetes mellitus. Semin. Thromb. Hemost. 39, 477–488