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Molecular docking analysis of selected bioactive components of *Glycyrrhiza glabra* against bronchial asthma

Research Article

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Abstract

Bronchial asthma is a chronic inflammatory disease of the lung by the combined action of various cytokines. As a result of the strong inflammatory response increased infiltration of cytokines result, damages respiratory epithelium, Hyperplasia of the trachealis muscle, and increased mucous production. *Athimathura choornam* is a promising drug used in all inflammatory conditions. Objective: To explore the efficacy of the Siddha formulation *Athimathura choornam*, an anti asthmatic drug using computational molecular docking analysis. Method: Based on the phytochemical study the active principles present in the plant *Athimathuram* were retrieved. 3D structure of the targets were retrieved from the repository and purified before the initiation of docking using the software. The potency of the drug was screened based on the binding of the ligands Asparagine, Liquiritin, and Glabridin with targets mentioned. These results were compared with the standard drugs such as Cetirizine, Salicylic acid, Diclofenac, Ibuprofen, and Celecoxib. Results and Conclusion: Liquiritin has 9 interactions (90%) similar to that of Ibuprofen, 14 interactions (90%) similar to that of Citrazinehence , 5 interactions (100%) similar to that of salicylic acid hence it has promising COX 1 inhibition ,Histamine 1 blocking activity and Prostaglandin Synthase inhibition activity. Asparagine has 3 interactions (60%) similar to Celecoxib, has promising COX 2 inhibition activity. Glabridin has 2 interactions (50%) similar to that of Diclofenac hence it has promising IL6 inhibition activity.

Key Words: *Bronchial asthma, Athimathura choornam,* Histamine 1 receptor, Prostaglandin H2 synthases, TNF alpha, IL6, COX1 and COX 2.

Introduction

Pathophysiologically, Bronchial asthma is a heterogenous and complex chronic inflammatory disorder of the lungs in which various cytokines coordinate inflammation. Major symptoms seen are epithelial disruption, airway smooth muscle hypertrophy and hyperplasia, increased mucus secretion, thickening of Basement membrane, increased cytokine production, and chronic, infiltration of inflammatory cells (1)(2) . Inhalation of corticosteroids is considered the most effective drug in controlling Bronchial asthma. Following are some of the important factors that may trigger the symptoms of asthma namely, allergens, exercise, cold exposure, chemical sensitizers, air pollutants, and respiratory viral infections (3).

The inflammatory infiltrate in asthma is multicellular in nature and characteristically involves T cells, eosinophils, macrophages, monocytes, mast cells, and Neutrophils (4). Every cytokine may have overlapping cell regulatory action and function through complex cytokine networks. It is perceived as a T helper cell type 2 (Th2) disease with a cytokine profile that is characterized by interleukin 4 (IL-4), IL-5, and IL-13. Tumor necrosis factor (TNF)-alpha is a Th1 cytokine, which has been concerned in asthmatic airway inflammation in vitro and in vivo studies (5). Mast cells perform a life-threatening part in the pathogenesis of allergic asthma. Mast cells secrete and release histamine in response to allergic reactions. Histamine plays a role in airway obstruction via smooth muscle contraction (6), producing leakage in the microcirculation of lungs (7), bronchial secretion, and airway mucosal edema (8). . Prostaglandins are synthesized from the metabolism of Arachidonic acid. Prostaglandin E2 /H2 shows high immunoreactivity in case of allergic asthma (9). . Cox 1 and Cox 2 enzymes seem to be increased as a result of inflammation of respiratory epithelium as seen in Bronchial asthma. They contribute to the formation of excess synthesis of prostaglandins in asthma (10). Any anti-asthmatic drug must possess anti-inflammatory action so as not to trigger the occurrence of asthma by inhibiting the release of cytokines IL-2, IL-4, and IL-6, the movement of leukocytes(11).

The (*Athimathuram*) *Glycyrrhiza glabra* or licorice powder and its extract are extremely useful in treating sore throat, cough, and bronchial phlegm

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(12).Licorice root contains various sugars (up to 18%), flavonoids, sterols, amino 36 acids, gum, starch, essential oils, and saponins. *Athimathura chooranam* is a familiar drug used in the Siddha system consisting of *Glycyrrhiza glabra* as the key ingredient possesses anti-allergic, antioxidant, and blood purifier properties and is used for many indications such as skin fissures, veneral rashes, acute itching and insect bite (13). *Athimathura chooranam* is one of the important drugs to treat Bronchial Asthma (*Ilaippu Noi*) in Siddha system of medicine.

The human genome project has been completed and advanced protein purification, crystallography, and nuclear magnetic resonance spectroscopy techniques have been advanced(14). This results in the discovery of many structural details of proteins and protein-ligand complexes. The molecular docking approach can be used to assign the interaction between a small molecule and a protein at the atomic level, which permits us to describe the performance of small molecules in the binding position of target proteins as well as to clarify vital biochemical processes (15). Many biological reactions get activated by binding a small molecular ligand to a protein. Medicines exert their pharmacological reactions depending only upon their effective binding to their receptor's active site (16). The binding mode of ligands with their receptors is very important in designing the most efficient formulations. Molecular docking can therefore widely be used in the study of many Siddha herbal formulations to explain the principle of action of Siddha medicines. Based on this method we can justify the mechanism and efficacy of *Athimathura chooranam* compared with some standard drugs in treating Bronchial asthma.

Materials and Methods

Test compounds

Based on the literature survey, the test compounds selected for docking against the target protein model are Asparagine, Liquiritin, and Glabridin which are the potent bio active components of *Athimathura Choornam*

Receptors / Target protein Selected for Docking

Receptors used to predict activity as Anti-asthma are,

Table 1: Receptors / Target protein Selected for Docking

Name of the Protein	PDB code	Standard antagonist
Histamine 1 receptor	3RZE	Cetirizine
Prostaglandin H2 synthases	1igx	Salicylic acid
TNF alpha	2AZ5	Diclofenac
IL6 Interleukin	1P9M	Diclofenac
Cyclooxygenase I	3KK6	Ibuprofen
Cyclooxygenase 2	6COX	Celecoxib

Data of the 3D crystal receptor structures used for molecular docking analysis are obtained from the Protein Data Bank (PDB) obtained from the site http://www.rscb.org/pdb.

Methodology

Docking calculations were carried out using Auto Dock 4. Gasteiger partial charges were added to the ligand atoms. Non-polar hydrogen atoms were merged, and rotatable bonds were defined. Docking calculations were carried out for test drug **Asparagine, Liquiritin , Glabridin and standard Cetirizine, Salycilic acid, Diclofenac, Ibuprofen and Celecoxib against target protein** model. Essential hydrogen atoms, Kollman united atom type charges, and solvation parameters were added with the aid of AutoDock tools (17). Affinity (grid) maps of ×× Å grid points and 0.375 Å spacing were generated using the Autogrid program (17). AutoDock parameter set- and distance-dependent dielectric functions were used in the calculation of the van der Waals and the electrostatic terms, respectively. Docking simulations were performed using the Lamarckian genetic algorithm (LGA) and the Solis & Wets local search method (18). Initial position, orientation, and torsions of the ligand molecules were set randomly. All rotatable torsions were released during docking. Each docking experiment was derived from 2 different runs that were set to terminate after a maximum of 250000 energy evaluations. The population size was set to 150. During the search, a translational step of 0.2 Å, and quaternion and torsion steps of 5 were applied.

Table 2: Ligands selected from the Test compound and it's 2D and 3D structures

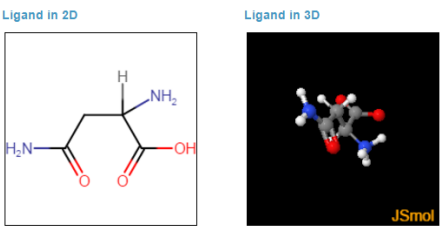
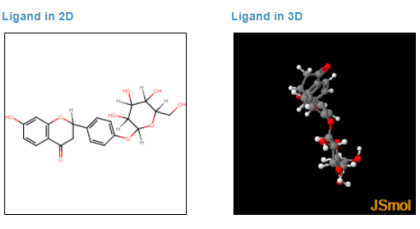
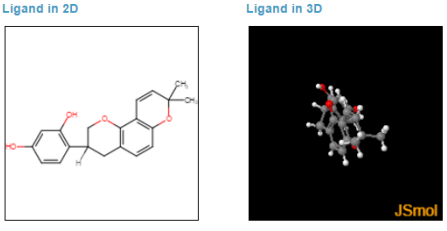
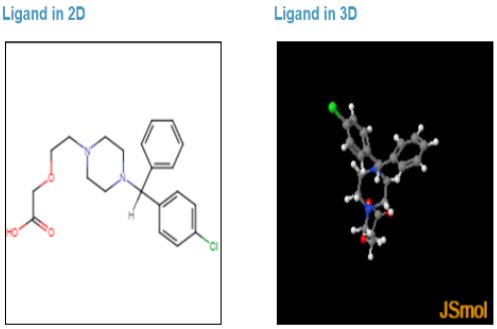
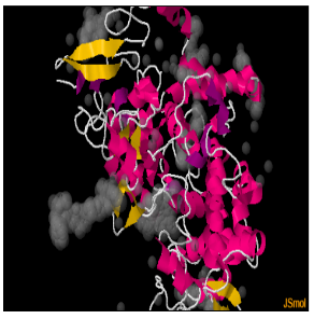
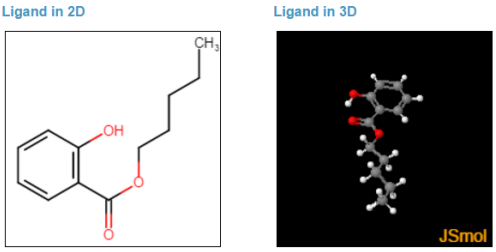
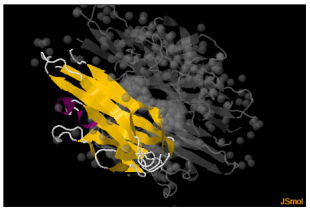
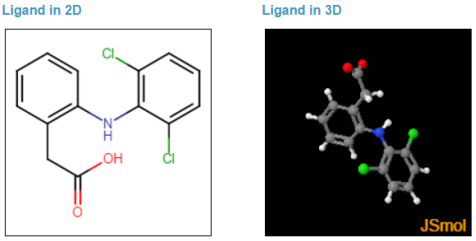
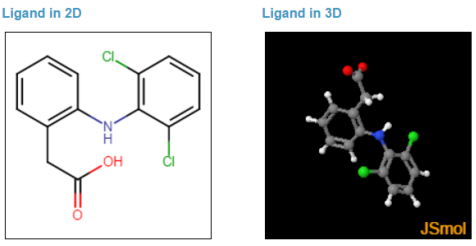
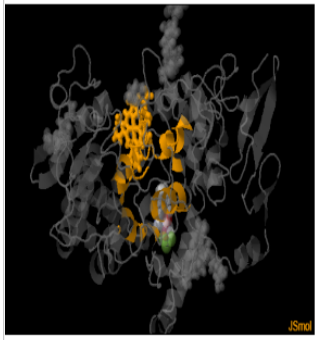
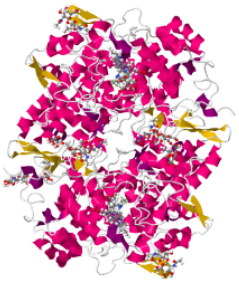
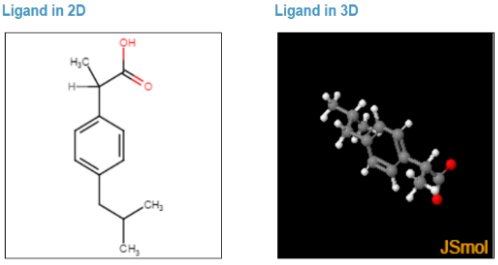
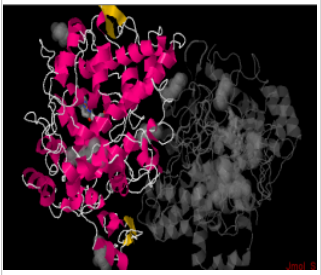
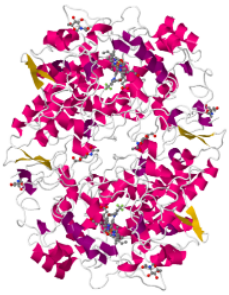
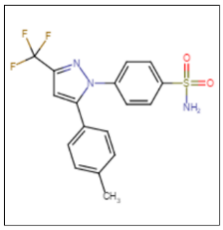
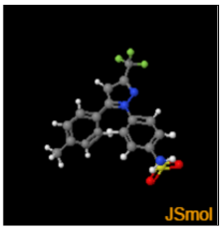
Asparagine	Liquiritin	Glabridin
		

Table 3: 2D and 3D structures of the target protein Selected for Docking and standard antagonist:

Target protein	3D structure	2D Structure	Standard antagonist 3D and 2D structures
Histamine 1 receptor-3RZE			 Cetrizine
Prostaglandin H2 synthases - 1igx			 Salicylic acid
TNF alpha -2AZ5			 Diclofenac sodium
IL6 Interleukin - 1P9M			 Diclofenac sodium
Cyclooxygenase 1 - 3KK6			 Ibuprofen

Cyclooxygenase 2 - 6COX				 Celecoxib

Results:

Table 4: Histamine 1 inhibition activity of Ligands of Test and Standard compounds

Rank	Amino Acid interaction	Compound	Amino Acid Sequence														
	15	Citrazine	84 ASN	103 TRP	107 ASP	108 TYR	111 SER	158 TRP	179 LYS	194 THR	424 PHE	428 TRP	431 TYR	432 PHE	435 PHE	454 ILE	458 TYR	
2	8	Asparagine	107 ASP	108 TYR	111 SER	179 LYS	428 TRP	431 TYR	435 PHE	458 TYR								
2	8	Glabridin	107 ASP	108 TYR	111 SER	112 THR	178 ASP	179 LYS	198 ASN	428 TRP	431 TYR	432 PHE	436 PHE	450 HIS	454 ILE			
1	14	Liquiritin	84 ASN	103 TRP	107 ASP	108 TYR	111 SER	112 THR	158 TRP	178 ASP	179 LYS	424 PHE	428 TRP	431 TYR	432 PHE	435 PHE	454 ILE	458 TYR

Table 5: Prostaglandin Synthase inhibition activity of Ligands of Test and Standard compounds

Rank	Amino Acid interaction	Compound	Amino Acid Sequence						
	5	Salicylic acid	35 PRO	38 TYR	40 PRO	54 ARG	55 TYR		
0	0	Asparagine	468 LYS	474 PRO	499 ASP				
2	3	Glabiridin	38 TYR	40 PRO	42 GLN	55 TYR	68 ASN	70 THR	468 LYS
1	5	Liquiritin	35 PRO	38 TYR	40 PRO	42 GLN	54 ARG	55 TYR	68 ASN

Table 6: TNF Alpha Receptor inhibition activity of Ligands of Test and Standard compounds

Rank	Amino Acid interaction	Compound	Amino Acid Sequence					
	5	Diclofenac	57 LEU	59 TYR	61 GLN	119 TYR	151 TYR	
1	4	Asparagine	59 TYR	60 SER	61 GLN	119 TYR	120 LEU	151 TYR
1	4	Glabridin	59 TYR	61 GLN	119 TYR	120 LEU	151 TYR	
1	4	Liquiritin	59 TYR	61 GLN	119 TYR	120 LEU	151 TYR	

Table 7: IL 6 inhibition activity of Ligands of Test and Standard compounds

Rank	Amino Acid interaction	Compound	Amino Acid Sequence																
	4	Diclofenac	66 LYS	168 ARG	169 SER	172 GLU													
2	1	Asparagine	63 ASN	64 LEU	66 LYS	86 LYS	93 GLU												
1	2	Glabridin	36 ILE	40 ARG	54 LYS	167 LEU	168 ARG	171 LYS	172 GLU										
2	1	Liquiritin	32 ILE	36 ILE	39 LEU	91 LEU	94 PHE	95 GLU	97 TYR	98 LEU	101 LEU	115 VAL	119 THR	122 LEU	123 ILE	166 ILE	167 LEU	170 PHE	174 LEU

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Table 8: Cox 1 Inhibition activity of Ligands of Test and Standard compound

			Cyclooxygenase 1 Receptor													
Rank	**Amino Acid interaction**	**Compound**	**Amino Acid Sequence**													
	10	**Ibuprofen**	205 PHE	209 PHE	348 TYR	352 LEU	381 PHE	385 TYR	387 TRP	518 PHE	530 SER	534 LEU					
3	**6**	**Asparagine**	205 PHE	209 PHE	228 VAL	344 VAL	375 ASN	377 ILE	381 PHE	385 TYR	530 SER	534 LEU					
2	**8**	**Glabridin**	205 PHE	209 PHE	228 VAL	344 VAL	348 TYR	349 VAL	352 LEU	381 PHE	385 TYR	527 ALA	530 SER	534 LEU			
1	**9**	**Liquiritin**	205 PHE	209 PHE	344 VAL	348 TYR	349 VAL	352 LEU	378 ASN	381 PHE	385 TYR	518 PHE	523 ILE	527 ALA	530 SER	531 LEU	534 LEU

Table 9 : Cox 2 Inhibition activity of Ligands of Test and Standard compounds

		Cyclooxygenase 2 Receptor					
Rank	**Amino Acid interaction**	**Compound**	**Amino Acid Sequence**				
	5	Celecoxib	54 GLN	55 TYR	56 LYS	57 CYS	67 GLU
1	3	Asparagine	53 ASP	54 GLN	55 TYR	67GLU	
3	1	Glabridin	37 CYS	38 SER	40 PRO	56 TYR	68 ASN
2	2	Liquiritin	55 TYR	67 GLU	68 ASN		

Out of three compounds Liquiritine has 14 interactions (90%) similar to that of the standard Cetirizine. similarly other compounds Asparagine and Glabridin has 8 interactions (53%) similar to that of the standard Cetirizine, hence all three compounds has promising Histamine 1 blocking activity.

Liquiritin has 5 interactions (100%) similar to that of the standard salicylic acid. similarly other compound Glabridin has 60% percentage similar interaction to that of the standard hence both compounds has Prostaglandin Synthase inhibition activity. Compound Asparagine has no Prostaglandin Synthase inhibition activity.

Glabridin has 2 interactions (50%) similar to that of the standard Diclofenac . similarly other compound Liquiritin and asparagine has 25% percentage similar interaction to that of the standard hence all three compounds has promising IL6 inhibition activity

Asparagine, glabridin and Liquiritin has 4 interactions (90%) similar to that of the standard Diclofenac hence all three compounds has promising TNF alpha inhibition activity.

Liquiritin has 9 interactions (90%) similar to that of the standard Ibuprofen . similarly other compound Glabridin has 80% percentage and compound asparagine has 60 % similar interaction to that of the standard hence all three compounds has promising COX 1 inhibition activity.

Asparagine has 3 interactions (60%) similar to that of the standard Celecoxib . similarly other compound Liquiritin has 40% percentage and compound glabridin has 20 % similar interaction to that of the standard hence all three compounds has promising COX 2 inhibition activity.

Discussion

Bronchial Asthma is the most prevalent chronic illness and is a serious non communicable disease (NCD) that affects both children and adults. Asthma symptoms, which can include any combination of coughing, wheezing, shortness of breath, and tightness in the chest, are brought on by inflammation and restriction of the tiny airways in the lungs (19). Studies reported that it has a significant anti-inflammatory activity and potential antioxidant activity (20,21).Compounds Asparagine, Liquiritin, and Glabridin were phytocompounds selected from *Athimathura chooranam* which possesses anti inflammatory , anti allergic and anti asthmatic , anti allergic and anti inflammatory, anti oxidant properties respectively were selected as a Ligand for docking study (22,23,24). Target proteins selected for the docking study were Histamine 1 receptor, Prostaglandin H2 synthases, TNF alpha, IL6 Interleukin, Cyclooxygenase I and Cyclooxygenase 2. And their respective antagonists Cetirizine, Salicylic acid , Diclofenac, Ibuprofen and Celecoxib were selected as a standard for this study.

Variability in the pattern of inflammation is seen in every case of Bronchial asthma, thus indicating phenotypic differences that may influence treatment responses. It has been demonstrated that H1-antihistamines can reduce NF-κB expression and some inflammatory reactions in connected cells (25).

There may be an imbalance in vascular diseases where PGHS-dependent vasoconstrictors predominate. Precise functions for PGHS-1 and PGHS-2 in regulating vascular function are still developing, When thinking about the use of particular PGHS-2 inhibitors for the treatment of a number of chronic disorders, such as inflammatory diseases and cancer (26).

A key player in the pathophysiology of various inflammatory diseases is tumour necrosis factor (TNF). Intracellularly, TNF is produced, primarily by activated macrophages. After proteolysis, the TNF-converting enzyme transforms the precursor TNF into soluble TNF. The physiologically active homotrimer TNF is subsequently created when this soluble TNF oligomerizes. TNF-alpha and TNF-beta are two distinct but closely related forms of TNF. Both TNFs work by binding to their respective TNF receptors I and II (TNFRI and TNFRII), which are found on practically all cell types (except erythrocytes) (27,28, 29).

Depending on the immune response environment, research have demonstrated that interleukin 6 (IL-6) is a multifunctional cytokine with both pro-inflammatory and anti-inflammatory activity (30).

The enzymes that produce prostaglandins are called cyclooxygenase (COX). There are two types of COX enzymes, COX-1 and COX-2. Both enzymes produce prostaglandins that promote inflammation, pain, and fever. The enzyme cyclooxygenase (COX) is responsible for producing the prostaglandins, which causes inflammation (31).

This study reveals that anti-inflammatory, anti-allergic and anti-oxidant activity plays an important role in the treatment of Bronchial Asthma. From the bioactive compounds derived from *Athimathura chooranam* ,Liquiritine has excellent Histamine 1 blocking activity similar to that of cetirizine. It has excellent Prostaglandin Synthase inhibition activity similar to that of standard salicylic acid and it has a promising COX 1 inhibition activity similar to that of standard Ibuprofen. Other compounds are also having the activity but less interactions when compared to Liquiritine. Glabridin has promising IL6 inhibition activity similar to that of standard Diclofenac. Other compounds are also having the activity but less interactions when compared to Glabridin. Asparagine has promising COX 2 inhibition activity similar to that of standard Celecoxib. Other compounds are also having the activity but less interactions when compared to Asparagine. Asparagine, glabridin and Liquiritin has promising TNF alpha inhibition activity similar to that of standard Diclofenac. Hence all three compounds has promising effect Histamine 1 blocking activity, Prostaglandin Synthase inhibition activity , IL6 inhibition activity, TNF alpha inhibition activity, COX 1 and COX 2 inhibition activity. Among the three compounds Asparagine has no Prostaglandin Synthase inhibition activity. So *Athimathura chooranam* has a potent effect against Bronchial Asthma.

Conclusion

Based on the results of the computational analysis the compounds such as Asparagine, Liquiritin, and Glabridin present in the formulation *Athimathura Chooranam* significantly binding with a target proteins similar to that of standard proteins. Hence the bio active compounds possess significant inhibition of COX 1& 2, Prostaglandin synthases, Histamine 1, TNF alpha and IL 6 inhibition activity where it was concluded that this formulation may have promising against bronchial asthma.

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