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INSILICO EVALUATION OF CITRIC ACID FROM *MYRCIARIA DUBIA* FOR ANTI-ULCER POTENTIAL AGAINST M3 MUSCARINIC RECEPTORS USING PATCH DOCK

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Abstract:
Medicinal plants have long played important roles in the treatment of diseases; their efficacy and some of the findings have led to the production of plant-based medicines. However, conscious efforts need to be made to properly identify, recognize and position medicinal plants in the design and implementation of these strategies. Synthetic drugs are used for the treatment of ulceration but these drugs produce several side effects. So herbal medicines are considered as better alternatives for the treatment of peptic ulcer.
Peptic ulcer is common among adults in modem society. The physical morbidity and economic incapacity associated with this disease justify continued interest in its epidemiology. Molecular docking technique is the famous in structural bioinformatics to solve the problems in protein and ligand interaction studies. Molecular docking was carried out for test and standard (citric acid and Oxyphenonium) and combined with target protein M3 muscarinic receptor (4DAJ) with the help of Patch dock server. The test compound showed good binding affinity and better pharmacokinetic parameters. The findings of the present study attest that Myrciaria Dubia exhibit better anti-ulcer potential when compared to the standard Ox phenonium as the docking energy is more for Myrciaria Dubia than the Oxyphenonium. Further studies are suggested in order to produce a potent compound to treat Peptic Ulcers.
Keywords: *Peptic Ulcers, Patch dock, Muscarinic Receptors, Citric acid, Oxyphenonium*

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1.INTRODUCTION:

Medicinal plants have long played important roles in the treatment of diseases all over the world [1]. Medicinal plants play vital roles in disease prevention and their promotion and use fit into all existing prevention strategies. However, conscious efforts need to be made to properly identify, recognize and position medicinal plants in the design and implementation of these strategies. There is increasing evidence that in several chronic disorders an increase in production of free radicals or reactive oxygen species (ROS) play a critical role. High reactivity of free radicals causes changes in most of cellular components, leading to lipid peroxidation. Medicinal plants are a source for a wide variety of natural antioxidants and are used for the treatment of diseases throughout the world [2]. Synthetic drugs such as proton pump inhibitors, H2 receptors, cytoprotectants, demulcents, anti-cholinergic, antacids and prostaglandin analogues are used for the treatment of ulceration but these drugs produce several side effects. So herbal medicines are considered as better alternatives for the treatment of peptic ulcer [3]. As herbal medicines are considered as safe for the treatment of ulcers with lesser adverse effects, economical, effective, relatively less toxic, extensive research is carried out in search for potent antiulcer agents of plant origin [4, 5].

Peptic ulcer is common among adults in modem society. The physical morbidity and economic incapacity associated with this disease justify continued interest in its epidemiology. Studies from the West reveal that 5-10% of the adult population can expect to develop a peptic ulcer during their life time [6-8].

H. pylori infection and the use of Nonsteroidal anti-inflammatory drugs (NSAIDs) are the predominant causes of peptic ulcer disease. A history of episodic or epigastric pain, relief of pain after food intake, and night-time awakening because of pain with relief following food intake are the most specific findings for peptic ulcer [9].

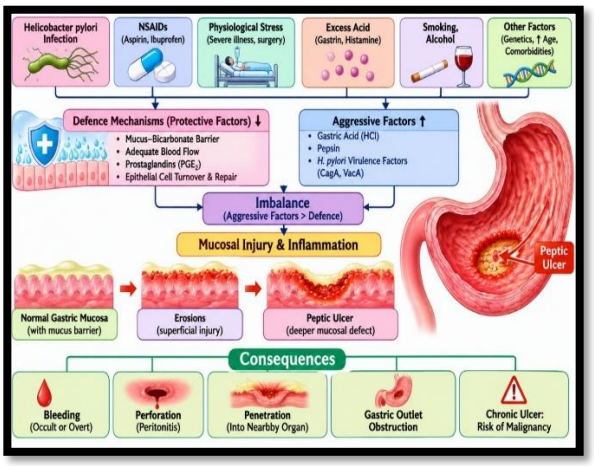


Figure:1-Pathophysiology of Peptic Ulcers

Molecular docking technique is the famous in structural bioinformatics to solve the problems in protein and ligand interaction studies. A molecule is characterized by a pair (A; B), in which A represents a collection of atoms, and B represents a collection of bonds between pairs of atoms. Information used for kinematic and energy computations is associated with each of the atoms and bonds. Each atom carries standard information, such as its Vander Waals radius. Three pieces of information are associated with each bond: (i) the bond length is the distance between atom centers; (ii) the bond angle, is the angle between two consecutive bonds; (iii) whether the bond is rotatable or not. Since bond lengths and angles do not affect significantly the shape of a molecule, it is common practice to consider them fixed. Thus, the degrees of freedom of the molecule arise from the rotatable bonds.

The three-dimensional embedding of a molecule defined when we assign values to its rotatable bonds is called the conformation of the molecule. Ligands typically have 3 to15 rotatable bonds, while receptors have 1,000 to 2,000 rotatable bonds. The

dimension of the combined searched space makes the docking problem computationally intractable.
In the context of docking, energy evaluations are usually carried out with the help of a scoring function and developing these is a major challenge facing structure-based drug design [41]. A large number of current scoring functions are based on force fields that were initially designed to simulate the function of proteins [10, 11]. A force field is an empirical fit to the potential energy surface in which the protein exists and is obtained by establishing a model with a combination of bonded terms (bond distances, bond angles, torsional angles, etc.) and non-bonded terms (Vander Waals and electrostatic). The relative contributions of these terms are adjusted for the different types of atoms in the simulated molecule by adjusting a series of parameters. Some scoring functions used in molecular docking have been adapted to include terms such as solvation and entropy [12].

Many docking algorithms [13-30] have been developed in the last three decades. However, only a few algorithms are currently available as a free web service [the pioneer was the Clus Pro server [31]]. The algorithms mostly differ in the method for searching the six-dimensional transformation space that they apply, and in their evaluation of the resolved complexes. The majority of these algorithms are computationally too heavy for large-scale experiments. Patch Dock [32], a very efficient algorithm for protein–small ligand and protein docking.

2.MATERIALS AND METHODS:

2.1 CAMU-CAMU -Plant profile:

Botanical Name: Myrciaria dubia
Common Names: camu camu,
Scientific classification:
Kingdom: Plantae
Order: Myrtales
Family: Myrtaceae
Genus: Myrciaria
Species: M. dubia



Figure:2-Camu Camu

Chemical constituents: It is considered the greatest natural source of vitamin C worldwide. It is also a promising source of many phenolic compounds, including flavonoids and anthocyanins. catechins, delphinidin 3-glucoside, cyanidin 3-glucoside, ellagic acid, and rutin; other analysis revealed the presence of gallic and ellagic acids, suggesting that the fruit possesses hydrolysed tannins like gallo- and/or ellagitannins [33].

Uses-Camu camu is used for viral infections including herpes, cold sores, shingles, and the common cold. It is also used for eye conditions including cataracts and glaucoma. Other uses include treatment of asthma, “hardening of the arteries” (atherosclerosis), chronic fatigue syndrome, depression, gum disease (gingivitis), headaches, and osteoarthritis. Some people use camu camu to increase energy and maintain healthy gums, eyes, and skin; and as an antioxidant and immune system stimulant.

2.2. Docking Study:

2.2.1. Hardware Components: In present work all, the calculations were carried out with high frequency computational analysis such as molecular modeling, energy minimizations, design and optimization of lead molecules, protein ligands interaction studies by molecular docking etc., a Hi-end server (Pentium IV 3.4 MHz's, AMD Athlon 64-bit, Dual processor with 1 GB RAM) manufactured by HCL Corporation, Pondicherry, India was used.

2.2.2. Software Components: Most of the Softwares used were either Windows or Linux plat form based which were well accepted and referred in various publications at high rated research journals. Academic license was obtained for the commercial software used in the present study by requesting the concerned suppliers. The software used in the present study was briefly detailed below.

2.2.3. Protein preparation-Protein data bank: Protein Data Bank (PDB) is repository for 3 -D Structural data of proteins and nucleic acid their data typically obtain by X-ray crystallography or NMR spectroscopy are submitted by biologist and biochemist from around the world are released into the public domain and can be accessed for free. The mission of the PDB is to maintain a single protein data bank archive of macromolecular structural data that is freely and publicity available to the global community (www. rcsb. org/pdb). In the present study the crystal structure of muscarinic receptor was obtained from protein data bank (PDB) code is 4DAJ the protein structure is shown below

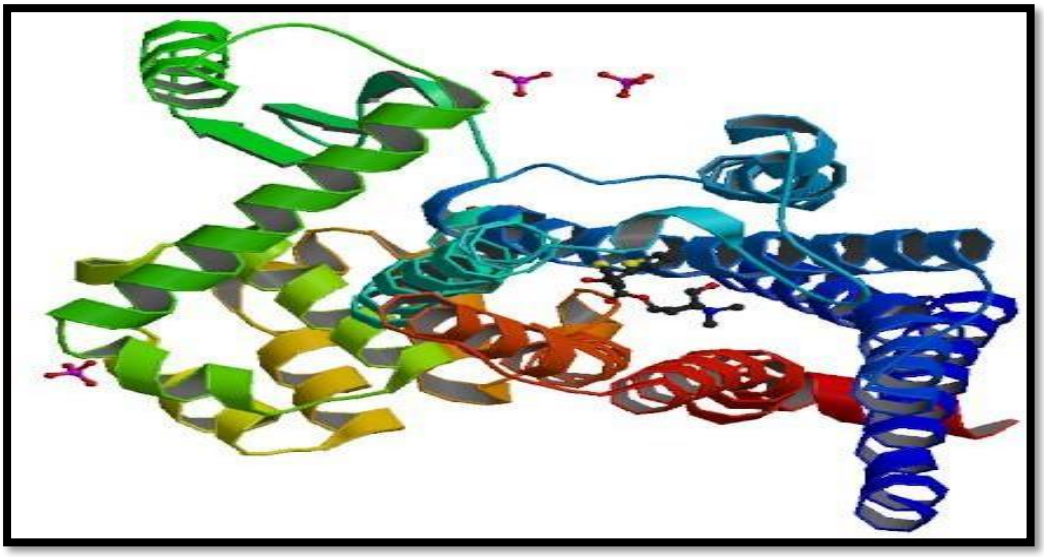


Figure:2.1-Structure of Muscarinic receptor

2.2.4. Ligand Preparation: The SMILES format of ligands was downloaded from PubChem database or from online SMILES translator tool. SMILES format of each compound was uploaded into Molinspiration web server to estimate the Lipinski properties of ligands. Finally, the files were saved in the pdb (protein data bank) format using SMILE translator online tool.

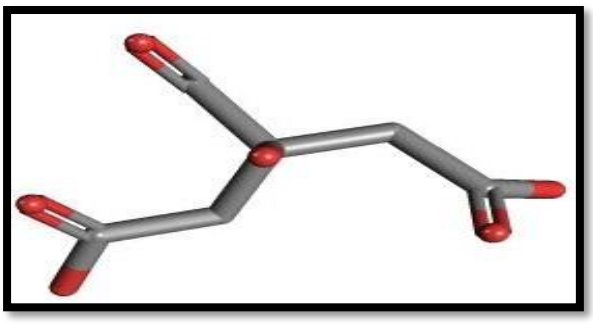


Figure:2.2.3D Structure of citric acid

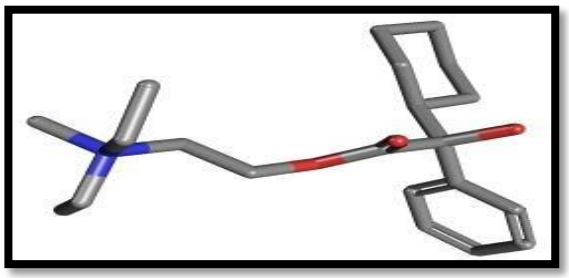


Figure:2.3.3D Structure of Oxyphenonium

2.2.5. Drug Docking: Docking of test (code) with the protein (code) was conducted with the help of advanced and automated molecular drug docking web server patch dock. (Http; esbioinl03d.cs.tau.ac.il/patch dock) an online tool for protein docking designed for the purpose of the identification of interaction sites between the drug and protein.

Input fields:

1.Receptor molecule; It is possible to specify the PDB code of the receptor molecule /upload file in pdb format each code is a four-character pdb Id.

2. Ligand molecule: - Same as receptor molecule.

3. E-mail address: - The link with the results of the request is sent to this address. Using this link one can view the docking results.

4. Clustering RMSD: - The value of the RMSD used for clustering. The higher the value the smaller the number of results the recommended values and it for protein docking.

5. Complex type: - We have used default complex type in case of enzyme -inhibitor complex type the algorithm restricts the search space to the cavities of the enzyme molecule.

Output:

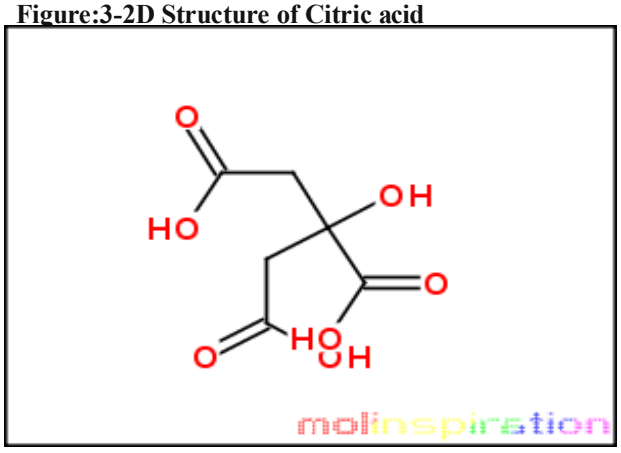
1. Solution number: No of the solution
2. Score: Geometric shape complementary score. The solution is sorted according to this score.
3. Area: Approximate interface area of the complex
4. ACE: Atomic contact energy (zhang et.al)
5. Transformation: 3D transformation
6. PDB files of the complex: The predicated complex structure in PDB format.

3.Drug Designing and Validation:

3.1Molinspiration

Molecular properties of the selected compounds are read using Molinspiration software to satisfy Lipinski's rule of five, which is essential for rational drug design. all the compounds showed no violation of all the five rules, not more than five hydrogen bond donors, not more than ten hydrogen bond acceptors, molecular weight less than 500, PC less than five and rotatable bonds less than ten.

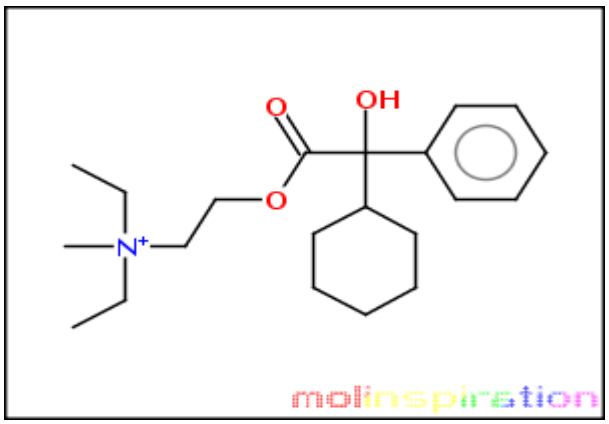
Figure:3-2D Structure of Citric acid



Molinspiration property engine v2016.10

miLogP	-1.98
TPSA	132.12
natoms	13
MW	192.12
nON	7
nOHNH	4
nviolations	0
nrotb	5
volume	151.76

Figure:3.1- 2D Structure of Oxyphenonium



Molinspiration property engine v2016.10

miLogP	0.29
TPSA	46.53
natoms	25
MW	348.51
nON	4
nOHNH	1
nviolations	0
nrotb	9
volume	359.64

Table 1: Molinspiration calculation of drug likeliness of compounds:

S.NO	Compound	Mol.wt	Hydrogen bond donor	Hydrogen bond acceptor	No of Rotating bonds	LOP	Violation
1	Citric acid	192.12	4	7	5	-1.98	0
2	Oxyphenonium	348.51	1	4	9	0.29	0

3.2. Drug Docking:

Docking of Oxyphenonium and citric acid on M3 Muscarinic receptor (4DAJ) The crystal structure of muscarinic receptor such as 4DAJ (PDB id) retrieved from RCSB PDB; the pdb file was edited and used for docking. The 4DAJ was docked with standard drug Oxyphenonium (Table 1). Among all the 20 docked runs, Oxyphenonium shown lowest docking energy value i.e., -131.63 kcal/mol is selected as a standard for comparative docking. Oxyphenonium and citric acid are docked on to 4DAJ. All the docking calculations were carried out using the program Patch Dock 4.0. The program searches for best conformation and best place of binding of the ligand within a fixed protein structure.

Table-2: Docking energy values of 4DAJ with Citric acid and Oxyphenonium

S. No	Receptor	Drug	Canonical SMILE	ACE
1	M3 Muscarinic(4DAJ)	CITRIC ACID	C(C(=O) O) C(CC(=O) O) (C(=O) O) O. O	-124.6
2	M3 Muscarinic(4DAJ)	OXYPHENONIUM	CC[N+] (C)(CC) CCOC(=O) C(C1CCCCC1) (C2=CC=CC=C2) O	-131.63

3.3. Evaluation Of Pharmacokinetic Parameters: - Swiss ADME

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. In that context, computer models constitute valid alternatives to experiments. Here, we present the new SwissADME web tool that gives free access to a pool of fast yet robust predictive models for physicochemical properties, pharmacokinetics, drug-likeness and medicinal chemistry friendliness.

Table -3: Pharmacokinetic Profile of Citric Acid

Drug	Lipid solubility	Aqueous solubility	GI absorption	BBB Permeation	CYP3A4 inhibitor	CYP2D6 Inhibitor
Citric acid	-1.02	0.57	Low	No	No	No

4.DISCUSSION:

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies. Synthetic drugs produce several side effects, so herbal medicines are considered as better alternatives for the treatment of peptic ulcer. Hence in the present study we have selected Myrciaria dubia which is a rich source of citric acid. Until now no reports were found for this plant that will be used as gastroprotective, hence in the present study aims for evaluation of gastroprotective effect of Myrciaria dubia by Insilco method.

For the molecular docking we have to select the drug and a target protein. In the present study we have selected citric acid from Myrciaria dubia acts as test compound. As citric acid is an antibacterial no

literature was found that citric acid acts on specific receptor and used in the treatment of peptic ulcers. Hence, we have selected citric acid in the present study as a test compound.

Previously the muscarinic receptors were identified only on the surface of enterochromaffin like cells which is a major cause of ulcers. Later the M3 type of muscarinic receptors was also identified in the parietal cells of stomach. So, the most abundant receptors in the git were the muscarinic which is one of the major causes of peptic ulcers, hence in the present study we have selected M3 muscarinic receptor as a target protein.

Molinspiration is used for complete drug designing and drug validation. In these studies, we used citric acid and Oxyphenonium in order to inhibit the 4DAJ protein structure in Insilco methods. Table -1 represents drug designing and validation. The molecular properties of the drug showed that (Mw: 192.12 and Log p: -1.98). Based on the complete drug likeness score the designed drug obeys the basic rules of drug designing. Molinspiration is developed based on the drug likeness score.
Hence the designed drug clearly represents that there are no errors present. We try to dock the chemical candidates with the modelled 4DAJ target protein.
In Drug docking studies, we used patch dock server in order to bind the modelled protein and the designed drug. The results show a binding value of (-124.6) of the Target protein with citric acid. Whereas, Oxyphenonium combined with the target protein shows a binding value of (-131.63). The results elucidated in Fig (3 &3.1), show that the designed drug shows a higher binding affinity when compared with Oxyphenonium. These findings of the new drug will help the patient to have a quality life for their survival.

In addition, analysis of pharmacokinetic properties such as the partition coefficient and Water solubility (QPlogS) of the evaluated compounds are within the range. All the pharmacokinetic parameters are within the acceptable range defined for human use, which collectively indicated that the screened compounds could be taken forward, for further analysis.

5.CONCLUSION:

Peptic ulcer disease has been a major cause of morbidity and mortality. Even though the synthetic drug treatment is available because of adverse effects and the importance of natural treatment directed us to study the anti-ulcer potential of Myrciaria dubia. Molecular docking was carried out for test and standard (citric acid and Oxyphenonium) and combined with target protein M3 muscarinic receptor (4DAJ) with the help of Patch dock server. In the present study we described the docking studies to identify inhibitors against 4DAJ.The test compound showed good binding affinity and better pharmacokinetic parameters. The findings of the present study attest that Myrciaria dubia exhibit better anti-ulcer potential when compared to the standard Oxyphenonium as the docking energy is more for Myrciaria dubia than the Oxyphenonium. Hence, we conclude that the results obtained from this study may be worthwhile, to carry out further in vitro and in vivo studies to design novel and potential inhibitors against 4DAJ.

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