Antihistaminic Action Of Phytochemicals Present In Herbal Siddha Formulation Swasakudori Via In Silico Docking Study Targeting Human Histamine Receptors

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Introduction

Bronchial asthma

Bronchial asthma is a chronic noncommunicable disease in which the airways are inflamed which results in hyperactivity of the bronchial tree and causes obstruction of the airway to a variable extent.(Ukena D et al., 2008) Bronchial asthma is mostly brought on by the following four mechanisms: (Bateman ED 2008 etal.,)

- Bronchial smooth muscle Tightening
- Edema of the airway walls
- Bronchiole clogging with mucus
- Permanent lung alterations

Asthma symptoms including coughing, wheezing, chest tightness, and shortness of breath are brought on by this. These sensations are frequently worst at night or after exercise. Asthma symptoms can deteriorate due to other common factors. Viral infections (colds), dust, smoke, fumes, weather changes, grass and tree pollen, animal fur and feathers, potent soaps, and perfumes are just a few examples of the many different triggers that might affect a person.(Global burden). Bronchial asthma can affect children and adults, whereas Children have a higher incidence and prevalence of asthma, but adults have a higher morbidity and mortality rate. (Dharmage et al., 2019). According to disability-adjusted life years, asthma ranks 28th among the major causes of disease burden. Asthma affects over 300 million people worldwide, and by 2025, another 100 million people may be at risk.(Global asthma report 2019). Main Etiology of Asthma is well understood with the main role of Histamine secretion and Degranulation of mast cells , which is an iniator of all the clinical features encountered in bronchial asthma(Gayathri et al.,2019)

Histamine

Histamine, a long-established chemical mediator released by mast cells in the immediate allergic response, is thought

to play an important role in the pathophysiology of asthma. (white MV et al.,) Goblet cell hyperplasia and mucus hyperproduction, has been reported to be associated with the development of airway hyperresponsiveness and increased severity and mortality of bronchial asthma. Histamine is known to strongly stimulate goblet cell secretion (Yamauchi K, et a.,2019) Four types of histamine receptors, such as H1, H2, H3, and H4, have been identified in airway and lung tissue.(Jones JV et al.,1952)(Tucker et al., 1975) .H1 receptor-mediated smooth muscle bronchoconstriction is one of the best known biological effects of histamine in the respiratory system.(Curry JJ et al., 1946) Histamine induces plasma extravasation from postcapillary venules by affecting the bronchial microcirculatory system. In addition, histamine plays an immunological role in the action of dendritic cells, B cells, and Th1 and Th2 lymphocytes via cell surface H1 and H2 receptors.(Jutel M et al)

Siddha system

The Siddha system is an indigenous system of medicine that has plenty of high-quality herbal medicines with minimal adverse effects. Many people have got aware on using herbal medicines for various diseases. While quite popular in some areas of Tamil Nadu, similar to other complementary medical practises , little research has been done to confirm the mode of action, pharmacology, pharmacokinetics, potential short- and long-term benefits, or side effects, etc. Statistics are not available to support the effectiveness of this approach. These systems are being criticised or rejected by the scientific community as a result of these gaps. The present study is a modest approach to understand the scientific basis of one such siddha herbal preparation, Swasakudori used in the treatment of Bronchial asthma and other respiratory disorders(M krishna Rao et al., 2016). The main ingredients of Swasakudori are Calotrpis and Piper nigrum (Black pepper). Swasakudori when used along with Thalisapathrikudineer provides the best results and acts as a good bronchodilator. It relieves chest tightness and severe cough immediately after intake. It is also effective in upper respiratory infections and adenitis and Allergic Rhinitis. Calotropis gigantea and Piper nigrum are the main ingredients of the swasakudori tablet and Abies webbiana, an ingredient of the adjuvant.

Limonene , Piperic acid , Myricetin , Rutin, Calotropin and Abiesin are the phytochemicals extracted from the drug. Limonene markedly reduced the IL-5, IL-13, eotaxin, MCP-1, and TGF-β_1 levels in bronchoalveolar lavage fluid of *Dermatophagoides farinae*-induced airway hyper responsiveness animal model(Ryoji Hirota2012). Piperic acid has best anticholinergic action in Bronchospasm. (Vani Mamillapalli et al .,2019) . Myricetin is very effective in treating neonatal asthma.(Min Chen et al., 2020). Rutin may be effective in reducing the symptoms of asthma by inhibiting histamine release.(Jung, C.H et al., 2007).In this study, we aimed to prove the efficacy and Interpretation of the phytochemicals from these ingredients with the target Human histamine receptor (3RZE) inhibitor activity through molecular docking. Docking studies pave the way for establishing the efficacy of the drugs with the help of advanced software and technology by justifying the potency of the herbs being studied based on the binding of phytochemicals to targets present in proteins.

2.AIM & OBJECTIVE:

To establish the promising Bronchodilator activity of the selected ligands present in *the formulation swasakudori* targeting Human histamine receptors of smooth muscles of the airway. Binding of these ligands with the core amino acid (428 TRP)of the target by forming hydrogen bonds will delay the role of the Human histamine receptor with PDB – 3RZE These amino acid residues are functionally responsible for the binding of substrate and inhibitors.

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3.Materials and Methods:

3.1 Preparation of Target:

The perfect structure of the target protein Human histamine receptor was obtained from protein data bank after the protein cleanup procedure was finished. The Autodock program evaluated various lead molecule orientations in relation to the target protein, and the best dock pose was chosen based on the interaction study analysi

Table.1 Name of the Target:

PDB	Name of the Target
3RZE	Human histamineH1 receptor

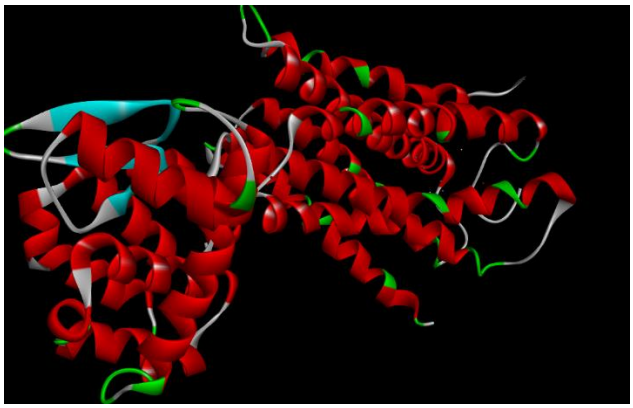
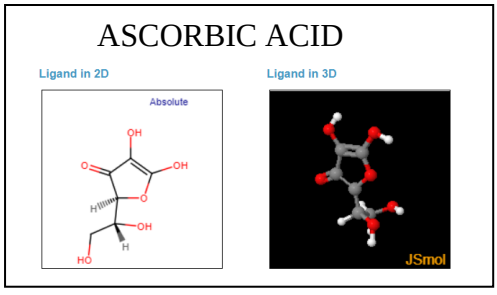
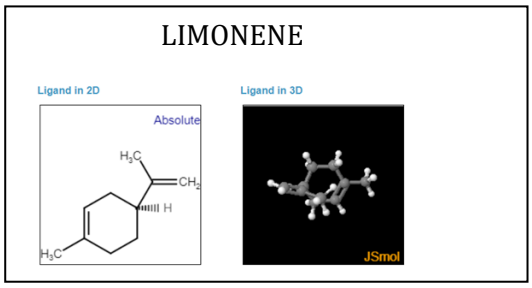


Fig . 1 3D- Structure of the histamine H1 receptor (PDB) - 3RZE

3.2 Preparation of Ligand:

The 3 D structure of Limonene , Piperic acid , Myricetin , Rutin , Calotropin and Abiesin were obtained . Hydrogen was added to the ligands .





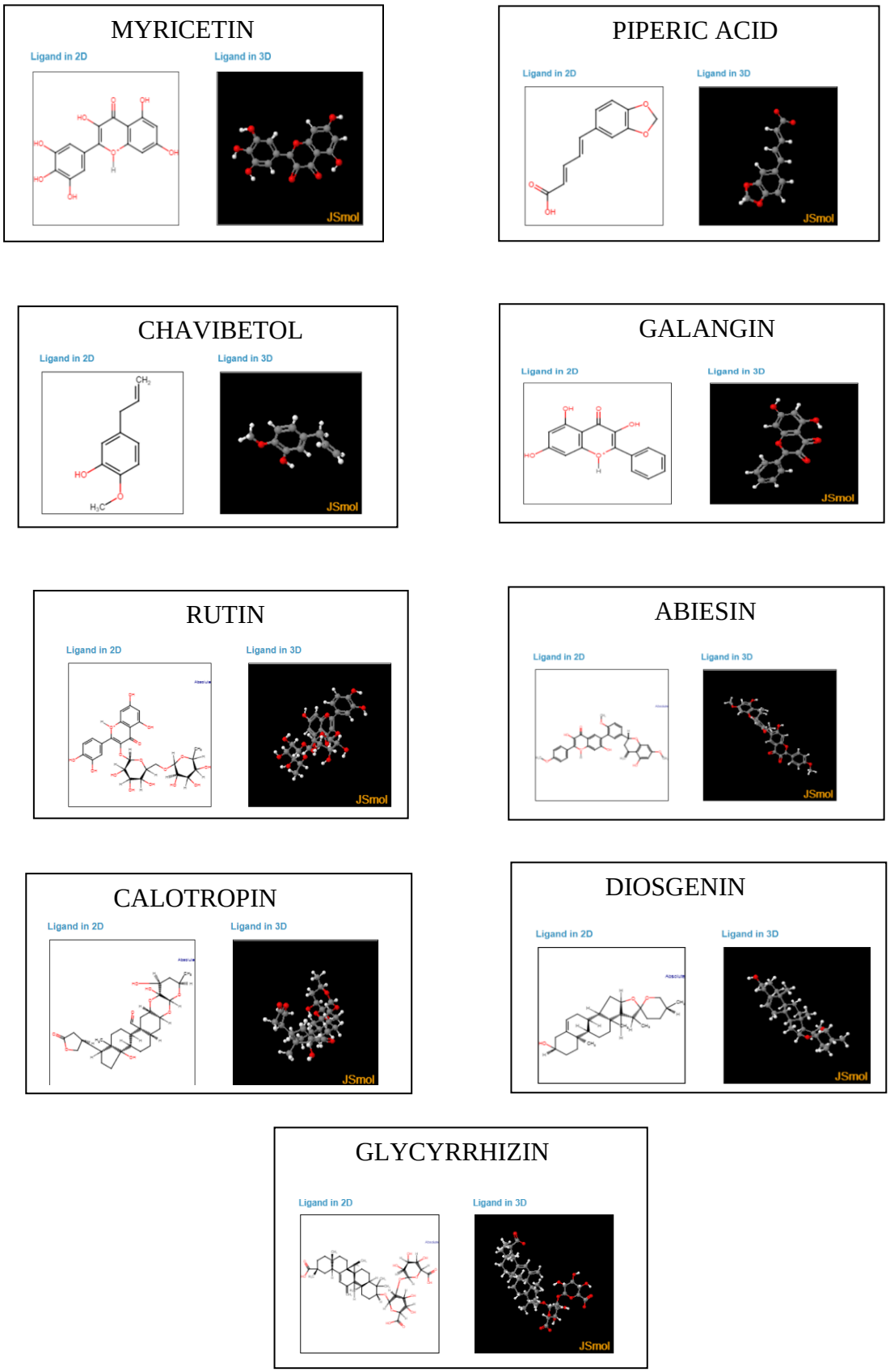


Fig.2: 2D & 3D Structure of Ligands Retrieved

3.3 Docking:

Docking calculations were performed for the recovered phytocomponents against the Phospholipases A2 target enzyme. Using AutoDock tools, necessary hydrogen atoms, Kollman united atom type charges, and solvation parameters were added (12). Using the Autogrid program, affinity (grid) maps with grid points and a spacing of 0.375 were created. The van der Waals and electrostatic terms were computed using AutoDock parameter set- and distance-dependent dielectric functions. The Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method were used to simulate docking (13). The ligand molecules' initial positions, orientations, and torsion were all set at random . All rotatable torsions were freed after docking. Each docking experiment was constructed from two separate runs, each of which was programmed to end after a maximum of 250000 energy assessments. The population was limited to 150 people. A translational step of 0.2, a quaternion step of 5, and a torsion step of 5 were all used during the search.

Results and discussion:

Based on the docking score(Nugroho AE et al., 2013), Ascorbic acid , Limonene , Chavibetol, Galangin, Rutin, Abiesin, Calotropin, Diosgenin, Glycyrrhizin, Myricetin, and Piperic acid were docked onto the histamine H1 receptor

Table .2: Summary of the molecular docking studies of compounds against histamine H1 receptor (PDB) - 3RZE

Compound	**Est. Free Energy of Binding** (kcal/mol)	**Est. Inhibition Constant, Ki(*** uM)(** nM)	**Electrostatic Energy** (kcal/mol)	**Total Intermolec. Energy (**kcal/mol)	**Interact. Surface**
Ascorbic acid	-6.31	23.85 *	-0.30	-5.60	467.447
Limonene	-6.22	27.78*	-0.00	-6.51	448.247
Chavibetol	-5.86	50.45 *	-0.01	-6.26	500.131
Galangin	-7.07	6.61 *	-0.03	-7.13	675.9
Rutin	-5.71	65.75 *	-0.18	-4.63	455.661
Abiesin	-6.83	9.87*	-0.52	-6.92	664.664
Calotropin	-6.98	7.64*	-1.41	-7.13	770.017
Diosgenin	-7.01	7.31 *	-0.05	-7.31	931.244

Glycyrrhizin	-7.24	4.96*	-0.78	-8.09	641.983
Myricetin	-8.38	721.09 **	-1.20	-7.89	699.278
Piperic acid	-7.58	2.76 *	-1.36	-8.47	568.961

Table . 3: Amino acid Residue Interaction of Lead against histamine H1 receptor (PDB) - 3RZE

Compounds	**Interactions**	**Amino acid residues**																
Ascorbic acid	1	108 TYR	111 SER	112 THR	115 ILE	199 PHE	424 PHE	428 TRP	432 PHE									
Limonene	1	111 SER	112 THR	115 ILE	158 TRP	198 ASN	199 PHE	424 PHE	428 TRP	432 PHE								
Chavibetol	1	111 SER	112 THR	115 ILE	158 TRP	198 ASN	199 PHE	424 PHE	428 TRP	432 PHE								
Galangin	1	107 ASP	108 TYR	111 SER	112 THR	158 TRP	194 THR	195 ALA	198 ASN	428 TRP	431 TYR	432 PHE	545 ILE					
Rutin	1	76 VAL	107 ASP	108 TYR	110 ALA	111 SER	112 THR	158 THR	179 LYS	194 THR	195 ALA	198 ASN	428 TRP	431 TYR				
Abiesin	1	84 ASN	87 TYR	103 TRP	107 ASP	108 TYR	111 SER	114 SER	115 ILE	178 ASP	179 LYS	191 LYS	198 ASN	199 PHE	424 PHE	428 TRP	431 TYR	
Calotropin	1	84 A	107	108	111	112	115	178	179	198	428	431	432					

		SN	ASP	TYR	SER	THR	ILE	ASP	LYS	ASN	TRP	TYR	PHE					
Diosgenin	1	84 ASN	103 TRP	107 ASP	108 TYR	111 SER	112 THR	115 ILE	179 LYS	198 ASN	428 TRP	431 TYR	432 PHE					
Glycyrrhizin	1	84 ASN	87 TYR	103 TRP	107 ASP	108 TYR	111 SER	112 THR	158 THR	178 ASP	179 LYS	191 LYS	194 THR	195 ALA	198 ASN	199 PHE	428 TRP	431 TYR
Myricetin	1	107 ASP	108 TYR	111 SER	112 THR	179 LYS	198 ASN	428 TRP	431 TYR	432 PHE	435 PHE	458 TYR						
Piperic acid	1	108 TYR	111 SER	112 THR	115 ILE	158 TRP	184 PHE	191 LYS	194 THR	198 ASN	424 PHE	428 TRP	432 PHE					

From the results it is evidenced that the estimated free energy of binding are Ascorbic acid (6.31 kcal/mol) , Limonene(-6.22 kcal/mol) , Chavibetol(-5.86 kcal/mol), Galangin(-7.07 kcal/mol) , Rutin (-5.71 kcal/mol), Abiesin(-6.83 kcal/mol) , Calotropin(-6.98 kcal/mol) , Diosgenin(-7.01) , Glycyrrhizin(-7.24 kcal/mol) , Myricetin(-8.38 kcal/mol) , and Piperic acid(-7.58 kcal/mol)Out of all Myricetin which is a compound retrieved from Abies spectabilis shows high affinity against the target.

Total number of hydrogen bonds present are Ascorbic acid(10), Limonene(0), Chavibetol(3), Galangin(8), Rutin(26), Abiesin(13), Calotropin(14), Diosgenin(4), Glycyrrhizin(24), Myricetin(14) and Piperic acid(5).Total number of rotatable bonds Ascorbic acid(2), Limonene(1), Chavibetol(3), Galangin(1), Rutin(6), Abiesin(6), Calotropin(2), Diosgenin(0), Glycyrrhizin(7), Myricetin(1) and Piperic acid(3)

GCMS analysis of Swasakudori tablets suggested that it possess effective anti-oxidant and anti-inflammatory activity (M. Ram Krishna Rao et al.,2016),Numerous experimental studies demonstrated that ascorbic acid has a relaxing impact on Tracheal smooth muscle that is mediated by a number of different mechanisms, including calcium channel blocking, stimulation of muscarinic and histamine H1 receptors, inhibition of 2-adrenoceptors, and inhibition of muscarinic receptors(Ghalibaf et al.,2023). Limonene, is one of the primary flavonoids, thought to reduce the generation of reactive

oxygen species, so lowering the inflammatory response. Limonene has a potential to reduce the airway inflammation by decreasing the levels of IL-5, IL-13, eotaxin, MCP-1, and TGF-β_1 in bronchoalveolar lavage fluid.(hirota R et al.,2012). Diosgenin is a naturally occurring saponin present in many herbal plants known to reduce allergy induced

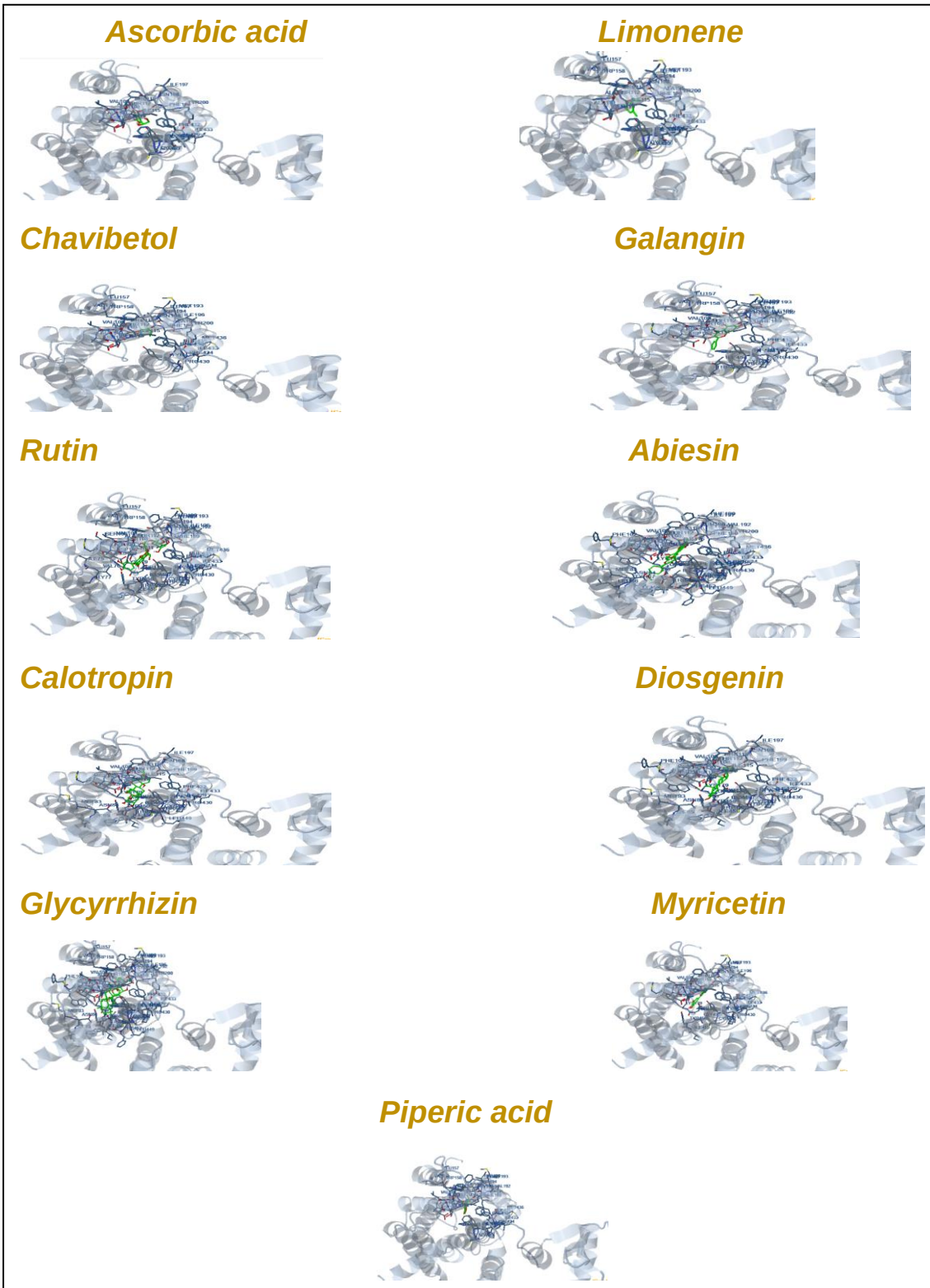


Fig.3: Docking pose with histamine H1 receptor (PDB) - 3RZE

intestinal inflammations and asthma(Junchao Y et al., 2017).

Rutin inhibits the release of histamine, Phospholipase A_2 PLA_2, and reduced recruitment of neutrophils and eosinophils into the lung thus has very useful bronchodialator action (Jung, C.H et al., 2007). Chavibetol(Sushma Pawar et al , 2022), Galangin(Hui-Hun Kim et al., 2015), Calotropin(Madhuri Kadiyala et al., 2013), Myricetin(Thanh Sang Vo et al 2020) and Piperic acid(Vani Mamillapalli et al., 2019) suppress of chemokine productions and reduce the endothelial vascular permeability thus down-regulate histamine-induced inflammatory reactions.

Conclusion:

Based on the results of the computational analysis it was concluded that the bio-active compound's like Ascorbic acid, Limonene, Chavibetol, Galangin, Rutin, Abiesin, Calotropin, Diosgenin, Glycyrrhizin, Myricetin and Piperic acid present in the herbal ingredients of the siddha formulation possess significant binding against the target histamine H1 receptor by interacting with active amino acid present on the active site . Thus it was concluded that Siddha herbal formulation Swasakudori may exert promising anti-allergic and anti-inflammatory activity by inhibition of histamine production .

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