EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN: 2663-0990 (Print) & ISSN: 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-6 | Issue-1 | Jan-Feb- 2024 | DOI: 10.36349/easjpp.2024.v06i01.006

Original Research Article

Assessment of Antidiarrheal Potency of *Psidium guajava* leaf: In-silico Validation

Pratik Singh[1*], Jitender K Malik[1], G. Pavan Kumar[1], Surendra Pratap Singh[1], Sunil Kumar[1], Gyan Singh[1], Vinay Siroliya[1]

[1]Faculty of Pharmacy, P.K. University, Shivpuri (M.P.)-India

Article History
Received: 07.01.2024
Accepted: 13.02.2024
Published: 15.02.2024

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: ***Background:*** Millions of people worldwide use alternative health care systems, and traditional medicines are a crucial part of such systems. In contrast to contemporary pharmaceuticals, which are single molecules that have undergone rigorous testing, structural optimisation, and toxicological clearance, traditionally used herbal remedies are multi-constituent medicines, the safety and efficacy of which are dependent on the experiences of the practitioners. More than 80% of contemporary medications are obtained directly from natural sources (plants, microorganisms, cells, etc.) or their molecules/compounds. Plants are becoming recognised as prospective sources for drug development. Diarrhoea and other gastrointestinal diseases are treated using a variety of conventionally used medicinal herbs. ***Aim & Objective:*** The focus of the current study is on testing *P. guajava* ethanolic leaf extract for its potent anti-diarrheal properties, which have been demonstrated in computer-aided simulation tests confirmed the plant's potential for antidiarrheal action. ***Method:*** A grid-based docking strategy was used to determine the binding using the Auto Dock software. Merck Molecular Force Field was used to build the 2D structures of compounds, convert them to 3D, and then energetically reduce them up to arms gradient of 0.01. (MMFF). ***Result:*** Based on previously proven effects of flavonoids and on diarrhea. Two flavonoids *i.e.* quercetin and quercetin-3-arbinoside which were found in the ethanolic leaf extract of *P.guajava* was selected as lead molecules for current investigation. So, in current study an attempt had been made to elucidate the proposed anti-diarrheal mechanism of the action of selected lead compound (flavonoids) against ***muscarinic- M3*** receptor by *in -silico* molecular docking. The result of molecular docking showing binding energy -5.81 & -4.32 kcal/mol for quercetin and quercetin-3-arbinoside respectively.

Keywords: *P. guajava,* anti-diarrheal**,** quercetin, quercetin-3-arbinoside & *in-silico* molecular docking.

INTRODUCTION

A person with diarrhoea experiences an increase in the amount of liquid in their stools. Diarrhoea is defined as the presence of 60–90% water. Individual differences exist in stools' consistency and frequency of evacuation. It's one of the illnesses that kills the most people, especially in underdeveloped nations. In third world nations, millions of individuals pass away annually [1-2]. Children are more vulnerable to this illness, which is the biggest cause of mortality in children, especially those under the age of five. Dehydration symptoms frequently start with irritable attitude changes and lack of typical skin stretchiness, and if the condition worsens, they might include reduced urine, skin colour changes, a higher heart rate, and a decrease in responsiveness [3-4]. It can be acute or chronic and can range in severity from mild to life-threatening and it has man causes and pathophysiologic mechanisms.

Types of Diarrhoea [5-6]

Diarrhoea occurs due to imbalance between secretion and reabsorption of fluids and electrolytes. Depending on the frequency and the duration of the diarrhoea episodes,

***Corresponding Author:** Pratik Singh
Faculty of Pharmacy, P.K. University, Shivpuri (M.P.)-India

The types of Diarrhoea include;

- Secretary diarrhoea can be caused by microbial toxins, vasoactive intestinal polypeptides, excessive bile acids, laxatives, unabsorbed fat.
- Mild diarrhoea in most cases is self-limiting and may subside within 1-2 days
- Chronic diarrhoea lasts for 14 days or longer and thus it needs more thorough diagnosis to determine the cause and enable selection of appropriate therapy.
- Severe diarrhoea caused by bacterial infections and other causes can lead to significant loss of fluid and electrolytes and therefore should be attended to promptly.

Causes of Diarrhoea [7]

- Food intolerance: especially lactose
- Bacterial infections: shigella, salmonella, E. coli
- Viral infections
- Functional bowel disorders
- Reaction to medicines: e.g. antibiotics and magnesium containing antacids
- Parasites: e.g. Entamoeba histolytica
- Intestinal diseases: inflammatory bowel syndrome (IBD), celiac disease
- Nutrient malabsorption due to some diseases
- Altered motility
- Secretory tumours of GIT e.g carcinoid, which secrets vasoactive intestinal peptide
- Emotional distress

Psidium guajava L. (guava) is a small tree known for its fruit flavor that is cultivated almost around the globe in tropical areas. Its fruit is amazingly rich in antioxidants, vitamin C, potassium, and dietary fiber [8]. In different parts of the world, this plant holds a special place with respect to fruit and nutritional items. The guava leaves contained numerous chemical constituents such as α-pinene, β-pinene, limonene, menthol, terpenyl acetate, isopropyl alcohol, longicyclene, caryophyllene, β-bisabolene, caryophyllene oxide, β-copanene, farnesene, humulene, selinene, cardinene and curcumene, mallic acids, nerolidiol, βsitosterol, ursolic, crategolic, and guayavolic acids, cineol, quercetin, 3-L-4-4-arabinofuranoside (avicularin) and its 3-L-4-pyranoside (essential oil), resin, tannin, eugenol, caryophyllene (1a α-, 4a α-, 7 α-, 7a β-, 7b α-)]-decahydro-1H-cycloprop-azulene, Guajavolide (2 α-,3 β-,6 β-,23-tetrahydroxyurs-12-en-28,20 β-olide; 1) and guavenoic acid (2 α-,3 β-,6 β-,23-tetrahydroxyurs12,20(30)-dien-28-oic acid, triterpene oleanolic acid, triterpenoids, flavinone-2 2'-ene, prenol, dihydrobenzophenanthridine and cryptonine [9-10].Guavas contain carotenoids and polyphenols, the major classes of antioxidant pigments giving them relatively high potential antioxidant value among plant foods. The pigment content as polyphenol, carotenoid and pro-vitamin A, retinoid sources than yellow-green ones. Traditionally *P. guajava* is mainly known for its antispasmodic and antimicrobial properties in the treatment of diarrhoea and dysentery. It also been used broadly as a hypoglycaemic agent. Numerous pharmacological studies have demonstrated the ability of this plant to revelation antioxidant, hepatoprotection, anti-allergy, antimicrobial, antigenotoxic, antiplasmodial, cytotoxic, antispasmodic, cardioactive, anticough, antidiabetic, antiinflamatory and antinociceptive activities [11].

Experimental Works

Flavonoids and alkaloids are well known to inhibit the release of autacoids and prostaglandins, thereby inhibiting secretion induced by castor oil [12]. As per literature survey Metwally AM etal;2010 demonstrated that ethanolic leaf extract contained flavonoidal constituents like Quercetin & Quercetin- 3-arbinoside, avicularin, guaijaverin, isoquercetin, hyperin, quercitrin, quercetin 3-O-gentiobioside & quercetin 4'-glucuronoide [13]. Therefore Quercetin & Quercetin- 3-arbinoside are selected as lead compound for molecular docking studies against *muscarinic M3* receptor for validation of antidiarrheal potential of *P.guajava* leaf.

Molecular Docking Studies

Ligand Preparation:

2D Structure of ligands like quercetin, and quercetin-3-arabinoside were drawn using ChemSketch [14], the two-dimensional structures of the prepared ligands were converted into their 3-D structures optimized with 3D geometry. The optimized structures were saved in PDB format for AutoDock compatibility. The basic structures of the prepared ligands were given below:

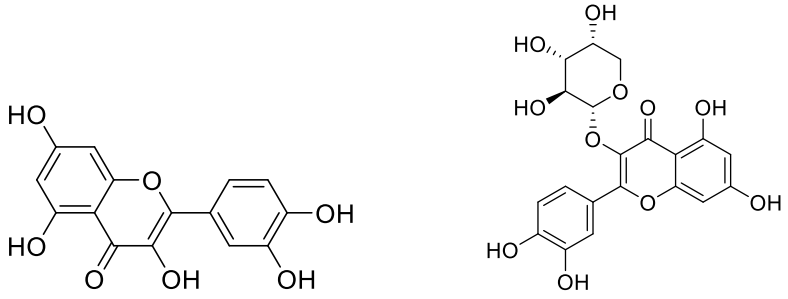


Figure 1: 2D structure of quercetin and quercetin-3-arabinoside

Preparation of the grid file

The regions of interest used by Autodock were defined by considering grid area by making a grid box around the active sites. Grid box plays a central role in process of docking as it is made to cover all the amino acids present in active sites necessary for binding other than those present in receptor. Grid box has 3 thumbwheel widgets which let us change the number of points in the x, y and z dimensions. The spacing and grid points is given in table 1 [15].

Table 1. Grid parameters used in current docking analysis of MUSCARINIC M3

S. No.	Receptor	x-axis	y-axis	z-axis	Spacing	x center	y center	z center
1	MUSCARINIC M3	50	50	50	0.386	-20.239	-49.843	178.453

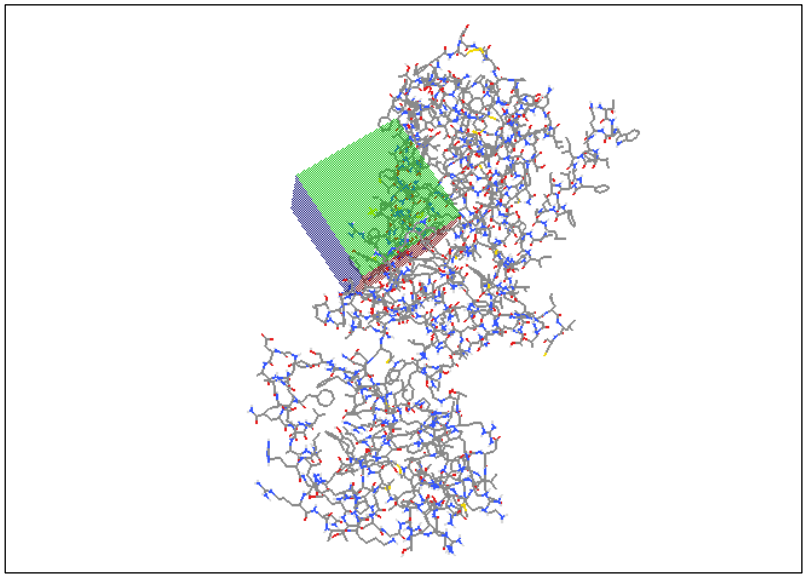


Figure 2: Grid box covering all active sites in MUSCARINIC M3 receptor

Preparation of the docking file

All the calculations were carried out by using Autodock 4.2 as docking tool. The visualization and other programs necessary for docking studies were performed out by means of Pymol, Chimera, DS visualizer, MMP Plus [16].

Docking Study

Crystal structure

The crystal structure of the protein consisting of MUSCARINIC M3 receptor is downloaded from the Protein Data Bank portal. All the primary information regarding receptor and structure (5zhp.pdb) registered in the Protein data bank was used [17]. The complex ligand was separated by using Chimera software.

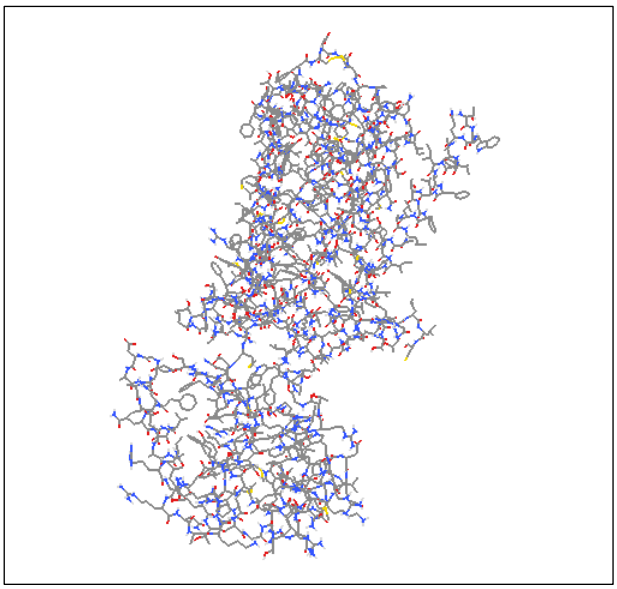


Figure 3: Crystal structure of MUSCARINIC M3 receptor (PDB ID-5zhp)

Processing of Protein

The downloaded receptor protein is having four chains, i.e. chain A, and B. Out of these two chains, chain A was selected for experimental purpose and other chains were removed from it. The bound ligand was separated from the macromolecular complex by using software Chimera [18-19].

Toxicity & ADME-T Studies

The ligand molecules viz. quercetin and quercetin-3-arabinoside were studied by online program OSIRIS, for prediction of presence of any toxic group as well as presence of any toxic group and ADME- T properties (20-22).

RESULT AND DISCUSSION

Millions of people worldwide use alternative health care systems, and traditional medicines are a crucial part of such systems. In contrast to contemporary pharmaceuticals, which are single molecules that have undergone rigorous testing, structural optimisation, and toxicological clearance, traditionally used herbal remedies are multi-constituent medicines, the safety and efficacy of which are dependent on the experiences of the practitioners. More than 80% of contemporary medications are obtained directly from natural sources (plants, microorganisms, cells, etc.) or their molecules/compounds. Plants are becoming recognised as prospective sources for drug development. Diarrhoea and other gastrointestinal diseases are treated using a variety of conventionally used medicinal herbs. In animal models of diarrhoea, several of these herbs have been scientifically shown effective. However, only few of these have undergone controlled clinical studies. The focus of the current study is on testing *P. guajava* ethanolic leaf for its potent anti-diarrheal properties, which have been demonstrated by computer-aided simulation tests confirmed the plant's potential for antidiarrheal action. The docking approach used in this work, in particular, gave helpful insight into the physiologically active isolates' bindings to several protein targets at the molecular and cellular level. These protein targets are known to play important roles in pharmacological pathways, including anti-diarrheal cascades.

Increased smooth muscle contraction (M3), gastric acid production from the parietal (oxyntic) cells of the stomach, and intestinal digesting enzyme release are all responses of the gut to muscarinic agonists. the effects of an antagonist on the oxyntic cell's M3 receptors when acting directly. Muscarinic antagonists block the M3 receptor-mediated contractions of the gastrointestinal tract brought on by Ach and other muscarinic agonists. However, they often aren't as efficient in reducing the contraction and motility that parasympathetic nerve stimulation causes (21). Irritable bowel syndrome (IBS), which is characterised by diarrhoea related to inflammation of the lower colon, including mild dysentery and diverticulitis. Existing drugs for the treatment of IBS, such as mebeverine, cimetropium and milverine have additional properties including Ca2+ channel blockade which contributes to their antispasmodic activity by ***Kenneth J etal;2001***. There is therefore a need for selective M3 receptor antagonists for the treatment of gastrointestinal tract disorders.

Based on previously proven effects of flavonoids and on diarrhea. Two flavonoids *i.e.* quercetin and quercetin-3-arbinoside which were found in the ethanolic leaf extract of *P.guajava* was selected as lead molecules for current investigation. So, in current study an attempt had been made to elucidate the proposed anti-diarrheal mechanism of the action of selected lead compound (flavonoids) against ***muscarinic- M3*** receptor by *in -silico* molecular docking. The grid parameter for docking analysis tabulated in the table 1. The result of molecular docking was tabulated in table 2, showing binding energy -5.81 & -4.32 kcal/mol for quercetin and quercetin-3-arbinoside respectively. The binding mode showed in fig.4-5 whereas 2D &3D binding interaction was shown in fig.7-12. Although quercetin and quercetin-3-arbinoside showed good interaction with selected ligand but highest binding interaction displayed by quercetin with ***muscarinic- M3*** receptor having conventional hydrogen bond interaction with VAL A:160, Tyr A:175 & MET A:156 as well as Pi- Sigma binding at MET A:187, LEU A: 159 whereas covalent bounding of quercetin at PHE A:163 with weak Vander wall's interaction at ILE A:161, PHE A: 105, PHE A:167. The binding interaction of quercetin-3-arbinoside on ***muscarinic- M3*** receptor displayed the conventional hydrogen bounding at TYR A:175,VAL A:160 along with PHE A: 163(covalently),LEU A: 159 (Pi-sigma) & PHE A:105,ASP A:164,ILE A:161, PHE A: 167(weak Vander wall's interaction).The pharmacokinetic profiling of the quercetin ligand has revealed that it is having good pharmacokinetic profile associated without the presence of major toxic effects like reproductive effects, irritant effect, and tumorogenic properties, but shows the presence of some mutagenicity. The pharmacokinetic and toxicity profiling results of flavonoids was shown in fig. 7.

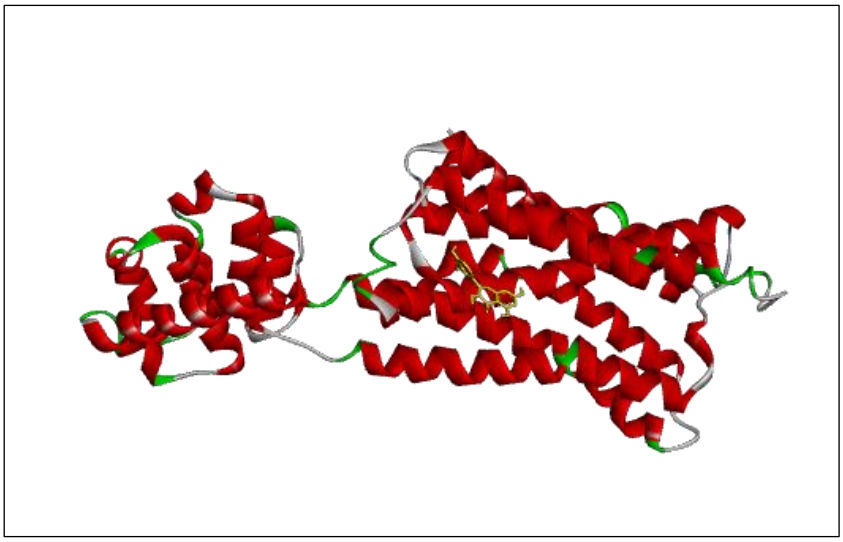


Figure 4: Binding mode of quercetin within the active site of human MUSCARINIC M3 receptor

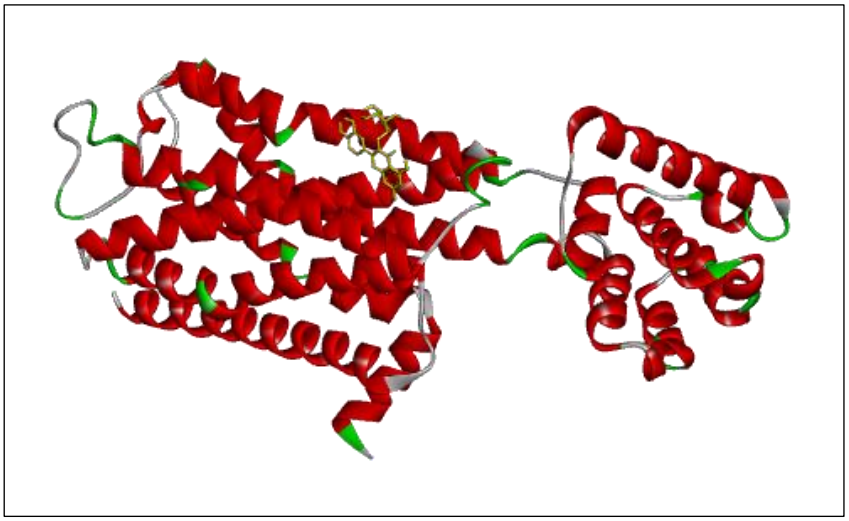
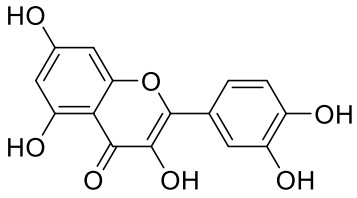
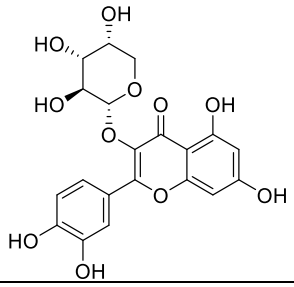


Figure 5: Binding mode of quercetin-3-arabinoside within the active site of human MUSCARINIC M3 receptor

Table 2: Results of docking of ligands like quercetin and quercetin-3-arabinoside against human MUSCARINIC M3 receptor

Sl. No	Compound Name	Structure	Binding Energy (Kcal/mole)
1	Quercetin		-5.81
2	Quercetin-3-arabinoside		-4.32

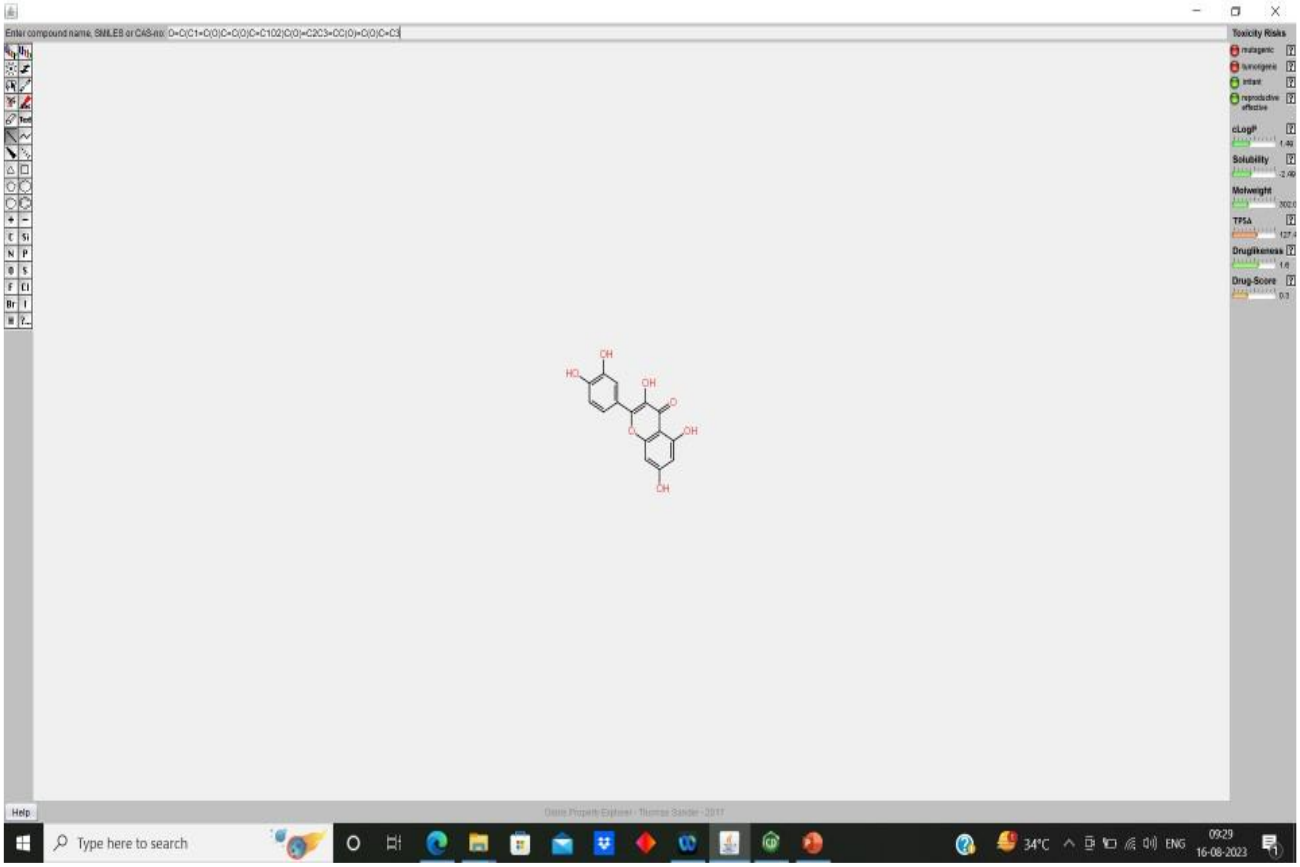


Figure 6: Pharmacokinetic and toxicity profiling of quercetin

Interaction

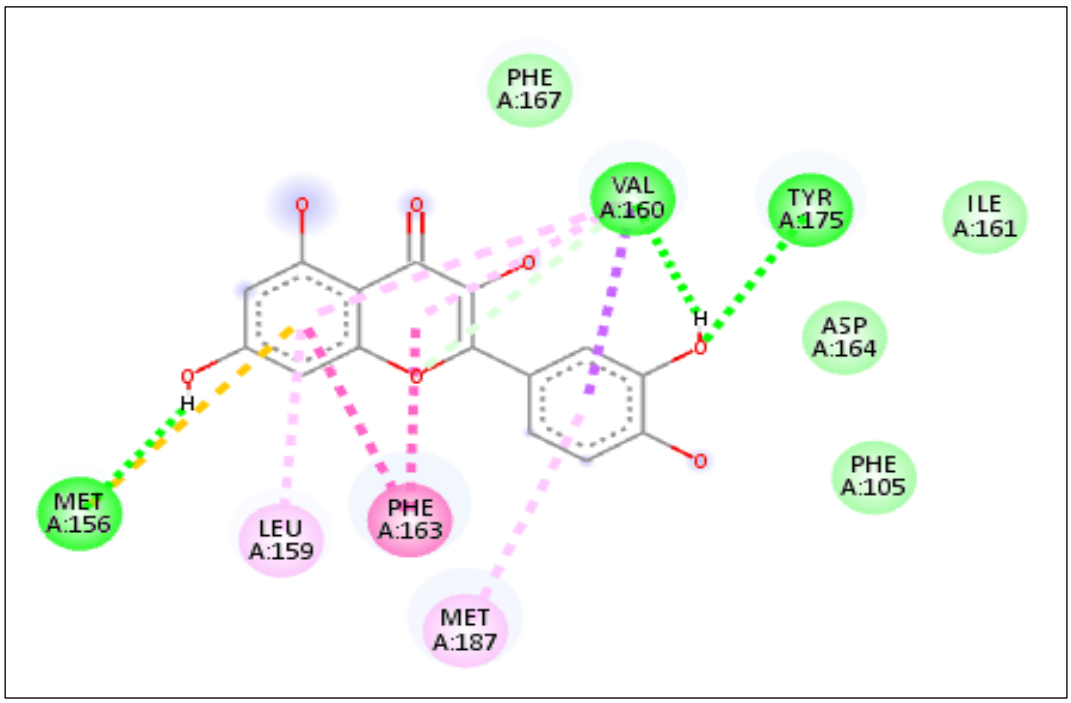


Figure 7: Two-dimensional binding mode of quercetin within the active site of human MUSCARINIC M3 receptor

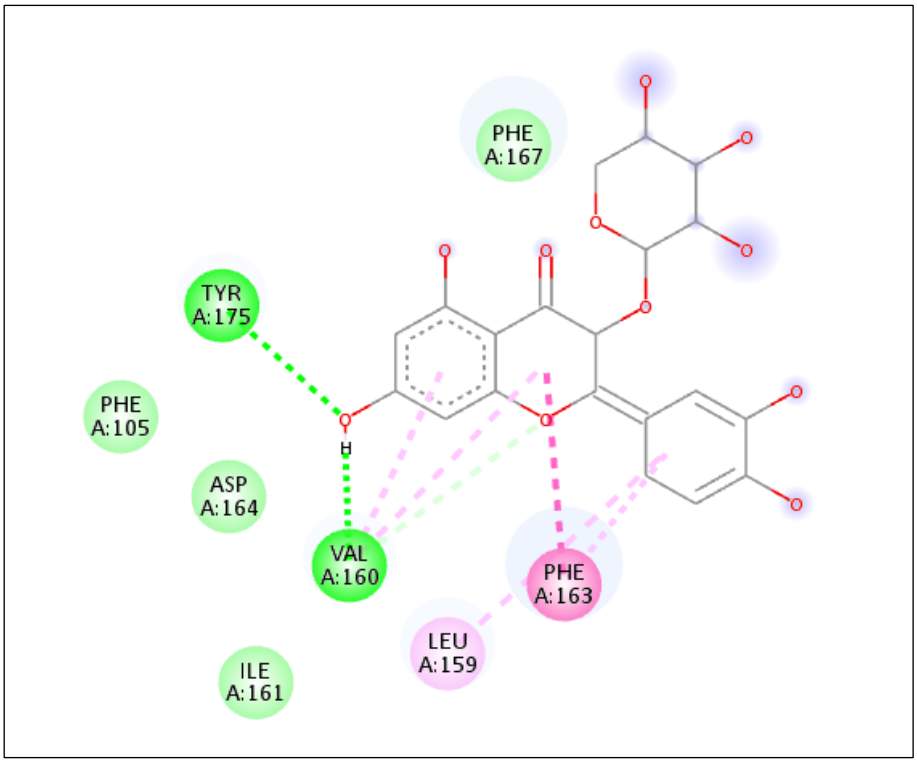


Figure 8: Two-dimensional binding mode of quercetin-3-arabinoside within the active site of human MUSCARINIC M3 receptor

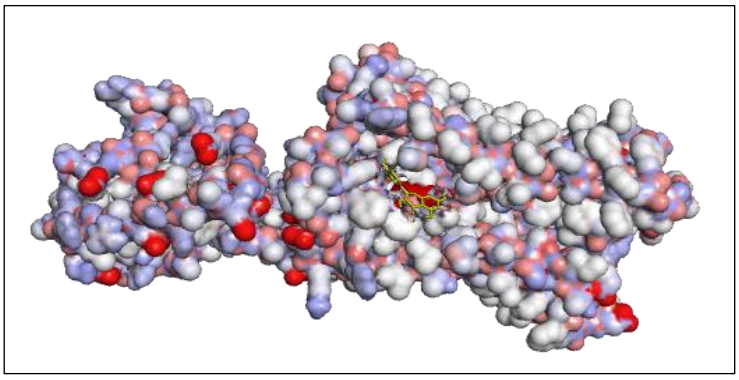


Figure 9: Three-dimensional binding conformation of quercetin within the active site of human MUSCARINIC M3 receptor

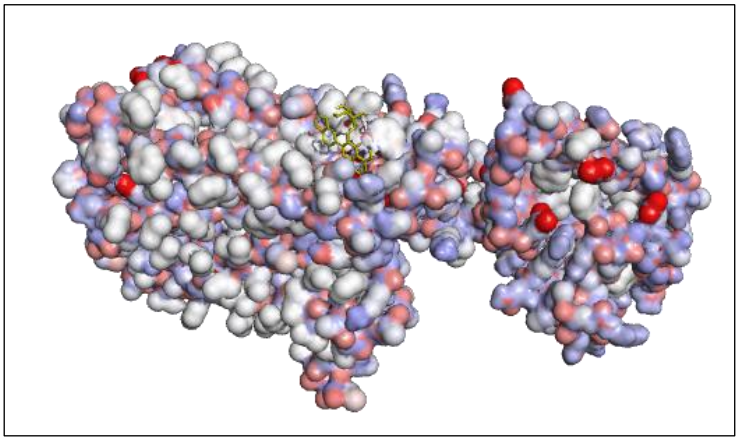


Figure 10: Three-dimensional binding conformation of quercetin-3-arabinoside within the active site of human MUSCARINIC M3 receptor

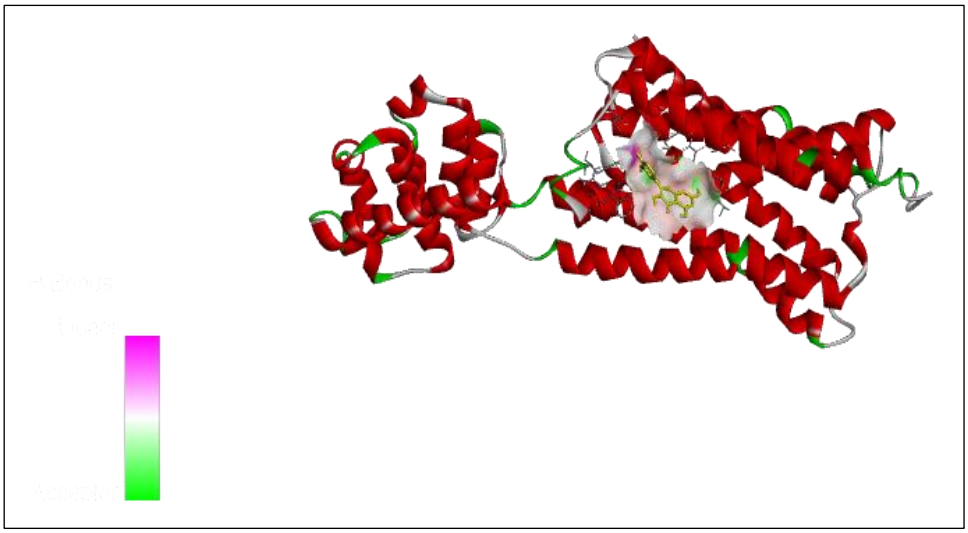


Figure 11: Three-dimensional binding mode of quercetin within the active site of human MUSCARINIC M3 receptor

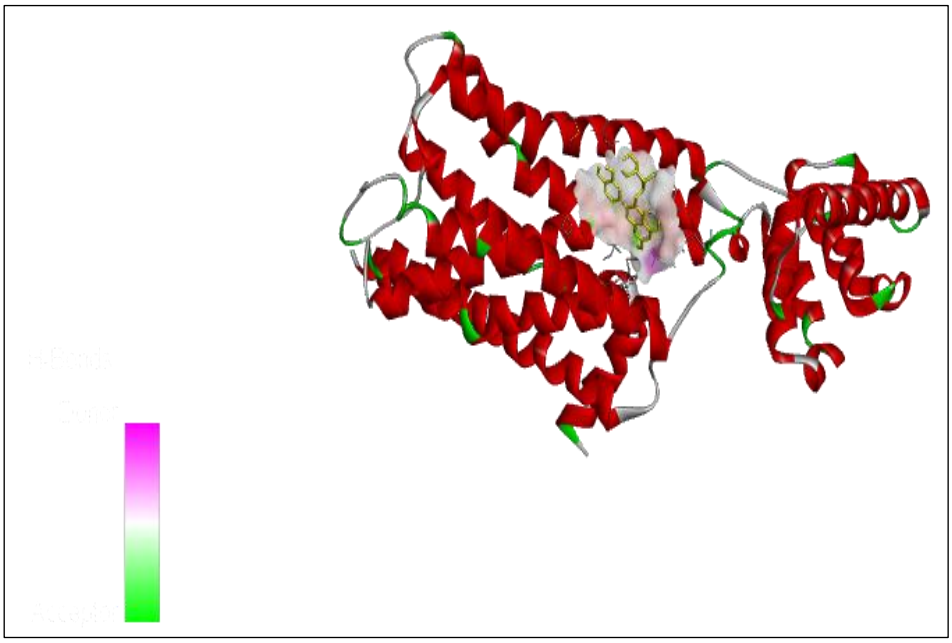
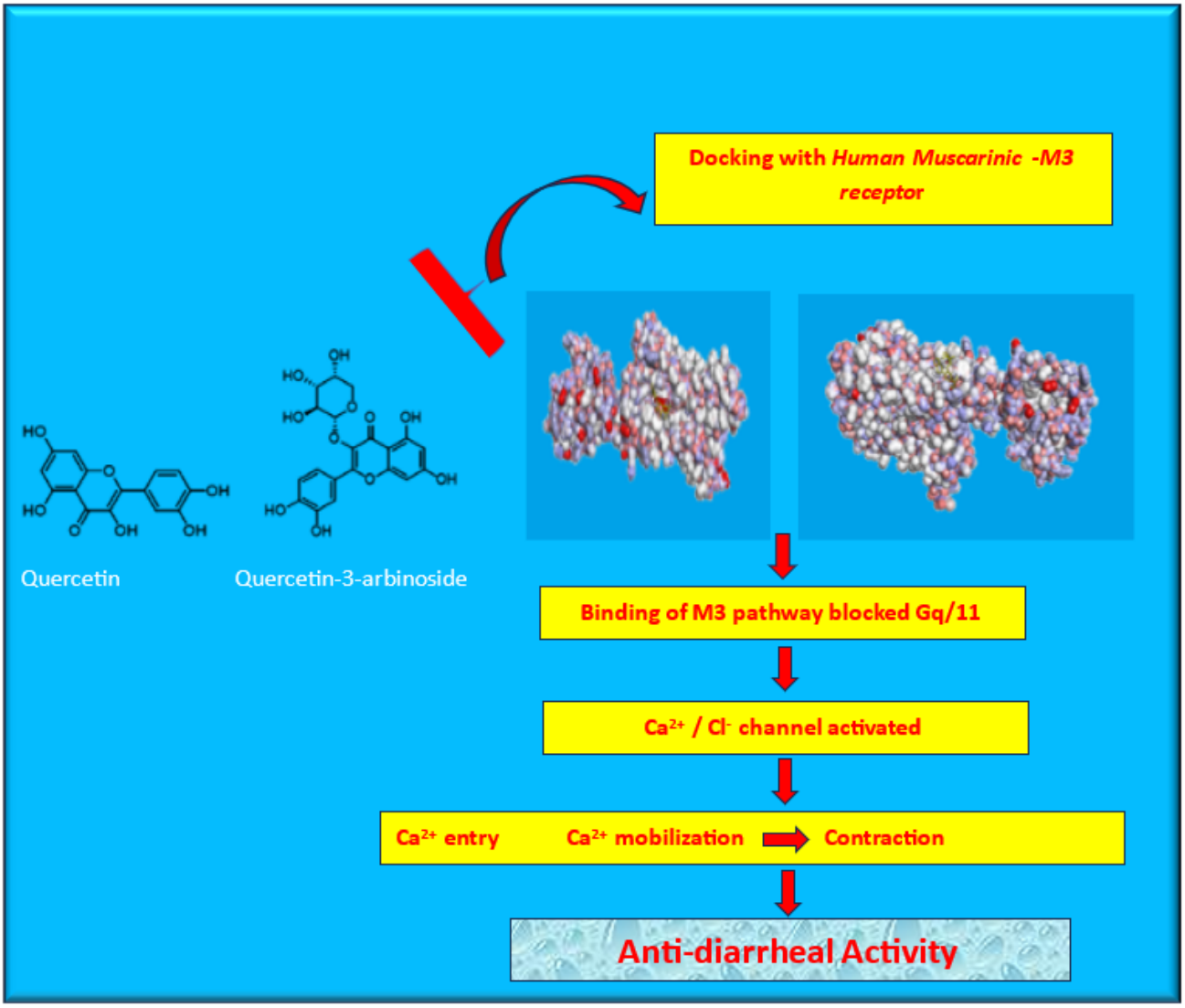


Figure 12: Three-dimensional binding mode of quercetin-3-arabinoside within the active site of human MUSCARINIC M3 receptor

Divulgence of Investigation

According to a computational analysis, some phytoceuticals have strong interactions with key ***human muscarinic-M3*** receptor binding sites and may have inhibitory effects on diarrhoea. Additionally, the Flavonoids is thought to impair intestinal motility, water and electrolyte secretions by interfering with the activity of autacoid and prostaglandins. Phytoceuticals also have no overt adverse effects, a superior safety profile, and good absorption. Proposed mechanism of action of active flavonoids constituents of *P.guajava* leaf are shown pictorially as follow:



REFERENCE

1. Bryce, J., Boschi-Pinto, C., Shibuya, K., & Black, R. E. (2005). WHO estimates of the causes of death in children. *The lancet*, *365*(9465), 1147-1152.
2. Kosek, M., Bern, C., & Guerrant, R. L. (2003). The global burden of diarrhoeal disease, as estimated from studies published between 1992 and 2000. *Bulletin of the world health organization*, *81*, 197-204.
3. Keusch, G.T., Fontaine, O., Bhargava, A., Boschi-Pinto, C., Bhutta, Z.A. & Gotuzzo, E. (2006). Diarrheal diseases. In: Jamison, J.T., Breman, J.G., Measham, A.R., Alleyne, G., Claeson, M. & Evans, D.B. editors. *Disease control priorities in developing countries.* 2nd ed. Washington, DC: World Bank; 371–87. [Google Scholar]
4. Bryce, J., Black, R. E., Walker, N., Bhutta, Z. A., Lawn, J. E., & Steketee, R. W. (2005). Can the world afford to save the lives of 6 million children each year?. *The Lancet*, *365*(9478), 2193-2200.
5. Orne-Gliemann, J., Perez, F., Leroy, V., Newell, M. L., & Dabis, F. (2003). A decade of child health in developing countries. *Cahiers d'études et de recherches francophones/Santé*, *13*(2), 69-75.
6. Peterson, R. S., Owens, P. D., Tetlock, P. E., Fan, E. T., & Martorana, P. (1998). Group dynamics in top management teams: Groupthink, vigilance, and alternative models of organizational failure and success. *Organizational behavior and human decision processes*, *73*(2-3), 272-305.
7. Ali, O. M. (2016). Assessment of knowledge and attitude towards diarrheal disease in children under-five years in Shendi town. *International Journal of Research—Granthaalayah*, *4*(3), 80-84.
8. Kamath, J. V., Rahul, N., Kumar, C. A., & Lakshmi, S. M. (2008). Psidium guajava L: A review. *International Journal of Green Pharmacy (IJGP)*, *2*(1).
9. Oliver-Bever, B. E. P. (1986). *Medicinal plants in tropical West Africa*. Cambridge university press.
10. bin Zakaria, M. (2010). *Traditional Malay medicinal plants*. ITBM.
11. Joseph, B., & Priya, R. M. (2011). Phytochemical and biopharmaceutical aspects of Psidium guajava

(L.) essential oil: a review. *Research Journal of Medicinal Plants*, *5*(4), 432-442.
12. Gutiérrez, R. M. P., Mitchell, S., & Solis, R. V. (2008). Psidium guajava: A review of its traditional uses, phytochemistry and pharmacology. *Journal of ethnopharmacology*, *117*(1), 1-27.
13. Van Wyk, B. E., Oudtshoorn, B. V., & Gericke, N. (1997). *Medicinal Plants of South Africa*. Briza.
14. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as Potent Inhibitor of COVID-19 Main Protease: In-Silico Docking Approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, 1-7.
15. Malik, J. K., Soni, H., Sharma, S., & Sarankar, S. (2020). Hydroxychloroquine as potent inhibitor of COVID-19 main protease: Grid based docking approach. *Eurasian Journal of Medicine and Oncology*, *4*(3), 219-226.
16. Soni, H., Gautam, D., Sharma, S., & Malik, J. (2020). Rifampicin as potent inhibitor of COVID-19 main protease: In-silico docking approach. *Saudi Journal of Medical and Pharmaceutical Sciences*, *6*(9), 588-593.
17. Malik, J., Jhariya, D., Ahirwar, P., Sharma, S., Upadhyay, S., & Soni, H. (2024). Mechanistic insight anti-arthritis efficacy of bio-actives of Moringa oleifera: In-silico molecular docking. *Journal of Pharmacognosy and Phytochemistry*, *13*(1), 44-48.
18. Soni, H., Mishra, S., Mishra, R. K., & Mishra, S. R. (2022). Silibin as Potent Inhibitor of COVID-19 Main Protease: In-Silico Docking Approach. *Journal of Molecular Pharmaceuticals and Regulatory Affairs*, 1-7.
19. Soni, S., Malik, J. K., Sarankar, S. K., & Soni, H. (2019). Rutin as a potent inhibitor of dihydrofolate reductase: A computational design and docking. *EAS J. Pharm. Pharmacol*, *1*, 130-134.
20. Soni, N. U. P. U. R., Pardasani, K. R., & Mujwar, S. O. M. D. U. T. T. (2015). Insilico analysis of dietary agents as anticancer inhibitors of insulin like growth factor 1 receptor (IGF1R). *J Pharm Pharm Sci*, *7*(9), 191-196.
21. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, *25*(13), 1605-1612.
22. Broadley, K. J., & Kelly, D. R. (2001). Muscarinic receptor agonists and antagonists. *Molecules*, *6*(3), 142-193.

Cite This Article: Pratik Singh, Jitender K Malik, G. Pavan Kumar, Surendra Pratap Singh, Sunil Kumar, Gyan Singh, Vinay Siroliya (2024). Assessment of Antidiarrheal Potency of *Psidium guajava* leaf: In-silico Validation. *EAS J Pharm Pharmacol, 6*(1), 40-49.