In-Silico Pharmacological and Molecular Docking Studies of Natural Inhibitors form *Musa* Spp. On *Vaca* Gene a Vacuolating Cytotoxin Autotransporter

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Abstract. The integration of computational with various bioinformatics software/tools/webservers and the molecular docking process is the current key point method in reducing the time for drug discovery and drug development from a Bioactive compound. Current Insilico Study considers the Bioactive Phytoconstituents from *Musa* species for our drug discovery process by using bioinformatics software to find out the analogs by following their related physicochemical properties for the structure-based drug design. The gastric bacterium *H. pylori* infect the gastric mucosa, and its eradication is associated with the prevention of ulcer reoccurrence. The significant Protein target from this species was a challenging task for finding, targeting, treating. Offering hope from the Bioactive compounds considered in line with already FDA approved Drug molecules against peptic ulcer causative organism’s special protein vacuolating cytotoxin autotransporter translated from *vacA* Gene. The recent challenge is to identify a drug moiety that will effectively work on the target protein. Usually, the Phytocompounds will not significantly cause a side effect. Sitosterol with docking score of -6.417 kcal/mol has promised to serve as vacuolating cytotoxin autotransporter inhibitor by blocking the autotransporter protein to the periplasmic space avoiding the toxin passage between periplasmic membrane which will avoid the gastric infections from *H. pylori*.

Keywords: *Musa paradisica, Helicobacter pylori*, Chemspider, SwissADME, Swissprediction Vacuolating cytotoxin autotransporter, Molecular Docking.

1. Introduction

Molecular Docking Studies have given shape for the success of Drug discovery process in Pharmaceutical research for understanding the Bioactivity between Compound and biological systems. Integration of computational and experimental strategies has been of great value in the identification...
and development of novel promising compounds. [7] Broadly used in modern drug design, molecular docking methods explore the ligand conformations adopted within the binding sites of macromolecular targets.

*Musa* is one of the two or three genera in the family Musaceae. It includes bananas and plantains. Plantains refer to India as a coarse banana. It grows 10 to 40 feet in height and has broad green leaves that grow through a hollow stem bears flowers and fruit. [24] These group of plants are commonly cultivated in all areas in India. *Musa spp.* have been reported to have many biological activities. In Ayurveda, a traditional system of medicine is cited for limited to Florida, Egypt, Southern Japan, and South Brazil for the treatment of many disorders. [26, 27] *Musa spp.* leaves are useful in treating upper respiratory disorders. Some parts of roots could help in treating hemoptysis and anthelmintic. [1] Fruits can increase renal activities, reduces the risk of kidney cancer. It contains anti oxidizer and counteracts the noxious effects of the free radicals. [23,28] The *Musa spp.* have various biological activities such as antidiabetic, Antiulcerogenic, antiatherogenic, antidiarrheic, antitumoral, antimutagenic. [10] It has also been found to be effective in the treatment of migraine, hypertension, cholesterol, and hyperoxaluria. It can be used as an antidote for snakebite, asthma, burns, excessive menstrual flow, fever, gangrene, gout, haemorrhage, inflammation, insomnia, intestinal parasites. [11, 29] A potent drug molecule must reach its target in the body in sufficient concentration, and stay there is a bioactive form long enough for the expected biologic events to occur. [3] Drug development involves assessment of absorption, distribution, metabolism and excretion (ADME) increasingly earlier in the discovery process, at a stage when considered compounds are numerous but access to the physical samples is limited. [6] In that context, computer models constitute valid alternatives to experiments. [8]

Molecular descriptors are mostly a set of chemical information from chemical structures which are considered in the field of chemoinformatics field one typical example is the Molecular Fingerprint which says about the sequence of bits present or absent of a chemical feature of the compounds. Few of the simple physicochemical descriptors like the molecular weight of a compound, molecular refractivity index of a bioactive substance, number count of specific atom types presence and polar surface area, etc. [3] Along with the chemical information of Bioactive compounds and biological targets molecular docking has become an increasingly important tool for the drug discovery process. The docking process involves two necessary steps: prediction of the ligand conformation as well as the different geometry conformations with the already know Pharmacopore features. It is the most likely process of knowing the binding site in the receptor can significantly increase the efficiency of ligand-target interactions during molecular docking.

1.1 Peptic Ulcer

A peptic ulcer is a disease caused on the lining on stomach or duodenum, at the start of the small intestine position. One in every 10 American will develop an ulcer in their life. The Common current cause of peptic ulcer is a bacterial infection other kinds of cause is drugs nonsteroidal anti-inflammatory agents (NSAIDs), like aspirin and ibuprofen for long-term use. [12] In a few cases, cancerous tumours can also cause ulcers in certain few cases in stomach and pancreas, and it is clear that spicy food or stress are not the causatives of ulcers. [12, 13] Researchers have found *Helicobacter pylori* one of the causatives for peptic ulcer which weaken the mucous coating of the stomach and duodenum for protection. Creating this mucous lining damage make the sensitive inner lining of the stomach to getexposer to acids and bacteria, creating irritate on the tissue lining followed by sore or ulcer. *H. Pylori* can secrete enzymes which assist for the existence in the stomach by neutralizing the acid. [13] Commonly, bacterial infections are treated with Antibiotics and some types of acid production suppressants are used two types of acid-suppressing drugs might be used like H2-blockers and proton pump inhibitors. [12, 25]

2. Methods

2.1 Pharmacological Prediction
The Pharmacological Physicochemical properties prediction Figure 2 of a given compound which consist of both primary and secondary properties include SMILES, Molecular formula, Molecular weight, No. of Rotatable Bonds, No. of hydrogen acceptors and donors, and TPSA. Secondary properties include Consensus Log Po/w, Leadlikeness, Lipinski, Bioavailability score which are shown in Table 1[16].

2.2 Ligand- Receptor preparation
The in-silico ligand and the receptor studies were obtained from ChemSpider, and PDB, respectively. The 3D structure of the receptor i. e. 2QV3 and five ligands Sitosterol, Fucostanol, Clionasterol, DL-Tryptophan and L-5-hydroxy-Tryptophan were prepared for docking using Molecular Operating Environment (A drug discovery software platform). Removal of hetero atoms molecules like water and non-amino acid groups and the addition of hydrogen atoms are crucial steps during ligand and receptor preparation [13, 15]

2.3. Molecular docking
The molecular docking is usually initiated with the preparation of ligand and receptor, further setting the docking grid and choosing the active site of the receptor along with Ligand conformation through energy refinement process for the best binding pose during ligand and receptor interaction as shown in Figure 2. Results are of affinity binding values are saved in Portable Document Format. Bioactive compounds and the selective receptor interactions were analyzed through Molecular Operating Environment’s platform that provides integrated visualization [7, 8]

2.4. Vacuolating cytotoxin autotransporter PDB-ID: 2QV3
Vacuolating cytotoxin autotransporter is a type of protein which interacts selectively with one or more biological molecules in other organisms biological system (Target host) to pathogenesis (creation of abnormalities within the host at detectable statues). This activity is because of the expression of the vacA gene of *H. pylori*, which code for the structure and function of vacuolating cytotoxin autotransporter. The creation of a pore from an autotransporter pathway leading the multiple alterations with the host mucosal cell line, and finally turning the host cell line to cause of peptic ulcer disease and reoccurrence of more host cell line damage by the secreted gastric juices. [13, 18] Here, we present a 2.4-A crystal structure of the vacA p55 domain, which has a vital role in mediating VacA binding to host cells. The structure is predominantly a right-handed parallel beta-helix, a feature that is characteristic of autotransporter passenger domains but unique among known bacterial protein toxins. Notable features of vacA p55 include disruptions in beta-sheet contacts that result in five beta-helix subdomains and a C-terminal domain that contains a disulphide bond. Analysis of vacA protein sequences from unrelated *H. pylori* strains, including m1 and m2 forms of vacA, allows us to identify structural features of the vacA surface that may be important for interactions. [14, 18]

| Sl. No. | Bioactive Compounds      | Target                                      | FDA Approved DRUG | % Similarity |
|--------|--------------------------|---------------------------------------------|-------------------|--------------|
| 1.     | Sitosterol               | PDB-ID 2QV3 Vacuolating cytotoxin autotransporter | Allylestrenol     | 91.2         |
| 2.     | Fucostanol               |                                             | Menthol           | 92.9         |
| 3.     | Clionasterol             |                                             | Allylestrenol     | 91.2         |
| 4.     | DL-Tryptophan            |                                             | L-Tryptophan      | 100          |
| 5.     | L-5-hydroxy-Tryptophan   |                                             | Oxitriptan        | 100          |
3. Result and Discussion

Table 2. Bioactive Ligands along with Different Molecular Descriptors and Pharmacological Properties

| Ligands | Predicted Physicochemical Pharmacological properties |
|---------|-----------------------------------------------------|
| Sitosterol | Formula: C_{29}H_{50}O  |
|          | SMILES: [H][C@@][1(CC[C@@][2](H))[C@@]3(H)]CC=C4C |
|          | Molecular weight: 414.71 g/mol |
|          | Mol. Wt: 414.71 g/mol |
|          | No. Rotatable bond: 6 |
|          | No. of H-bond acceptors: 1 |
|          | No. of H-bond donors: 133.23 |
|          | Molar Refractivity: 20.23 A^0 |
|          | TPSA: 5.05 |
|          | Log P_{a/w} (iLOGP): 9.34 |
|          | Log P_{a/w}(XLOGP3): 6.73 |
|          | Log P_{a/w}(MLOGP): -7.90 |
|          | Log S (ESOL): Poorly soluble |
|          | Solubility class: Yes; 1 violation : MLOGP > 4.15 |
|          | Lipinski: 0.55 |
|          | Bioavailability score: No; 2 violations; MW > 350, XLOGP3 > 3.5 |
|          | Leadlikeness: 6.30 |
|          | Synthetic accessibility: 6.30 |
Fucostanol

Formula: C_{29}H_{52}O_{2}
SMILES: CC[C@@H](C(C)C)CC[C@@H]([C@H]1CC[C@@H]2[C@H]1CC[C@@H](N[C@@H](C2)O)C
Mol.Wt: 416.72 g/mol
No. Rotatable bond: 6
No. of H-bond acceptors: 1
No. of H-bond donors: 1
Molar Refractivity: 20.23 Å^2
TPSA: 5.17
Log Pa/w (iLOGP): 8.32
Log P a/w(XLOGP3): 6.88
Log Pa/w(MLOGP): -7.27
Log S (ESOL): 8.32
Solubility class: Poorly soluble
Lipinski: Yes; 1 violation: MLOGP > 4.15
Bioavailability score: 0.55
Leadlikeness: No; 2 violations: XLOGP3 > 3.5, MW > 350
Synthetic accessibility: 5.38

Clionasterol

Formula: C_{29}H_{50}O
SMILES: CC[C@@H](C(C)C)CC[C@@H]([C@H]1CC[C@@H]2[C@H]1CC[C@@H](N[C@@H](C2)O)C
Mol.Wt: 414.71 g/mol
No. Rotatable bond: 6
No. of H-bond acceptors: 1
No. of H-bond donors: 1
Molar Refractivity: 133.23 Å^2
TPSA: 5.07
Log Pa/w (iLOGP): 9.34
Log P a/w(XLOGP3): 6.73
Log Pa/w(MLOGP): -7.90
Log S (ESOL): 9.34
Solubility class: Poorly soluble
Lipinski: Yes; 1 violation: MLOGP > 4.15
Bioavailability score: 0.55
Leadlikeness: No; 2 violations: XLOGP3 > 3.5, MW > 350
Synthetic accessibility: 6.30

DL- Tryptophan

Formula: C_{11}H_{12}N_{2}O_{2}
SMILES: OC(=O)[C@H](Cc1c[nH]c2c1ccc2)N
Mol.Wt: 204.23 g/mol
No. Rotatable bond: 3
No. of H-bond acceptors: 3
No. of H-bond donors: 3
Molar Refractivity: 57.36 Å^2
TPSA: 79.11 Å^2
Log Pa/w (iLOGP): -1.06
Log P a/w(XLOGP3): -1.66
Log Pa/w(MLOGP): -0.68
Log S (ESOL): Very soluble
Solubility class: Yes; 0 violations
L-5-hydroxy-Tryptophan

| Sl. No. | Ligand               | Amino Acids with Distance in Å | RMSD in Å | Rough Docking Score in kcal/mol | Refinement energy in kcal/mol | Final Docking Score in kcal/mol |
|--------|----------------------|-------------------------------|-----------|---------------------------------|-------------------------------|---------------------------------|
| 1.     | Sitosterol           | GLY(3.9)                      | 1.9409    | -10.5921                        | -31.9434                      | -6.417                          |
| 2.     | Fucostanol           | ASN(4.1)                      | 1.3366    | -10.1892                        | -15.4823                      | -4.637                          |
| 3.     | Clionasterol         | SER(3.6), ASN(4.5)            | 1.7175    | -10.9913                        | -29.1627                      | -5.965                          |
| 4.     | DL-Tryptophan        | THR(3.0), SER(3.1)            | 2.2974    | -9.7871                         | -21.9120                      | -4.928                          |
| 5.     | L-5-hydroxy-Tryptophan | ARG(2.9)                     | 1.6200    | -13.7376                        | -15.5269                      | -4.567                          |
Figure 2. Molecular Interaction of Sitosterol with 2QV3_Vacuolating cytotoxin autotransporter

Figure 3. Comparison in Energy Score of five Bioactive compounds against 2QV3_Vacuolating cytotoxin autotransporter

3.1 Docking Score
Ligands could be more potent and likely to interact more significantly to receptor 2QV3_Vacuolating cytotoxin autotransporter based on the predicted free-energy of binding, i.e., Docking Score predicted value shown in Table 3 and comparison of energy graph in Figure 3. In this study, the Five natural Inhibitors have shown promising Binding affinity which is expressed in terms of Docking out of which Sitosterol with -6.417 kcal/mol has promised to serve as Vacuolating cytotoxin autotransporter inhibitor from the Musa spp. to prevent gastric infections with H. pylori. [17, 18, 30]
4. Conclusion

By the main Docking Energy Scoring when involved during in-Silico Docking Studies with Triangle matching Receptor-Ligand Movement with the Rigid receptor 2QV3 Vacuolating cytotoxin autotransporter along with five selected bioactive compounds have shown a significantly good Ligand along with the Percentage of structural similarity of FDA drugs Table 1 with an affinity towards the targeted protein of vacA gene product Vacuolating cytotoxin autotransporter, which could be further studied using Molecular Dynamics simulation and docking since it can even reveal the dynamic activity of target and receptors. Through this In-silico pharmacological and molecular docking studies will provide a strong recommending analysis for selected five Bioactive compounds will be able to become potent drug molecule against H. pylori in the clinical trial of a drug discovery process. Based on the Docking energy score predicted results, one can mostly conclude that the Bioactive compound Sitosterol from the Musa spp. will further promise to inhibit the activity of vacA gene function and its structural output vacuolating cytotoxin autotransporter.

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References

[1] Obolskiy D, Pischel I, Siriwatanametanon N, Heinrich M and Garcinia mangostana LA phytochemical and pharmacological review. Phytotherapy Research An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives 23 1047-65
[2] Lipinski CA, Lombardo F, Dominy BW and Feeney PJ 2001 Eksperimental and computational approaches to estimate solubility and permeability in drug discovery and development settings Advance Drug Delivery Reviews 64 1-17.
[3] Onasanwo SA, Emikpe BO, Ajah AA and Elufioye TO 2013 Anti-ulcer and ulcer healing potentials of Musa sapientum peel Pharmacognosy Research 5 3
[4] Elango et al. J. Res. Educ. Indian Med., Jan.-March, 2007; 13, 63-69
[5] Shahin R, Mansi I, Swellmeen L, Alwidyan T, Al-Hashimi N, Al-Qarar’h Y and Shaheen O 2018 Ligand-Based Computer Aided Drug Design Reveals New Tropomycin Receptor Kinase A (TrkA) Inhibitors J Mol Graph Model 80 327-352
[6] Nisha CM, Kumar A, Nair P, Gupta N, Silakari C, Tripathi T and Kumar A 2016 Molecular docking and in silico ADMET study reveals acylguanidine 7a a potential inhibitor of β-secretase Advances in Bioinformatics 1-6
[7] Laurie ATR and Jackson RM 2014 Q-SiteFinder: an energy-basedmethod for the prediction of protein-ligand binding sitter Bioinformatics 21 1908-1916
[8] Manjulika Y, Sanjukta C, Sharad KG and Watal G 2014 Alternative Therapeutics Unit, Drug Development Division, Medicinal Research Lab, Department of Chemistry, University of Allahabad
[9] Swathi D, Jyothi B, Sravanthi C 2011 Pharmacognostic studies and Pharmacological actions of Musa Paradisiaca A Review Paper International Journal of Innovative Pharmaceutical Research 2 122-5
[10] Ehiowemwenguan G, Emoghene AO, Inetianbor JE 2014 Antibacterial and phytochemical analysis of Banana fruit peel OSR Journal of Pharmacy 4 18-25
[11] Podolski JL 1996 Recent advances in peptic ulcer disease: H. pylori infection and its treatment Gastroenterology Nursing 19 128-136.
[12] Soll AH 1996 Medical treatment of peptic ulcer disease: Practice guidelines Journal of the American Medical Association 275 622-628
[13] National Institutes of Health, Office of the Director. 1994 NIH consensus statement: Helicobacter pylori in peptic ulcer disease 12 Bethesda, MD.
[14] Nerkar G, Shena AK, Poorvashree JE and Hemant UC 2012 In silico screening synthesis and pharmacological evaluation of novel quinazolines as NMDA receptor inhibitors for anticonvulsant activity Inter. J. Pharm. Pharm Sci. 4 449-53
[15] Zubair MS and Subehan 2010 Molecular docking of lunacridine from Lunasia amara to DNA: its inhibition and interaction study correlated with the cytotoxic activity on P388 murine leukemia cells Indonesian Journal of Cancer Chemoprevention 1 108-17
[16] Agarwal PK, Singh A, Gaurav K, Goel S, Khanna HD, Goel RK 2009 Indian J Exp Biol 47 32-40.
[17] Foegeding NJ, Caston RR, McClain MS, Ohi MD, Cover TL 2016 An Overview of Helicobacter pylori VacA Toxin Biology Toxins 8 173
[18] Raghu PS, Antioxidant and Antiulcer activity of musa paradisica in rats Int. J. Pharma & Ind. Res 2
[19] Onasanwo SA, Emikpe BO, Ajah AA, Elufioye TO 2013 Anti-ulcer and ulcer healing potentials of Musa sapientum pecl, Pharmacognosy Research, 5
[20] Sidhu JS and Zafar TA 2018Bioactive compounds in banana fruits and their health benefits 2
[21] Rao T 2016 Antiulcer activity of musa paradisiaca (banana) tepal and skin extracts in ulcer induced albino mice, Malaysian journal of analytical sciences 20 1203-1216
[22] Maharani L, Apriasari I and Eko S 2014 Bioactive Compound and Antioxidant Activity of Methanol Extract Mauli Bananas (Musa sp) Stem International Journal of Bioscience, Biochemistry and Bioinformatics 4 2014
[23] Asif K, Rahman M, Annanya K 2019 Extraction and evaluation of phytochemicals from banana peels (musa sapientum) and banana plants (musa paradisica) malaysian journal of halal research journal (mjhr)2
[24] Bohr UR, Annibale B, Franceschi F, Roccarina D ad Gasbarrini A 2007 Extragastric manifestations of Helicobacter pylori infection–other Helicobacters Helicobacter 24 45-53
[25] Bishajit S, Asad U, Syed SI 2020 In Silico Analysis of Some Phytochemicals as Potential Anti-cancer Agents Targeting Cyclin Dependent Kinase-2, Human Topoisomerase Ila and Vascular Endothelial Growth Factor Receptor-2
[26] Laeliocattleya RA, Estiasih T, Griselda G, Muchlisiyah J 2013 The bioactive compounds and antioxidant activity of ethanol and ethyl ecetate extracts of Candi Banana (Musa paradisiaca) IOP Conf. Ser.: Earth Environ. Sci. 131
[27] Prabha P, Karpagam T, Varalakshmi B and Packiavathy AS. Indigenous anti-ulcer activity of Musa sapientum Pharmacognosy Research 3
[28] Onyema CT, Ofor CE, Okudo VC and Ogbuagu AS 2016 phytochemical and antimicrobial analysis of banana pseudo stem (musa acuminate) bjpr, 10
[29] Calister T 2018 In vivo Antiulcer Activity of Phospholipid–Based Complexes of Musa, Paradisiaca (Musaceae) Peel Extract for Improved Oral Drug Delivery Indian Journal of Novel Drug Delivery 11 20-29