Identification of *Curcuma longa* phytochemicals as a novel inhibitor of proteins involved in Allergic Rhinitis

Project report submitted to the Central University of Punjab

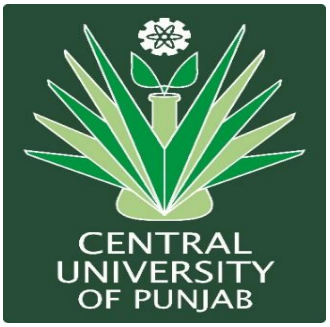
For the award of
M.Sc. life sciences (Biochemistry)
In
Biochemistry and microbial sciences
By

SWAGATA DAS

(Registration No.-16mslsbc13)

Under the Supervision of

Dr. Shashank K.



Department of Biochemistry and Microbial Sciences
School of Basic and Applied Sciences
Central University of Punjab
Bathinda, 151001

May, 2018

CERTIFICATE

I declare that the project entitled "**Identification of *Curcuma longa* phytochemicals as a novel inhibitor of proteins involved in Allergic Rhinitis*"***, has been prepared by me under the guidance of Dr. Shashank Kumar, Assistant Professor, Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab. No part of project has formed the basis for the award of any degree or fellowship previously.

Swagata Das
M.Sc. Life Sciences with specialization in Biochemistry,
Department of Biochemistry and Microbial Sciences,
School of Basic and Applied Sciences,
Central University of Punjab,
Bathinda-151001
Date-

CERTIFICATE

I certify that Swagata Das has prepared her Project entitled "**Identification of *Curcuma longa* phytochemicals as a novel inhibitor of proteins involved in Allergic Rhinitis**", for the award of M.Sc. Life Sciences with specialization in Biochemistry degree of Central University of Punjab, under my guidance. She carried out this work at the Department of Biochemistry and Microbial Sciences, School of Basic and Applied Sciences, Central University of Punjab.

(SHASHANK KUMAR)
M.Sc. Life Sciences with specialization in Biochemistry,
Department of Biochemistry and Microbial Sciences,
School of Basic and Applied Sciences,
Central University of Punjab,
Bathinda-151001
Date-

ABSTRACT

Identification of *Curcuma longa* phytochemicals as a novel inhibitor of proteins involved in Allergic Rhinitis

Name of the student	: Swagata Das
Registration Number	: 16mslsbc13
Degree for which submitted	: Master of Science
Supervisor	: Dr. Shashank K.
Department	: Biochemistry and Microbial Sciences
School	: Basic and Applied Sciences

Keywords: Anti-inflammatory potential, Ayurvedic product, *In silico*, Target proteins, Lead compounds, *In vitro*

Histamine and other chemical mediators play crucial role in the disease development condition of allergic rhinitis. Allergic rhinitis is a mild or severe allergic condition due to the interaction of allergens with the IgE antibodies leading to release of chemical mediators like histamine, leukotrienes, prostaglandins etc. Various drug therapies have been elucidated based on the target proteins or enzymes involved. Certain specific proteins like histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y) alongwith non specific proteins like adenylate kinase (2c9y), phospholipase C (4qj4) are mainly targeted. Commonly prescribed drugs are antihistamines and leukotriene receptor antagonists, which generally reduces the symptoms occurring due to the release of the chemical mediators. Yet, there are persistent and prevalent conditions whereby the release and accumulation of histamine is misinterpreted as allergy instead of a totally different condition called histamine intolerance resulting in histamine accumulation due to defected or mutated enzymes related in its metabolism. Now day's natural products are popular remedies against a number of diseases and allergic rhinitis is no exception. These products have been significantly reported due to the low/non-toxicity and cost effectiveness. Curcuma longa or turmeric is a common medicinal herb with

enormous medicinal properties including anti-inflammatory properties. Various phytoconstituents of turmeric were identified and considered for receptor-based molecular docking. The target proteins and their interactions with each of phytoconstituent present in turmeric were studied.

.

Swagata Das Dr. Shashank Kumar

ACKNOWLEDGEMENT

First and foremost I extend gratitude to my revered seminar advisor Dr. Shashank K., Assistant Professor, Department of Biochemistry and Microbial Sciences, for instilling in me the confidence and drive for doing an arduous task and having faith in my abilities to accomplish any assignment which I set out to do. I thank him also for being a guardian, a friend as well as an erudite scientist to troubleshoot the problems encountered during the scientific report presentation preparation.

I wish to thank Mr. Prem Prakash Kushwaha (Ph.D. scholar) and my classmates for their kind cooperation and support.

At last, I am thankful to all people who have helped and inspired me during my seminar preparation and wishing for my bright future.

Swagata Das

Date-

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Sr. No	Abbreviations	Expansion
1	ADT	Auto Dock Tool
2	AR	Allergic rhinitis
3	ARIA	Allergic rhinitis and its impact on asthma guidelines
4	BBB	Blood Brain Barrier
5	BLAST	Basic Local Allignment Search Tool
6	CADD	Computer-Aided Drug Design
7	cAMP	cyclic AMP
8	CD4+	Cluster of Differentiation
9	CNS	Central Nervous System
10	COX	Cyclooxygenase
11	DAG	diacylglycerol
12	DAO	diamine oxidase
13	DMSO	Dimethyl sulfoxide
14	DNA	Deoxyribonucleic acid
15	DPPH	2,2-Diphenyl-1-Picryl Hydrazyl
16	FASTA	Fast Adaptive Shrinkage Thresholding Algorithm
17	FcεRI	Fc region of immunoglobulin E
18	GLIDE	Grid-Based Ligand Docking with Energetic
19	GM-CSF	Granulocyte Macrophage Colony stimulating Factor
20	GRADE	Grading of Recommendation, Assessment, Development and Evaluation
21	HDC	Histidine decarboxylase
22	HME	Histidine methyl ester
23	HMT	Histamine methyltransferase
24	I	Inhibitor
25	IgE	Immunoglobulin E
26	IL	Interleukins
27	IP3	Inositol triphosphate
28	LB	Luria-Bertani
29	LOX	Lipooxygenase
30	LTRA	Leukotriene receptor antagonists
31	MCP-4	Monocyte Chemoattractant Protein-4
32	MR	Mixed rhinitis
33	MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)
34	NAR	Non allergic rhinitis
35	NCBI	National Center for Biotechnology Information

36	PC	Phosphatidylcholine
37	PDB	Protein Data Bank
38	PE	Phosphatidylethanolamine
40	PI3K	Phosphatidylinositol 3-Kinase
41	PIP2	Phosphatidylinositol-4,5 bisphosphate
42	PKC	Protein kinase C
43	PMTI	Phospholipid methyl transferase I
44	PMTII	Phospholipid methyl transferase II
45	PS	Phosphatidylserine
46	RANTES	Regulated on activation, normal T-cell expressed and secreted
47	RAST	Radioallergosorbent test
48	RCSB	Research Collaboratory for Structural Bioinformatics
49	ROS	Reactive Oxygen Species
50	SD	Standard Deviation
51	Th2	T helper cells
52	WHO	World Health Organization
53	ZOI	Zone of inhibition

1. Introduction

Ayurveda science is one of the oldest medical systems available in the world. As per the study, approximately more than 25% of modern medicines are obtained from natural sources. Natural products are molecules produced by plants or microorganisms. These are organic substances having small molecular weight. The past has been evident for the fact that natural products are pharmacologically important compounds that are usually taken as home remedies in treating various types of diseases ranging from common cold to life-threatening cancer (Patel, 2012). In this view, herbal products may do a lot. A number of traditional herbal medical practices have been accepted for the prevention, diagnosis, and treatment of numerous diseases such as diabetes, cancer, neurological disorders and cardiac dysfunction, etc. The advantage of these medicinal plants is hundred percent natural.

Plant-derived products were majorly used as foods or botanical potions and extracted powders which have been used successfully in cure and prevention of diseases throughout history. There has been an exponential growth in the field of herbal medicine in the last few years because of their fewer side effects and natural origin. The World Health Organization (WHO) has listed about 21,000 plants, which are used for medicinal purposes around the world (Kumar *et al.,* 2014). Natural products were screened as templates for structure optimization programs for designing novel drugs. In spite of the present day concern with synthetic chemistry for drug designing and manufacture, the role of plants in the treatment of disease and prevention is still enormous. Phytochemicals are bioactive metabolites present naturally in plants showing biological significance in plants, playing an essential role in the defence mechanism of plants by inhibiting or killing the interacting pathogen. A number of plants are known for their anti-inflammatory property including *Urtica dioica* or stinging nettle, pineapple plant that contains bromelain (a proteolytic enzyme) and many herbs and vegetables having the natural phytochemical quercitin (flavonoid). Moreover, certain other naturally derived components like N-acetylcysteine (a natural sulfur-containing amino acid derivative) and vitamin C had potential antihistamine and anti-inflammatory activities (Thornhill and Kelly, 2000).

The concept of natural products has its roots back in the 19th century. Common reported examples of drugs discovered initially this way is morphine (the active agent present in Opium) and digoxin, which is a heart stimulant originating from *Digitalis lanata (*Lahlou, 2013*).* Historically natural products have been a rich source of compounds that have a great importance in medicine, pharmacy and biology. A number of important new commercial drugs have been obtained from natural sources*.* Drugs of natural origin can be classified as original natural products, products derived semi-synthetically from natural products or synthetic products based on natural product model*.*

Allergic rhinitis (AR) commonly known as hay fever is a symptomatic disorder inducing an allergic response to specific allergens. It is a nose related disorder due to exposure to different types of allergens which may be seasonal or perennial. Seasonal allergens include pollens and moulds whereas perennial allergens include dust, mites, pests etc. It is a type of inflammation caused due to Ig-E mediated hypersensitivity reactions resulting in its four cardinal symptoms watery rhinorrhea (running nose), nasal obstruction, nasal itching and sneezing. Its prevalence is ever increasing throughout the globe. In the United States alone it is estimated to affect about 60 million people and among which 10-30% being adults and 40% being children (Min, 2010). In India itself, one out of six people suffers from AR.

The adequate treatment of allergic rhinitis is important due to its impact and perturbance in quality of life and its increased risk of asthma. The mast cells respond to the offending allergens which lead to inflammation which in turn is responsible for the release of chemical mediators including histamine, prostaglandins and leukotrienes. As a result, targeting these events of the hypersensitivity reactions became significant in treating allergic rhinitis. Many studies in AR and its possible therapeutics have been reported and ther0efore allergic rhinitis and its impact on asthma (ARIA) guidelines have been published (2001) and revised (2008). According to the revised ARIA guidelines, second generation H_1- antihistamines were preferred over the first generation H_1-antihistamines due to safety concern. Leukotriene

receptor antagonists (LTRA) also became commonly recommended drugs for the treatment of allergic rhinitis (Min, 2010). Histamines are responsible for symptoms like rhinorrhea, nasal itching and sneezing while leukotrienes increase resistance in nasal airway or nose blockade and vascular permeability. Among the most common drugs for the treatment of AR, fexofenadine, a second generation non-sedating H_1-antihistamine and montelukast, a leukotriene antagonist are popularly recommended (Walekar *et al.*, 2016). Although these drugs have higher effectiveness and lesser side effects, these seem to further decrease the quality of life. Due to this, it is important to search for natural or plant-derived alternatives for treatment of AR which are more effective and have lesser or no side effects. Although very less is known about the potent natural inhibitors of AR, some studies show that *Urtica dioica* or stinging nettle, bromelain (a proteolytic enzyme derived from the stem of pineapple plant), quercetin (a flavonoid found in many herbs and vegetables), N-acetylcysteine (a natural sulfur-containing amino acid derivative) and vitamin C had potential antihistamine and anti-inflammatory activities (Thornhill and Kelly, 2000). Similarly, *Curcuma longa* or turmeric also showed potential anti-inflammatory activity but its target sites of inhibition and the mechanism of action are still unknown and uncertain. In this study, we will emphasize on the anti-inflammatory and anti-allergic activities of *Curcuma longa* and its possible target sites for the treatment of allergic rhinitis.

Curcuma longa or turmeric is a commonly used curry spice as well as a traditional Chinese medicinal herb with a long history of anti-inflammatory, antioxidant, anti-carcinogenic, anticoagulant and antidiabetic activities. Its constituents mainly include three curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), volatile oils (natlantone, tumerone and zingiberone), proteins, sugar and resins (He *et al.*, 2015). Among these, the curcuminoids including curcumin and its derivatives are the major bioactive compounds. Curcumin, a pleiotropic molecule has the capacity of interacting with numerous molecular targets involved in inflammation. Thus curcumin plays a major role in the anti-inflammatory action of *Curcuma longa* (Jurenka, 2009). Thus, it is important to relate the role of curcumin and its anti-inflammatory activity for the possible treatment of common allergic diseases like allergic rhinitis.

1.1 Hypothesis

Various *in vitro* and *in vivo* studies are going on and a lot must be carried out in order to explore its absolute efficacy or medicinal potential. The present study has been designed to identify literature based active compounds in *C. longa* and validate it scientifically through *in silico* and *in vitro* studies for exploring the anti-inflammatory potential of turmeric for its therapeutic usage against allergic disorders or histamine intolerance.

1.2 Objective

To identify literature based active compounds present in *C. longa* as well as its scientific validation as a potential drug against allergic rhinitis through *in silico* and *in vitro* studies.

2. Review of literature

2.1 Allergic, non-allergic and mixed rhinitis

Allergic rhinitis (AR) which is widely recognized as hay fever is a symptomatic disorder inducing an allergic response to specific allergens. It was demarcated by symptoms including nasal blockage or congestion, sneezing, itching of the conjunctiva, rhinorrhea, lacrimation or teary eyes, pruritis of oropharynx and nasal mucosa, allergic shiners and fatigue. A person with an ancestry of the disease or the similar symptoms or with a personal history of diseases like asthma or eczematous dermatitis is most likely of having this disease. Although it may also have a genetic cause as recent researches and studies identified the gene FAM134B encoding a reticulophagy receptor protein having a role-play in the disease (Islam *et al*., 2017). Based on the types of allergens, AR can be classified as seasonal, occupational or perennial. Seasonal allergens mainly include pollens and moulds whereas perennial allergens include dust, mites, pests etc. Environmental pollution and diverse pollutants may also be a serious concern for the incidence of allergic rhinitis. Classification of AR is also based on whether the symptoms are intermittent or persistent, with categories of mild, and moderate to severe based on the guidelines of allergic rhinitis and its impact on asthma (ARIA). Its prevalence is ever increasing throughout the globe. About 60 million people are affected by it, of which 1-40% was adults and 2-25% was children (Brozek *et al*., 2017).

On the other hand, non-allergic rhinitis (NAR) is another symptomatic disorder but without a cause. It mainly involves sneezing, drippy nose, congestion and other symptoms similar to allergic rhinitis but without any apparent cause as any such allergic reaction is prominently observed or evident. But certain factors are known to trigger non-allergic rhinitis proving chronic or transient symptoms. These factors mainly include environmental, weather, occupational, certain medications or food and beverages and infections. Similarly, mixed rhinitis (MR) is a condition with combinable symptoms of allergic plus non-allergic rhinitis. Sometimes mixed rhinitis may be more common than allergic and non-allergic rhinitis. Sometimes MR is considered to have occurred when people having allergic rhinitis show its different symptoms of being

exposed to some environmental or other related irritants, strong odours and even smokes (Sin and Togias, 2011).

2.2 Allergic rhinitis is a type I hypersensitivity

Our body's incitive response for the offending allergen results in inflammation which depends upon a series of events for its initiation. These complex sequences of events include the release of certain chemical mediators which play a major role in the initiation of inflammatory responses in our body. Inflammation due to allergic reactions is mainly characterized by the mast cell activation which is Ig-E dependent along with the activation of CD4+ Th2 lymphocytes and the simultaneous influx of eosinophils. Allergic rhinitis also triggers an immune response particularly a type I hypersensitivity reaction (Min, 2010).

Type I reactions of hypersensitivity are mainly characterized by the release of chemical mediators during the mast cell or basophil degranulation which in turn acts on secondary effector immune cells like eosinophils, monocytes, T lymphocytes, neutrophils and platelets. These chemical mediators are classified as primary and secondary mediators. The primary mediators which are stored in the granules located in the cytoplasm are synthesized before the mast cell or basophil degranulation. These mediators mainly include histamine, neutrophil chemotactic factor, eosinophils chemotactic factor and heparin. On the other hand, secondary mediators like leukotrienes, cytokines, prostaglandins, platelet-activating factor and bradykinins are produced during degranulation process with their release on significant phospholipid breakdown of the biological membranes or after the activation of the target cell (White, 1990).

Histamine, which is a primary chemical mediator, released by a number of cells primarily acts in response to tissue damage by binding to nearby receptors inducing permeability of vessels and vasodilation. The presence of histamine in the mast cell granules along with other mediators which are pharmacologically active are generally noted. Several cytokines including GM-CSF, IL-6 and IL-2 along with others like IL-4, IL-5, IL-9, IL-13 etc are involved in the degranulation of basophil and proliferation of eosinophil along with the significant release of histamine (Bousquet *et al*., 1996). The

receptor subfamilies of these cytokines have structural similarity with an identical signal transducing subunit. Certain chemokines are also involved in inflammatory reactions which when bind to its particular receptor initiates a series of signal transduction cascade which in turn stimulates the release of contents of the cytoplasmic granules, histamine from basophils, proteases and certain cytotoxic proteins (Barnes, 2011).

On random exposure to an allergen, mainly the responses are categorized in mainly three ways: sensitization, early phase reaction, late phase reaction. The initial step or sensitization of allergen consists of events like the random exposure of antigen (allergen), it's subsequent processing by dendritic cells as well as its presentation to certain immune cells like T lymphocytes. This induces interaction of B lymphocytes with the T lymphocytes along with the activity of certain cytokines and co-stimulatory molecules. This interaction, in turn, triggers the B cells to synthesize specific IgE molecules. Further the exposure or vulnerability of the specific allergen by the sensitized individual triggers the second phase i.e. early phase reaction (Busse and Lemanske, 2001). The immediate or early phase reaction occurs immediately within a few minutes and can last for about 2-3 hour in a sensitized person on cross-linking of the allergen to mast cell surface bound Ig E. Next, the degranulation of mast cells upon its activation takes place which results in the release pre-formed primary mediators specifically histamine. The early phase reaction is succeeded by the late phase reaction about 4-6 hours later with a number of predominant symptoms including rhinorrhea, sneezing and specifically nasal congestion. A number of mediators including leukotrienes, prostaglandins, bradykinins, histamine released in the late phase are predominantly related to inflammation due to the activity of basophils, T lymphocytes and eosinophils. Additionally, the release of several cytokines and chemokines acts as chemoattractants for other chemokines like eotaxin, RANTES, MCP-4 as well as causes the influx of eosinophils, basophils and T lymphocytes towards the nasal mucosa. As a result of this infiltration of cells, late reaction phase has a continuation of symptoms due to their prolonged survival (Pawankar *et al.*, 2011).

Activation of mast cell and its subsequent degranulation are important events in the initiation of allergic reaction in allergic rhinitis. The cross-linking of allergen is the initiation step which further stimulates a cascade of reactions leading to degranulation of the cytoplasmic granules in mast cell releasing histamine and other mediators. The offending allergen crosslinks with the IgE antibody attached to the mast cell in the Fc receptor leading to aggregation of FcεRI which in turn activates a membrane attached protein tyrosine kinase. This enzyme helps in the phosphorylation of phospholipase C, which is involved in the conversion of phosphatidylinositol-4,5 bisphosphate or PIP2 to two compounds namely- inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 helps in the mobilization of intracellular calcium ions whereas DAG helps in the microtubule assembly and fusion of the granule to the membrane by activating the protein kinase C which works in conjugation with calcium ions for the assembly and fusion as shown in figure 2.1.

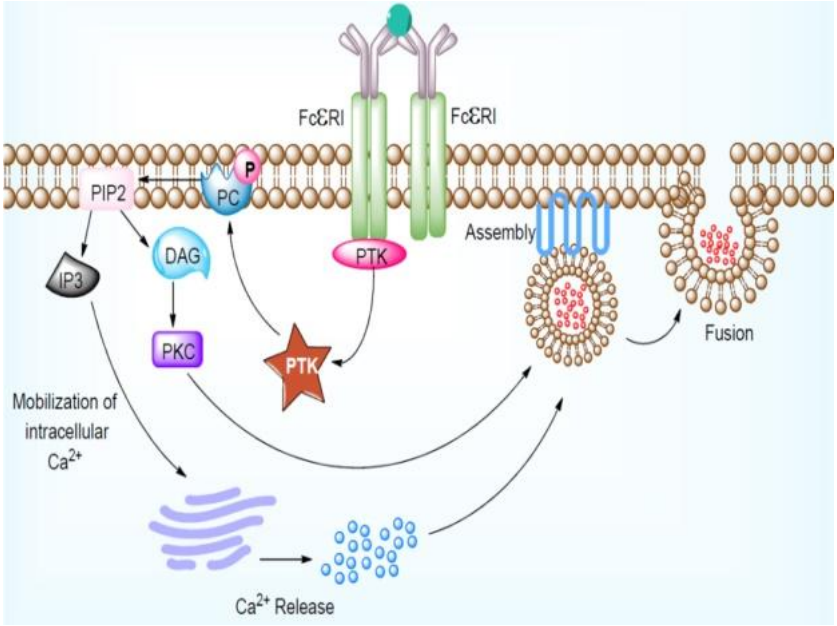


Figure 2.1- The events occurring post receptor ligand interaction in the FcεRI. PTK-Protein tyrosine kinase, PC-phosphatidylcholine, PIP2-phosphatidylinositol-4,5 bisphosphate IP3-inositol triphosphate, DAG-diacylglycerol, PKC-protein kinase C

The crosslinking also helps in conversion of phosphatidylserine (PS) to phosphatidylethanolamine (PE) after which PE gets methylated and converts into

phosphatidylcholine or PC with the help of two enzymes namely- phospholipid methyltransferase I (PMTI) and phospholipid methyltransferase II (PMTII). Next, this PC gets accumulated on the outer side of the membrane developing fluidity of plasma membrane which leads to the formation of calcium ion channels for calcium influx. This leads to the disintegration of PC into arachidonic acid and lysophosphatidylcholine by the activation of phospholipase A2, of which, arachidonic acid breaks down to form leukotrienes and prostaglandins by the lipooxygenase(LOX) and the cyclooxygenase(COX) pathway respectively (shown in figure 1.2).

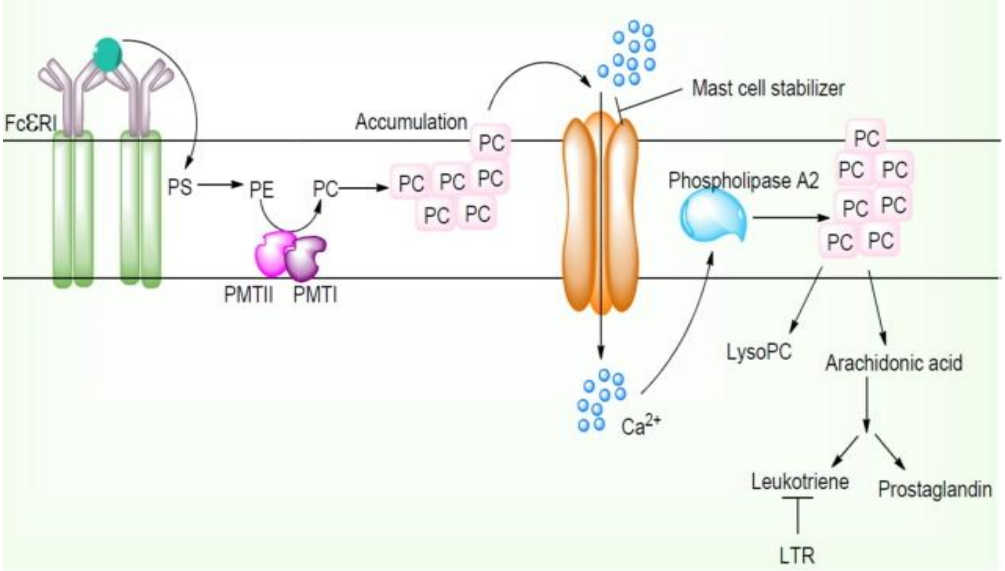


Figure 2.2- The events occurring post receptor ligand interaction. PS- phosphatidyl serine, PE- phosphatidylethanolamine, PC-phosphatidylcholine, LysoPC-lysophosphatidylcholine

Another simultaneous event that occurs due to the FcεRI cross-linkage is the activation of adenylate cyclase located in the membrane. The activated adenylate cyclase increases the cAMP (cyclic AMP) level by conversion of ATP to cAMP. Later the cAMP-dependent protein kinase reduces the cAMP level and also phosphorylates the granule membrane protein leading to change in membrane fluidity and permeability which helps the calcium ions and water molecules to enter. This influx of molecules results in subsequent swelling of the granules along with its fusion with the plasma membrane and ultimately releasing the potent mediators like histamine. On the other hand, the increase in the level of calcium ions mediates the conversion of

arachidonic acid to leukotrienes and prostaglandins along with its function in the fusion of granules to the membrane which is described diagrammatically in figure 1.3 (Owen and Punt, 2013).

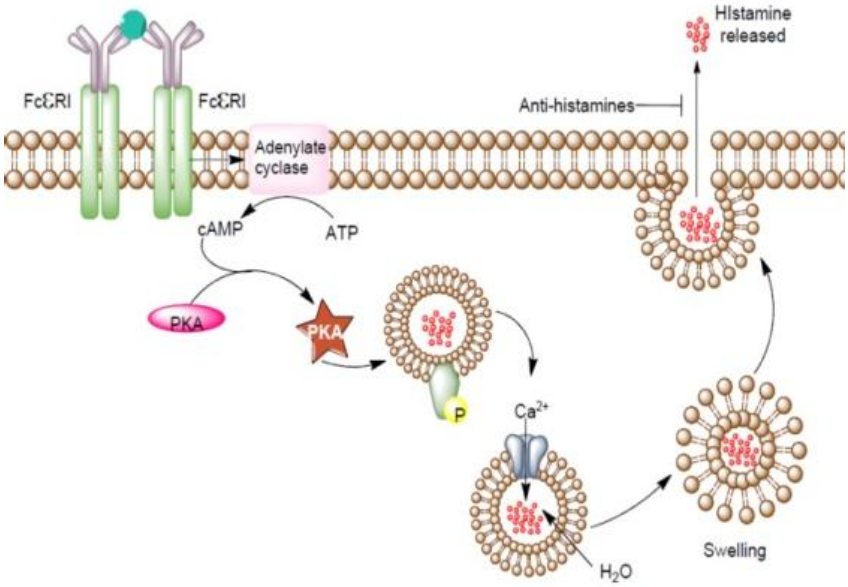


Figure 2.3- The biochemical events taking place simultaneously after the receptor ligand interaction. Activation of adenylate kinase converts ATP to cAMP which activates Protein kinase A(PKA) to ultimately release histamine from the granules.

2.3 Histamine Intolerance: another aspect of allergies

Histamine intolerance or pseudo-allergy, unlike allergies with IgE mediated histamine response to allergens, is a toxic histamine response by the body because of accumulation of excessive endogenous or exogenous histamine in the body. It is caused mainly due to the impaired breakdown of histamine or its overproduction. Defects in the enzymes involved in its synthesis and breakdown are responsible for its occurrence. It is a condition whereby the level of histamine is raised in the body either due to defective or mutated enzyme histidine decarboxylase responsible for its synthesis from the amino acid histidine or due to deficiency of enzyme diamine oxidase (DAO) responsible for the further breakdown of histamine. However, DAO is responsible mainly for the breakdown of exogenous or ingested histamine while another enzyme Histamine methyltransferase (HMT) is responsible for the degradation of endogenic histamine into its constituent components (Jernigan, 2015).

Histamine intolerance can be caused due to many factors that may lead to a rise in the levels of histamine. Sometimes, certain food chemicals are responsible for this condition to occur as a result of food allergy that causes inflammation. Fermented foods are primarily responsible for the production of these biogenic amines in excess due to activation of mast cells. Histamine is a biogenic amine and its accumulation may also result from the intake of various foods exogenously. Various foods resulting in increased histamine levels include fish, meat or dairy products, fermented foods, beer, wine etc (Leech, 2018). The symptoms of histamine intolerance mainly coincide with those of allergic rhinitis which is why it is often confused or misinterpreted as allergic rhinitis whereas on the other hand it is barely allergy and is involved in the increased level of histamine or its accumulation. Because of this, the treatments opted for show less or no improvement failing to target the enzymes involved in histamine intolerance.

2.4 Allergic rhinitis and its impact on asthma (ARIA) guidelines

Extensive studies in allergic rhinitis and its possible therapeutics have been reported and therefore during a workshop by World Health Organization, allergic rhinitis and its impact on asthma (ARIA) guidelines have been initiated in 1999. It was then published (2001) and updated (2008) and again revised in 2010 for following the GRADE (Grading of Recommendation, Assessment, Development and Evaluation) approach which was the first ever guideline in allergy based on scientific evidence. It mainly consisted of a detailed summary of the highlights and challenges related to the recommendations along with the potential and preferred recommendations for the well being and up-gradation of the lives of the common mass in relation to the decreased quality of life. Another revision of ARIA guidelines (2014) was considered significant in the development and management of AR.

It is a global health initiative with a sturdy purpose of educating, implementing and proper execution of allergic rhinitis disease management guidelines based on evidence in correlation to asthma. It classifies the diseases and categorizes it into four stages based on the severity-

(1) Intermittent (mild, moderate and severe)

(2) Persistent (mild, moderate and severe)

Based on these guidelines and severity of the disease, common drug treatments like oral H1- antihistamine, intranasal H1- antihistamine, leukotriene receptor antagonists and intranasal corticosteroids are recommended for treating allergic rhinitis either alone or in combination. According to the revised ARIA guidelines, second generation H_1- antihistamines were preferred over the first generation H_1-antihistamines due to safety concern. Leukotriene receptor antagonists (LTRA) also became another class of commonly recommended drugs for allergic rhinitis (Min, 2010).

Antihistamine comprises the most widely used and largest class of drugs for AR which mainly targets the blockade of H1 histamine receptors which are present in the nerve endings and the nasal vascular system. The presence of ethylamine moieties in the first generation antihistamine provides them easy access to the blood-brain being highly lipophilic (Rachelefsky, 1998). Due to this a number of its negative effects may be noticed including euphoria, blurred vision, dizziness, upset stomach, tremors etc. The apparent side effects commonly drowsiness makes it less potent and therefore were not recommended after the revised ARIA guidelines were published. In contrast, second generation antihistamines had a higher H1 receptor selectivity and so they are commonly recommended under the ARIA guidelines (Walekar *et al*., 2016). Similarly, other classes of drugs against AR are recommended either alone or in combination.

2.5 Pharmacological treatment options for allergic rhinitis

Although not a serious disorder, AR has mild or moderate symptoms initially. However, it may also turn to chronic and even worse are the risk of other serious issues like asthma or anaphylactic shock. It also decreases the quality of life as it interferes with the sleep pattern, school or work due to its prolonged symptoms. Thus it is necessary to undergo proper treatment. Antihistamines and leukotriene receptor antagonists are the most common and widely used pharmacological drug against allergic rhinitis. Many standard drug treatments have been proposed against allergic rhinitis, of which H1-antihistamines, intranasal glucocorticoids, and leukotriene-receptor antagonists are most common. Fexofenadine is a common second-

generation non-sedating H1-antihistamine with greater selectivity for the H_1 receptor (Walekar *et al.*, 2016). Montelukast and zafirlukast are two of the most commonly prescribed LTRA available worldwide (Dempsey, 2017). Recently, budesonide, triamcinolone acetonide, fluticasone propionate, mometasone furoate and fluticasone furoate are widely used intranasal glucocorticoids (Min, 2010). All these drugs are USFDA approved and are commonly used for the treatment of allergic rhinitis In addition to these, several other pharmacological treatment options like oral decongestants, newly inhaled steroids, intranasal mast cell stabilizers, intranasal anticholinergics, monoclonal anti- Ig E antibody treatment and immunotherapy. It is to be noted that different pharmacological treatment options are to be opted based on its symptoms and severity. Allergic testing, RAST testing or skin testing is another option for allergic rhinitis through which direct avoidance of allergen can be made.

Intranasal corticosteroids are common allergic drugs that provide inhibition of early phase response and late phase response by blocking cytokine secretion (IL-5, IL-13 and IL-4) as well as decreasing the level of eosinophils and Ig-E production in our body (Lee *et al*., 2001). It can be used for all symptoms but is especially effective in case of nasal obstruction and eye symptoms. Common corticosteroids include fluticasone furoate, mometasone furoate, fluticasone propionate, budesonide, beclomethasone dipropionate etc are commonly prescribed intranasal corticosteroids mainly used for nasal congestion (Min, 2010). Common oral decongestants like pseudoephedrine act mainly on the α- adrenergic receptors of the respiratory or nasal mucosa for the stimulation of vasoconstriction. It stimulates α- adrenergic receptors which increase the blood pressure as well as stimulate β-adrenergic receptors which enhances our heart pulse rate and its contractility. They are recommended to be used either alone or with antihistamines as a combination therapy for treatment of nasal congestion (Medscape). New inhaled steroids is a recent approach to the treatment of AR. Ciclesonide, a common example of this class is inhaled in an inactive state which later modifies to be pharmacologically active by certain esterases present in the upper as well as a lower respiratory tract and functions effectively. It is currently under clinical development for more efficient results in the treatment of AR (Braido *et al*., 2008). Cromolyn sodium, a common mast cell stabilizer is used against seasonal

allergic rhinitis. It is generally considered to block the histamine release along with the release of other mediators mainly by stabilizing mast cells during inflammation (Druce and Kaliner, 1998). It blocks the calcium ion influx during degranulation. But its biggest disadvantage is that it must be used continuously for weeks until the required efficacy is obtained (Lieberman, 1988). Intranasal anticholinergic drugs mainly function by inhibiting the interaction of acetylcholine to its specific receptors located on the mucous glandular tissue. This specifically reduces the nasal secretion of mucus and efficiently reduces rhinorrhea (Spector, 1999). Ipratropium bromide, an anticholinergic drug is particularly used for the treatment of rhinorrhea.

There are certain immunotherapeutic approaches discovered which work efficiently by enhancing the quality of life by reducing the symptoms overall by modification of the progression of the disease. Monoclonal anti- Ig E antibody treatment and immunotherapy are two efficient treatment options for allergic rhinitis. Monoclonal anti- Ig E antibody treatment uses humanized monoclonal anti-IgE antibody modified through recombinant DNA technology to block the interaction of IgE antibodies with the mast cells or basophils. It mainly works by binding itself to the IgE antibodies (ARIA, 2007). This approach efficiently reduces IgE level in the blood. Omalizumab is a monoclonal anti-IgE antibody which is used to decrease nasal symptoms in addition to reducing inflammation. It is especially helpful in case of seasonal allergic rhinitis with allergens like ragweed pollen, birch and other outdoor allergens (Braido *et al.,* 2008). Immunotherapy is another immunological therapeutic approach which involves desensitization of allergens such that the basic allergic mechanism is altered. Allergen extract doses are either injected subcutaneously or administered orally, sublingually or through nasal route. It is used for allergic treatment against seasonal allergens (pollens), animal dander, dust, hymenoptera etc. The advantage of using immunotherapy is the prolonged effect even after its subsequent discontinuation (Min, 2010).

2.6 Adverse effects of these drugs

Although pharmacotherapeutic drugs have higher effectiveness with instant or immediate results, the incidence of their adverse effects can also be equally observed

and encountered. Pharmacological drugs for allergic rhinitis have certain mild or severe side effects. These side effects may also be dependent on the dose, delivery or coordination of the drug administration or simply the attributes of the drug itself (Braido *et al*., 2008). According to revised guidelines of ARIA in 2007, the first generation antihistamines were not considered safe due to certain health issues faced by the patients as side effects. The ability of a drug to cross the blood-brain barrier has a significant effect on its action. First generation antihistamines are mainly small chemical components that circulate in the bloodstream until it easily crosses the blood-brain barrier having a greater impact on the CNS. Due to this a number of its adverse effects were visible including insomnia, blurred vision, anxiety, nervousness, hallucinations, tremors and depression (Aaronson, 1998).

Even some of the commonly used drug therapies have many disadvantages and the effects of which including gastrointestinal, nasopharyngeal, CNS, sensory or anticholinergic effects. There may also be certain general side effects including a headache, rash, gastric dyspepsia, dental pain, respiratory tract infections or dream abnormalities due to administration drugs of leukotriene receptor inhibitors. Oral antihistamines may have side effects like a headache, drowsiness, stomach upset or myalgias. Certain intranasal therapies including corticosteroids, antihistamines and mast cell stabilizers may also lead to a range of nasopharyngeal or sensory side effects like epistaxis, nasal irritation, cough, pharyngitis, loss of sense of taste or smell and headache. Similarly, certain oral decongestants may also cause adverse symptoms like dizziness, weakness, headache, insomnia, nervousness, palpitations and urinary retention (UMHS AR Guideline, 2013). Moreover efficient pharmacological as well as immunological therapies may also be cost intensive.

2.7 **Knowledge gap**

Natural products are used as remedies for various diseases and ailments. *Curcuma longa*, having a wide history of anti-inflammatory activity is a common medicinal herb being used as traditional medicine for a number of chronic diseases. Presence of active components like curcumin renders it anti-inflammatory property which might play important role for treatment of allergic diseases like allergic rhinitis. The mechanism of action of curcumin and its potential target sites of inhibition are not

studied or reported yet. Thus there is an immense necessity for the study of curcumin and other phytochemicals present in turmeric and its role in the possible treatment of allergic rhinitis as an alternative therapy which will be cost effective and with lesser or no side effects. Thus, the *in silico* scientific validation of *C. longa* as a potential source of treatment of AR is yet to be studied.

2.8 History of anti-inflammatory natural products against Allergic Rhinitis

The concept of natural products has its roots back in the 19th century. Classical examples of drug compounds discovered this way is morphine, the active agent in Opium, and digoxin, a heart stimulant originating from flower *Digitalis lanata (*Lahlou, 2013*).* Historically natural products have been a rich source of compounds that have a great importance in medicine, pharmacy and biology. A number of important new commercial drugs have been obtained from natural sources*.* Drugs of natural origin can be classified as original natural products, products derived semi-synthetically from natural products or synthetic products based on natural product model*.*

Many standard drug treatments have been proposed against allergic rhinitis, of which H1-antihistamines, intranasal glucocorticoids, and leukotriene-receptor antagonists are most common. Fexofenadine is a common second-generation non-sedating H1-antihistamine with greater selectivity for the H_1 receptor (Walekar *et al.*, 2016). Montelukast and zafirlukast are two of the most commonly prescribed LTRA available worldwide (Dempsey, 2017). Recently, budesonide, triamcinolone acetonide, fluticasone propionate, mometasone furoate and fluticasone furoate are widely used intranasal glucocorticoids (Min, 2010). All these drugs are USFDA approved and are commonly used for the treatment of allergic rhinitis.

2.9 Importance and advantages of natural anti-inflammatory products

The pharmaceutical drugs come with many pros and cons and most importantly its cons could be highly felt in comparison to the naturally derived products. The benefits of modern drugs could only be utilized primarily in developed countries, being highly cost intensive. Not being able to access the modern healthcare products, the developing countries still relied on ethnobotanical remedies as primary medicines.

Moreover, some of the significant pharmaceutical drugs come with many side effects (Ratini, 2017). Natural products considered to be the best source of drugs for past many years as these having no or very poor side-effects and easy availability in addition to their cost-effectiveness. Moreover, natural products have structural and chemical diversity. Natural products are a good source of active therapeutic agents.

2.10 Currently used natural anti-inflammatory products

Several plants are known for their anti-inflammatory activity due to the presence of many important phytochemicals. But most of them are not used clinically despite their potent activity. Although very less is known about the potent natural inhibitors of AR, some studies show that *Urtica dioica* or stinging nettle, bromelain (a proteolytic enzyme derived from the stem of pineapple plant), quercetin (a flavonoid found in many herbs and vegetables), N-acetylcysteine (a natural sulfur-containing amino acid derivative) and vitamin C had potential antihistamine and anti-inflammatory activities.

Moreover, some plants like *Curcuma longa* are shown efficient anti-inflammatory property but are not yet identified as a cure for AR. Thus the importance of natural compounds that can be effectively used for its treatment without any side effects is highly felt. There is a number of phytochemicals showing high efficiency against allergic rhinitis due to their anti-inflammatory property which is yet to be accepted as therapeutic or diagnostic agents. Potential identification and screening are utterly important in order of these phytochemicals to be recognized as therapeutic standards.

3. Material and methods

3.1 *In silico* potential screening

3.1.1 Protein preparation and modelling

The protein structures of target proteins histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) were downloaded from RCSB Protein Data Bank in .pdb format. The structure of another protein histamine H4 receptor was not available in PDB so the protein structure modelling was done using online servers like SWISS-MODEL. The protein sequence was initially obtained from NCBI FASTA format and searched for homology protein in BLAST for sequence recognition and alignment. A detailed 3d structure modelling was done by online server SWISS-MODEL and a QMEAN Z-score was checked for its checking its efficiency and % similarity. Next, structure analysis was carried out using model analysis tools like PDBSUM and SAVESERVER. The model analysis was checked using PROCHECK which analyses residue-by-residue geometry along with the general or comprehensive geometry to check the stereochemical protein quality and the modelled structure. PROVE gives us a calculated statistical Z-score deviation for the modelled protein. The Ramachandran plot is also calculated which is also available in the tools.

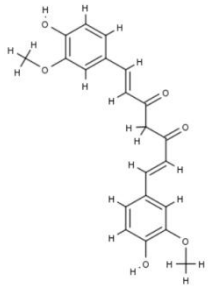
3.1.2 Retrieval of receptors

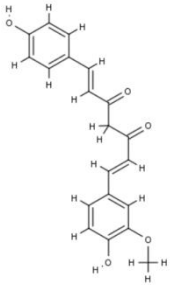
The crystal structure proteins histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) were retrieved from RCSB Protein Data Bank. Standard inhibitors such as doxepin, histidine methyl ester (HME), glutathione sulfonate, 2,4-thiazolidinedione and Galphaq binds with the targeted proteins histamine H1 receptor, histidine decarboxylase, leukotriene C4 synthase, adenylate kinase and phospholipase C respectively on the heterodimer interface. For the remaining proteins that were not complexed or inhibitor-bound structures originally as well as for the modelled protein structure, active site prediction was done using SiteMap in Maestro (Version 11.2.013). All the heteroatoms were removed leaving

only the residues of the receptor. Preparation of the target protein with ADT involved the addition of polar hydrogen to the macromolecule, an essential step to correct the calculation of partial charge. Finally, Gasteiger charges will be calculated for each atom of the macromolecule.

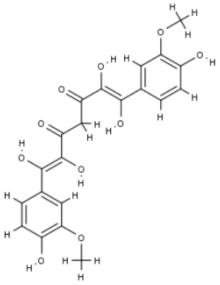
3.1.3 Ligand preparation

Different literature database has been searched for the presence of phytochemicals in curcumin powder. At the end of literature survey, we found the presence of 265 phytochemicals in curcumin powder of different origin and brand (Li *et al*., 2011). The structures of the phytochemicals are given below.

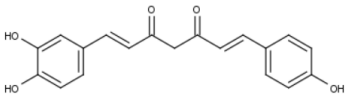
1 



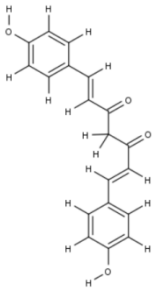
2



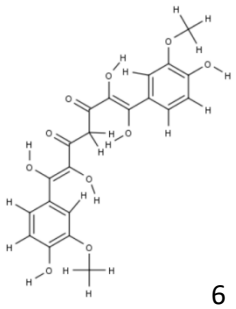
3



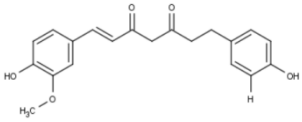
4



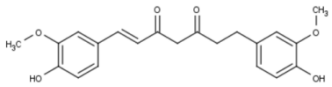
5



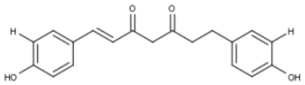
6



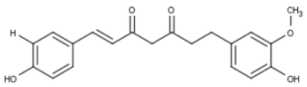
7



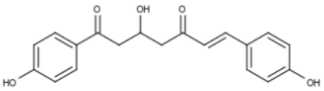
8



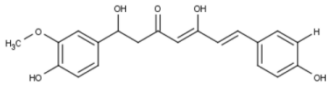
9



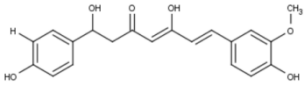
10



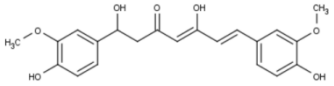
11



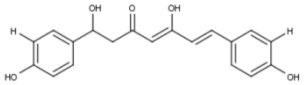
12



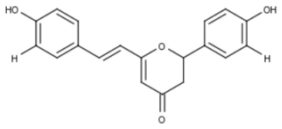
13



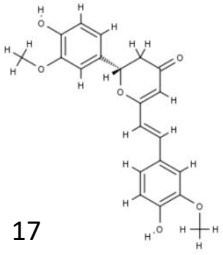
14



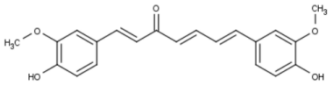
15



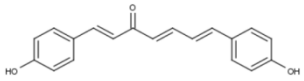
16



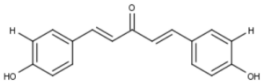
17



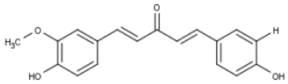
18



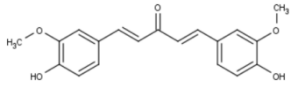
19



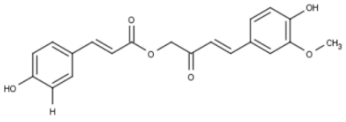
20



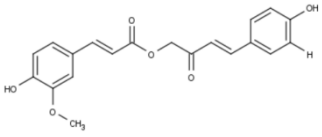
21



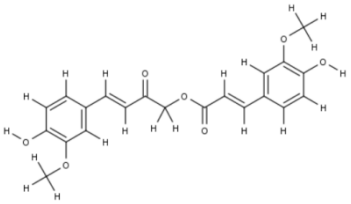
22



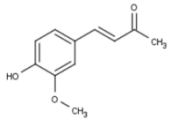
23



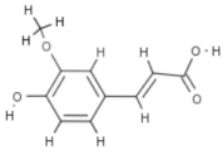
24



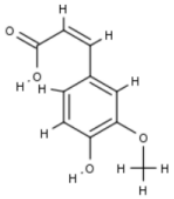
25



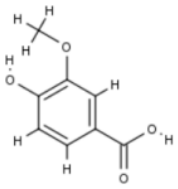
26



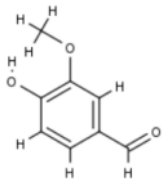
27



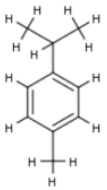
28



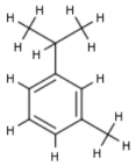
29



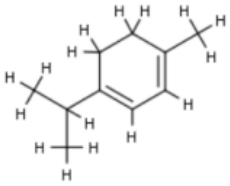
30



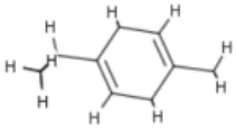
31



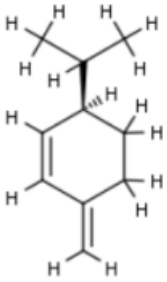
32



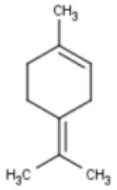
33



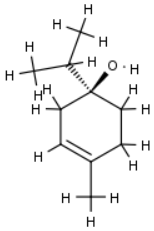
34



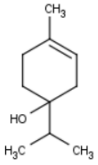
35



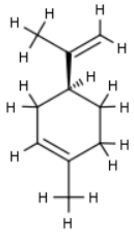
36



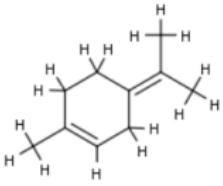
37



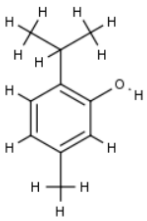
38



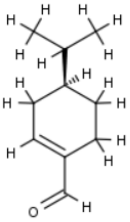
39



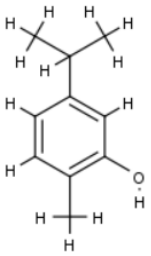
40



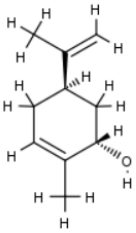
41



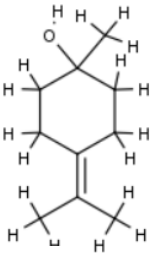
42



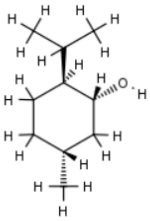
43



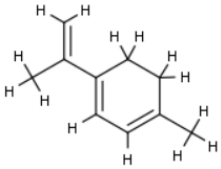
44



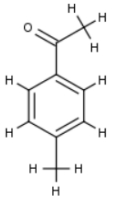
45



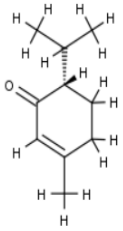
46



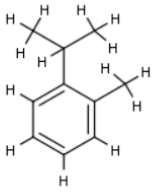
47



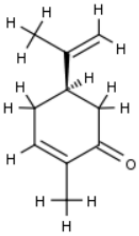
48



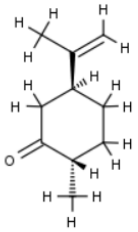
49



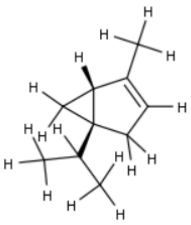
50



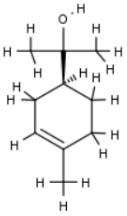
51



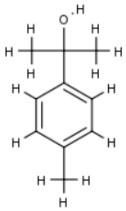
52



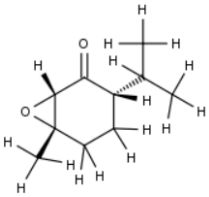
53



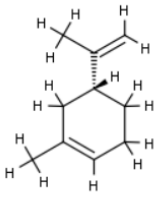
54



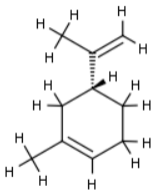
55



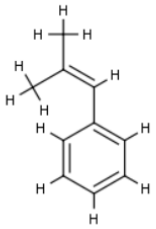
56



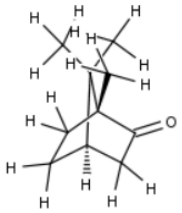
57



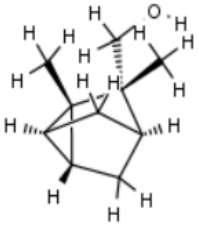
58



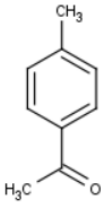
59



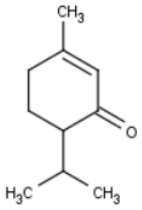
60



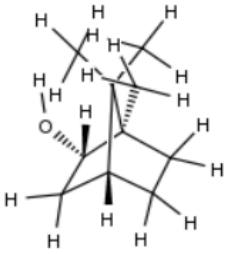
61



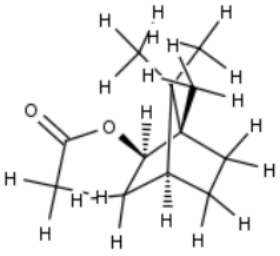
62



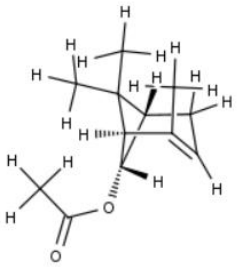
63



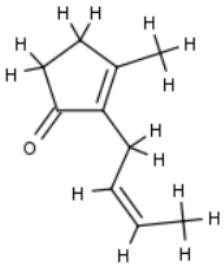
64



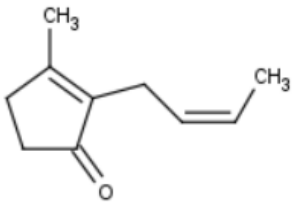
65



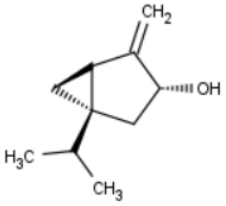
66



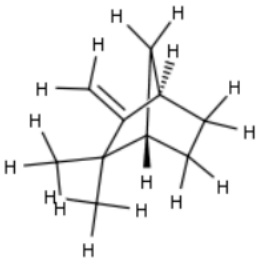
67



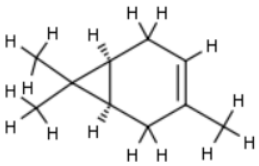
68



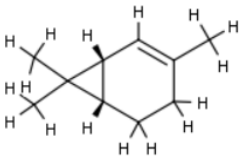
69



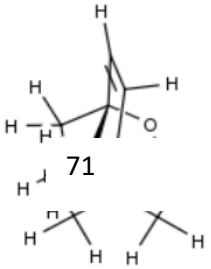
70



71

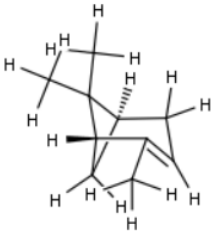


72

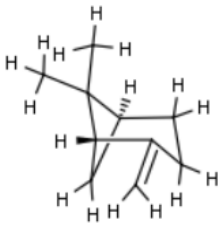


71

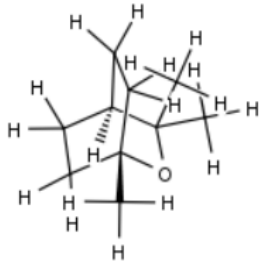
73



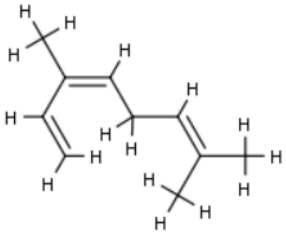
74



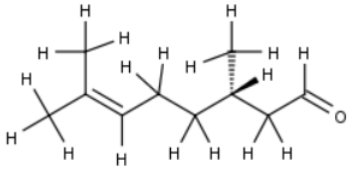
75



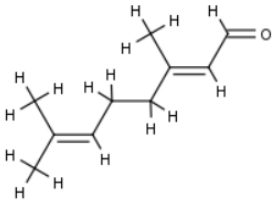
76



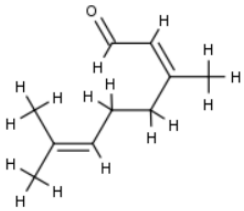
77



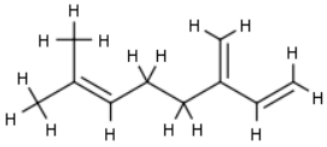
78



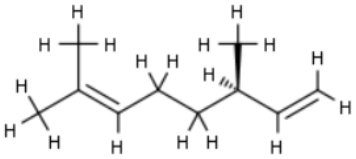
79



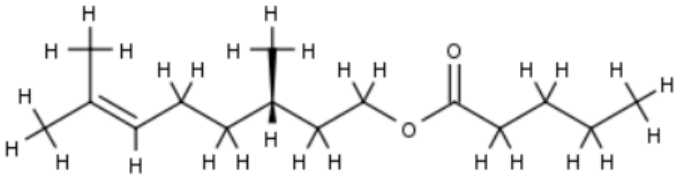
80



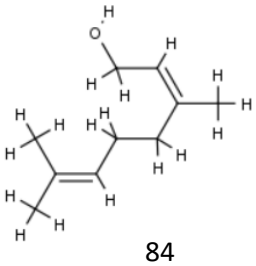
81



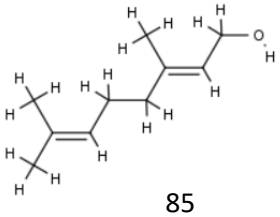
82



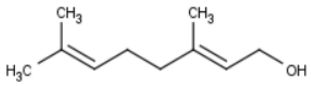
83



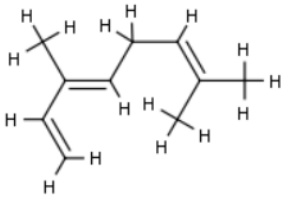
84



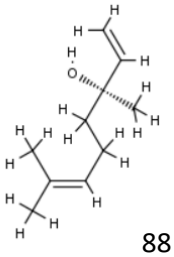
85



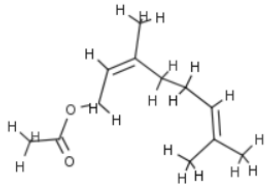
86



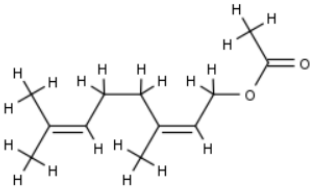
87



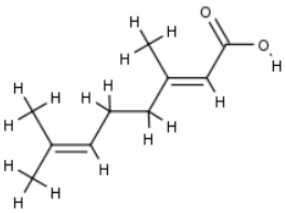
88



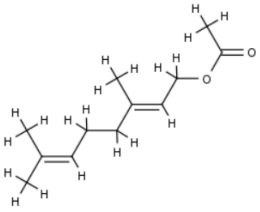
89



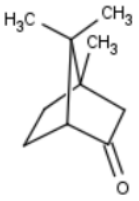
90



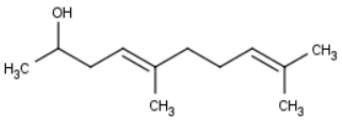
91



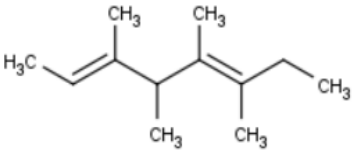
93



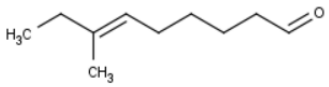
94



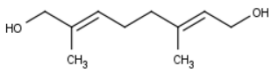
95



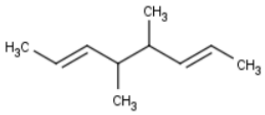
96



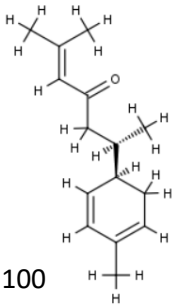
97



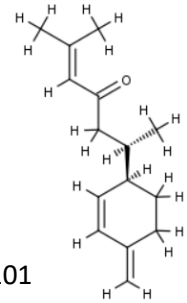
98



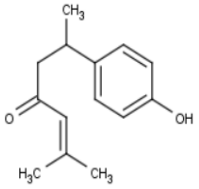
99



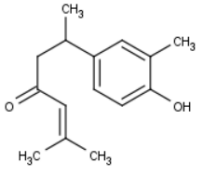
100



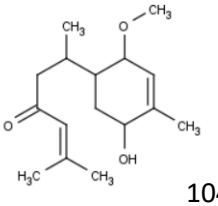
101



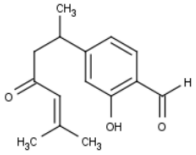
102



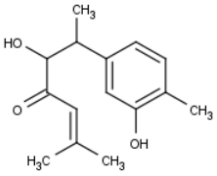
103



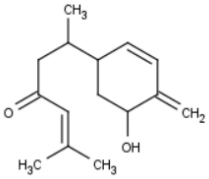
104



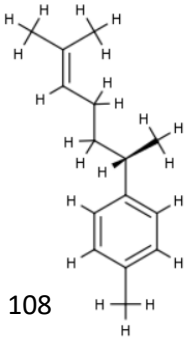
105



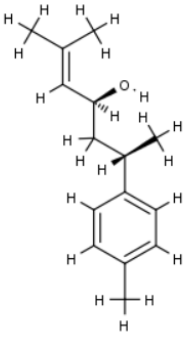
106



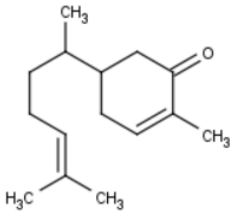
107



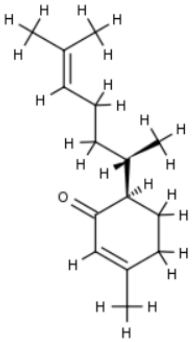
108



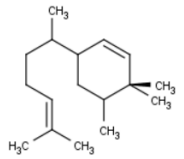
109



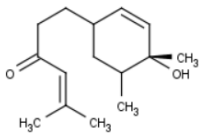
110



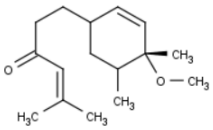
111



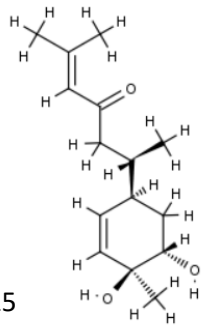
112



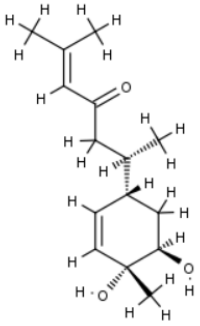
113



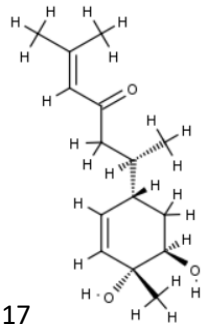
114



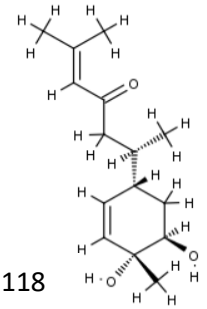
115



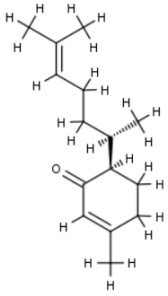
116



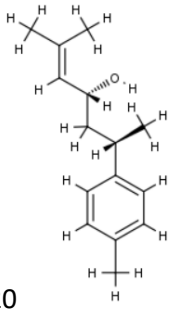
117



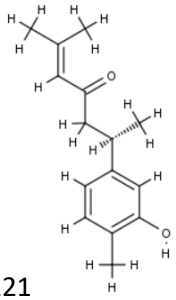
118



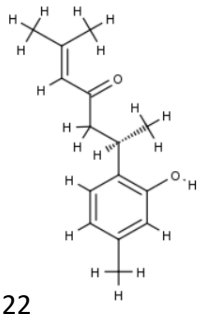
119



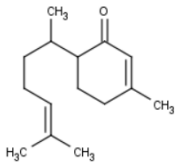
120



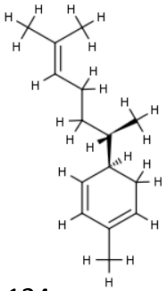
121



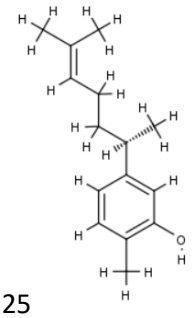
122



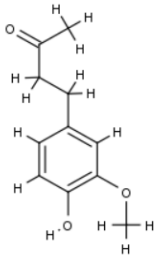
123



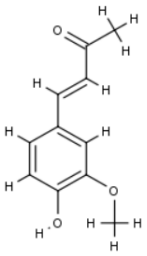
124



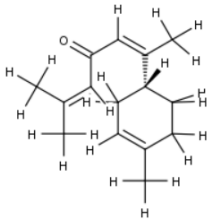
125



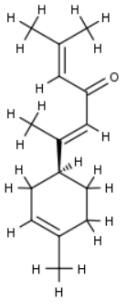
126



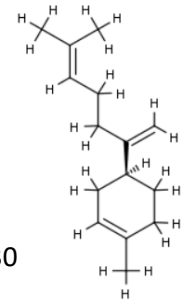
127



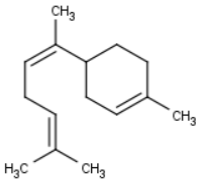
128



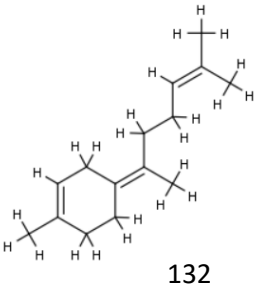
129



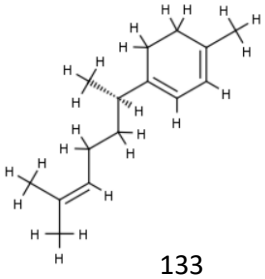
130



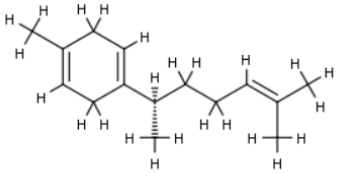
131



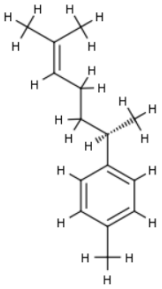
132



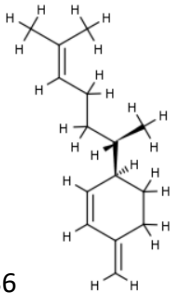
133



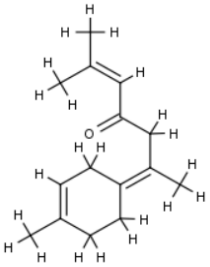
134



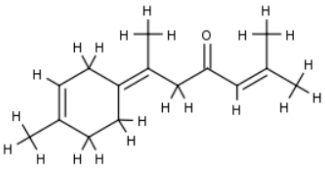
135



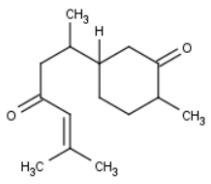
136



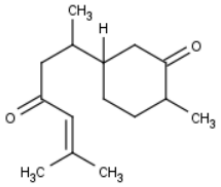
137



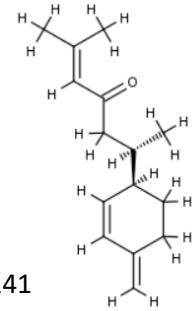
138



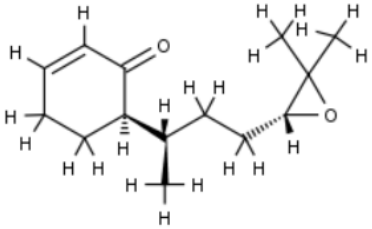
139



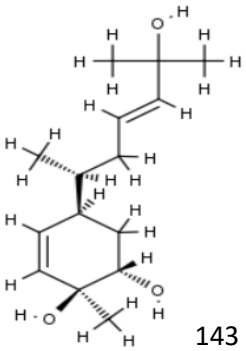
140



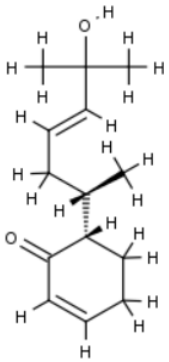
141



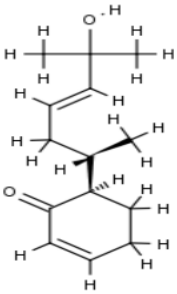
142



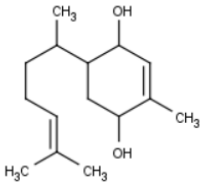
143



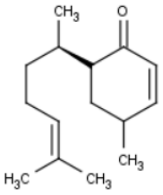
144



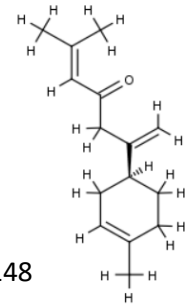
145



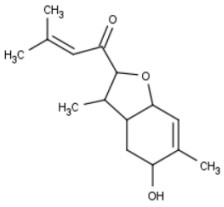
146



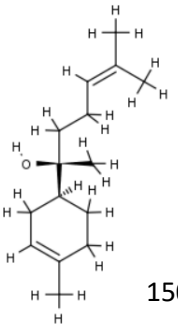
147



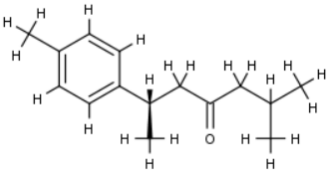
148



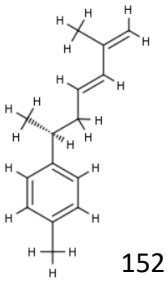
149



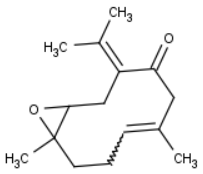
150



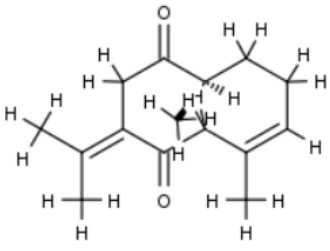
151



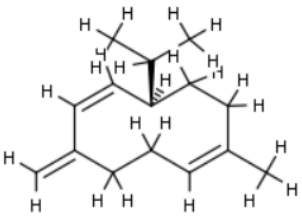
152



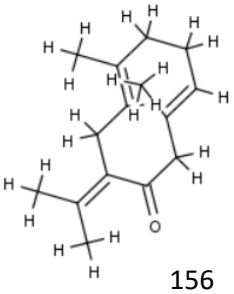
153



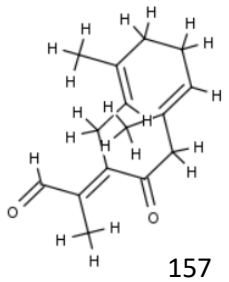
154



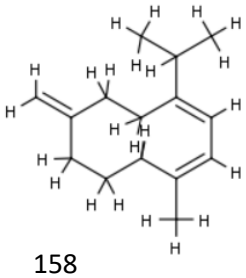
155



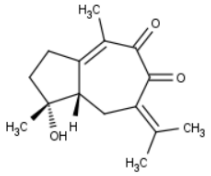
156



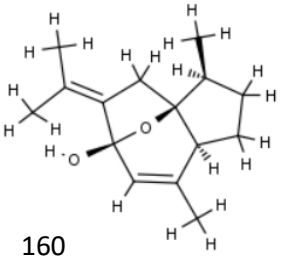
157



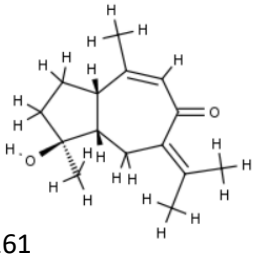
158



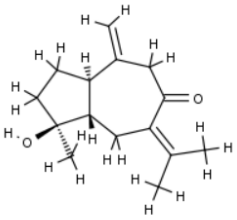
159



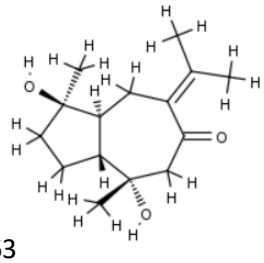
160



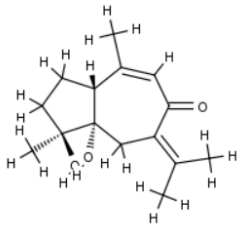
161



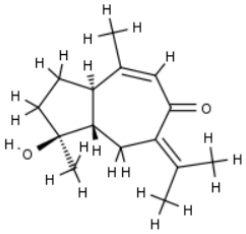
162



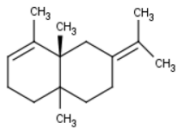
163



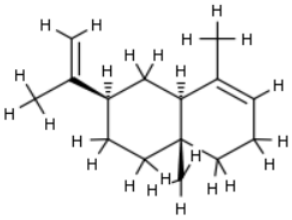
164



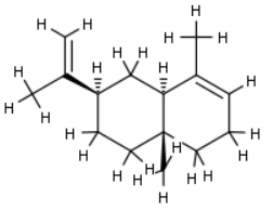
165



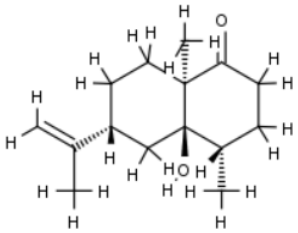
166



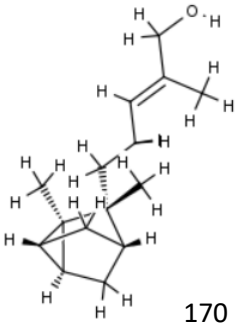
167



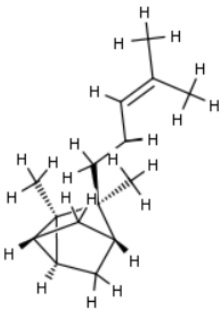
168



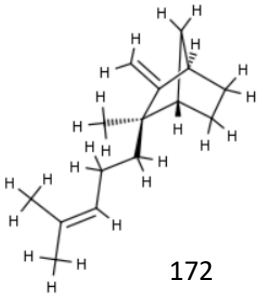
169



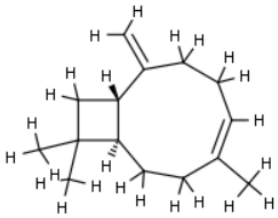
170



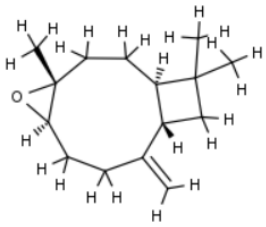
171



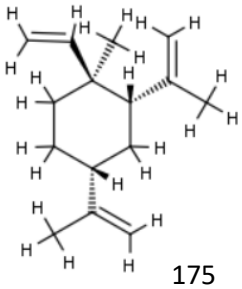
172



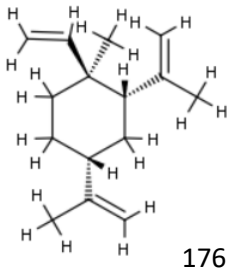
173



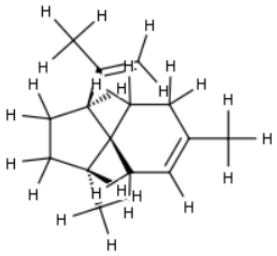
174



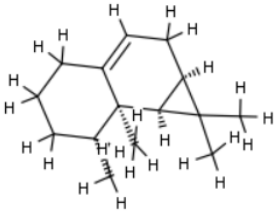
175



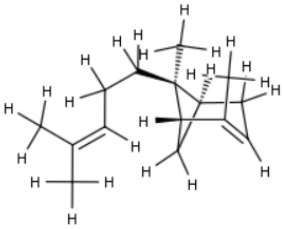
176



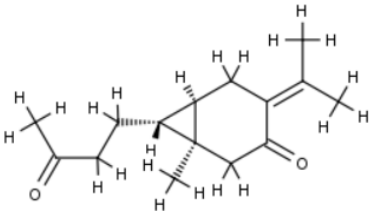
177



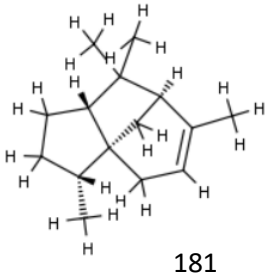
178



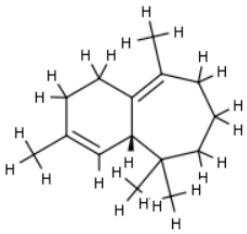
179



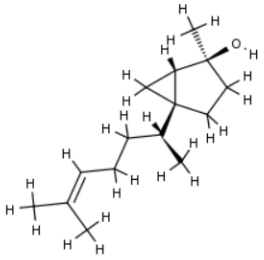
180



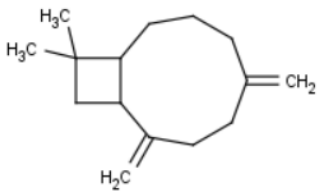
181



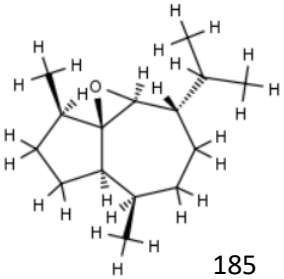
182



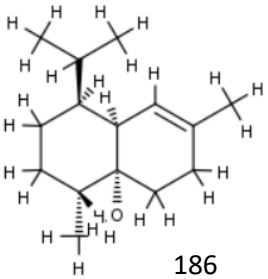
183



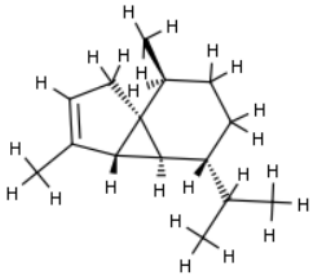
184



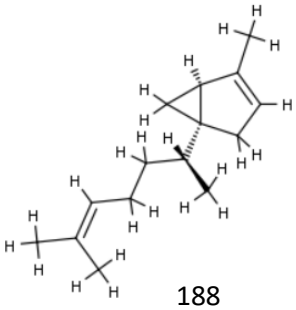
185



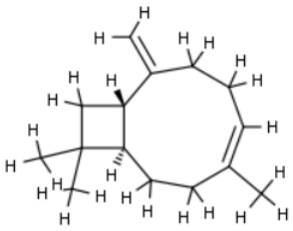
186



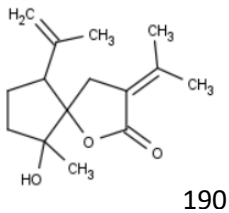
187



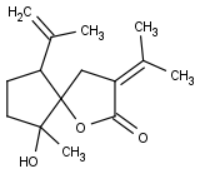
188



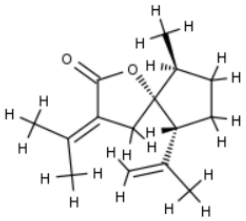
189



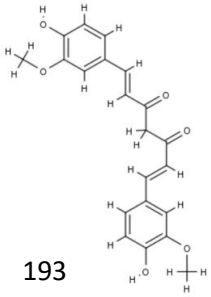
190



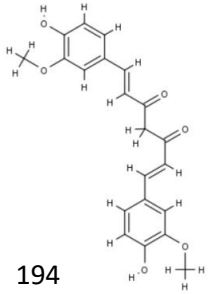
191



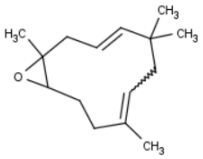
192



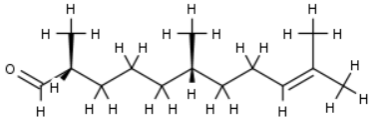
193



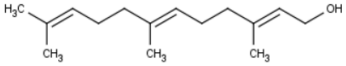
194



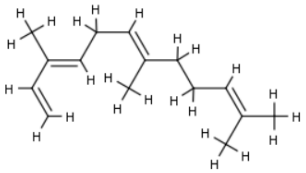
195



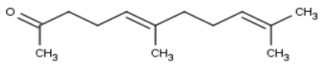
196



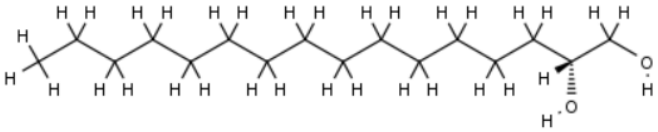
197



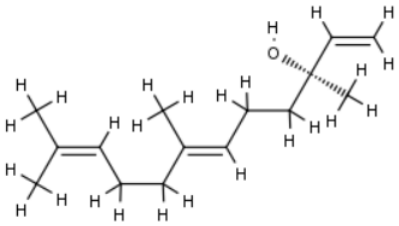
198



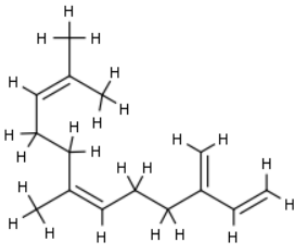
199



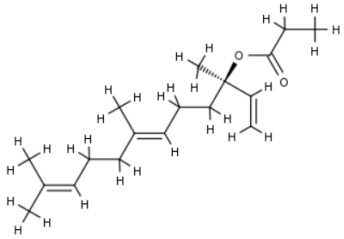
200



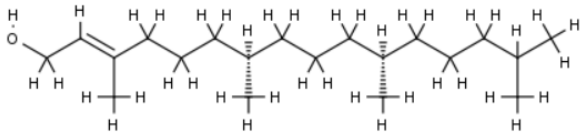
201



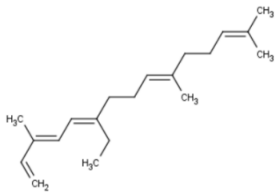
202



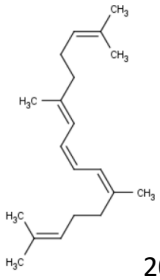
203



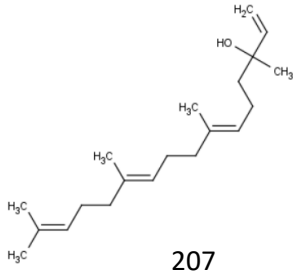
204



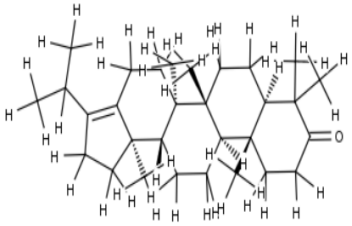
205



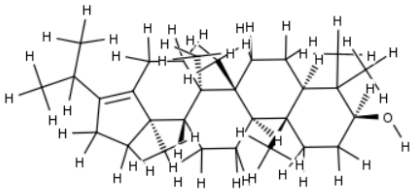
206



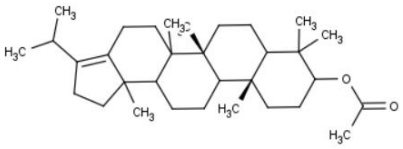
207



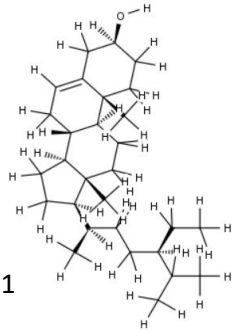
208



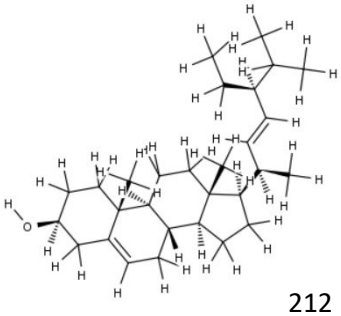
209



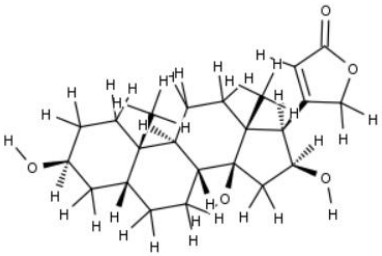
210



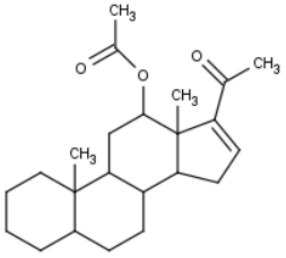
211



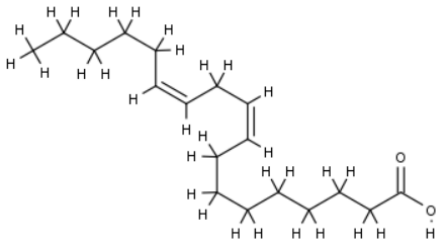
212



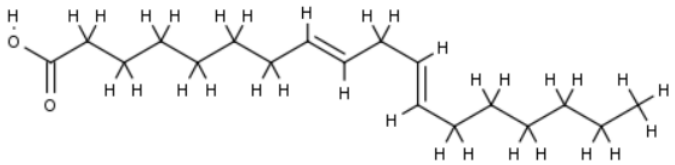
213



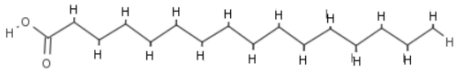
214



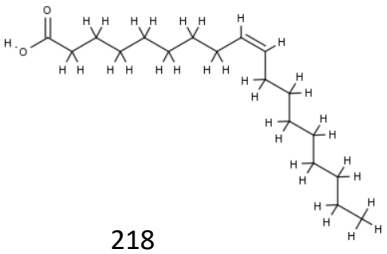
215



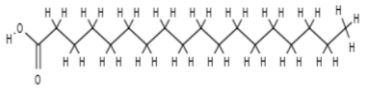
216



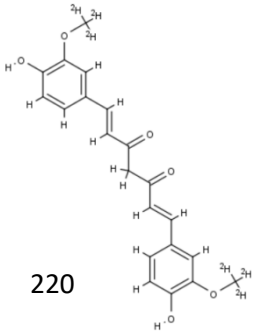
217



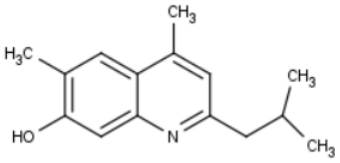
218



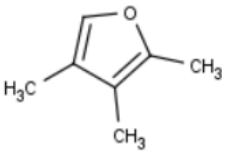
219



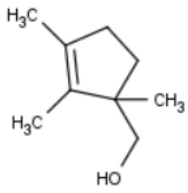
220



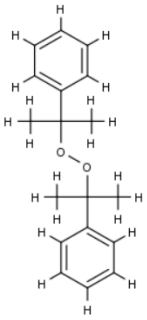
221



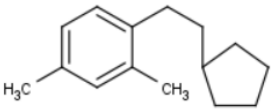
222



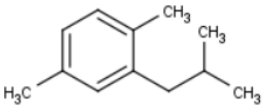
223



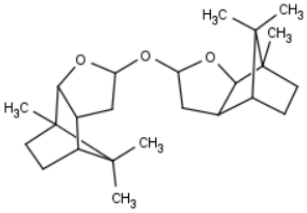
224



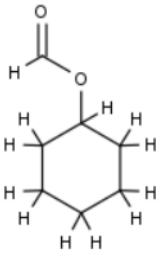
225



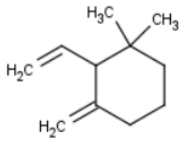
226



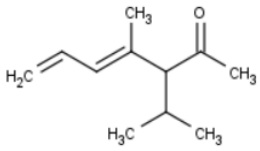
227



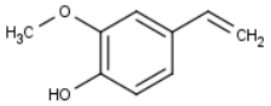
228



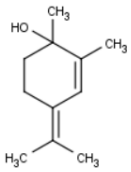
229



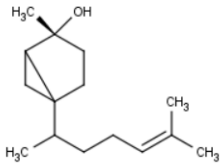
230



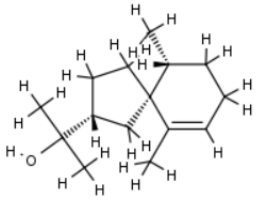
231



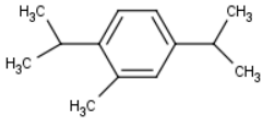
232



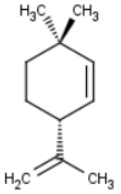
233



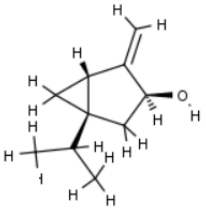
234



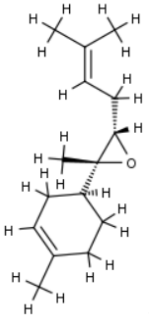
235



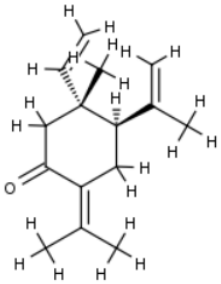
236



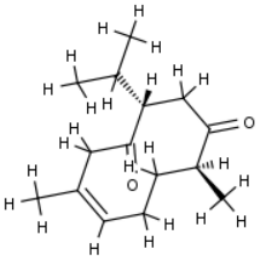
237



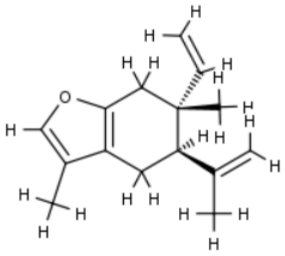
238



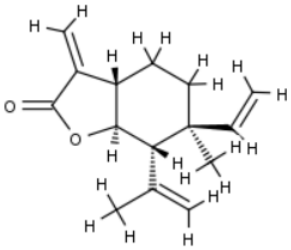
239



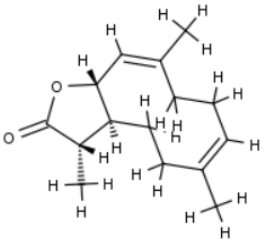
240



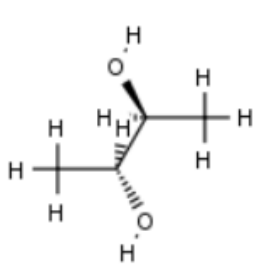
241



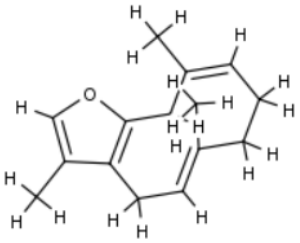
242



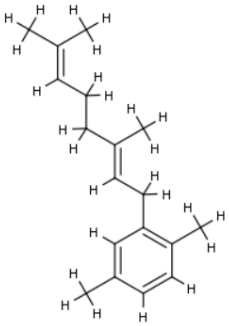
243



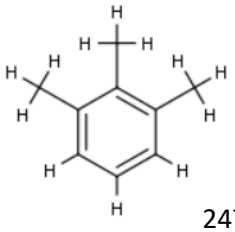
244



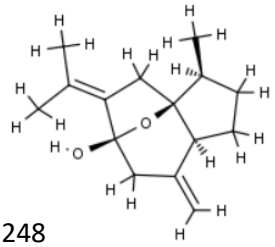
245



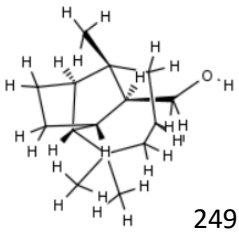
246



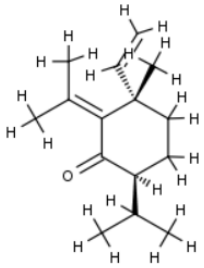
247



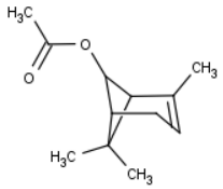
248



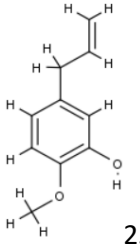
249



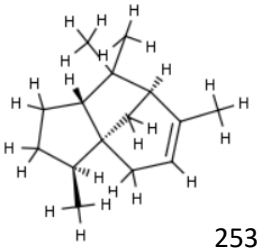
250



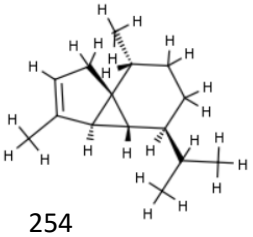
251



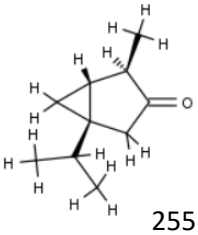
252



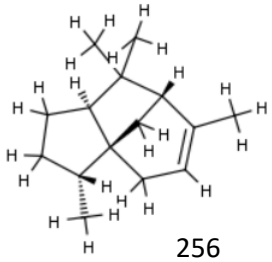
253



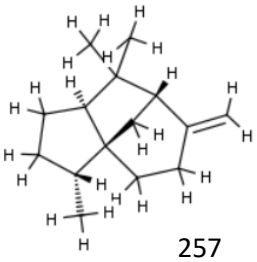
254



255



256



257

Figure3.1- The structures shown above are phytochemicals present in turmeric namely- (**1**) curcumin (curcumin I) (**2**) demethoxycurcumin (curcumin II) (**3**) 1-(4-hydroxy-3-methoxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione (**4**)1-(4-hydroxyphenyl)-7-(3, 4-

dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione (**5**) bisdemethoxycurcumin (curcumin III) (**6**) tetrahydroxycurcumin (**7**)5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one (**8**) 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (**9**) 1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione (**10**) 5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one (**11**) 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione (**12**) 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one (**13**) 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (**14**) 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (**15**) 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one (**16**) 1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene (**17**) Cyclocurcumin (**18**) 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one (**19**) 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (**20**) 1,5-bis(4-hydroxyphenyl)-penta-(1E,4E)-1,4-dien-3-one (**21**) 1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1, 4-pentadiene-3-one (**22**) 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one (**23**) 4''-(4'''-hydroxyphenyl)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate (**24**) 4''-(4'''-hydroxyphenyl-3-methoxy)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl)-propenoate (**25**) calebin-A (**26**) (*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (**27**) (*E*)-ferulic acid (**28**) (*Z*)-ferulic acid (**29**) vanillic acid (**30**) vanillin (**31**) *p*-cymene (**32**) *m*-cymene (**33**) *α*-terpinene (**34**) *γ*-terpinene (**35**)α-cedrene (**36**) *β*-phellandrene (**37**) *p*-mentha-1,4(8)-diene (**38**) terpinen-4-ol (**39**) 4-terpinol (**40**) limonene (**41**) terpinolene (**42**) thymol (**43**)phellandrol (**44**) carvacrol (**45**) (*E*)-carveol (**46**) *γ*-terpineol (**47**) menthol (**48**) 1,3,8-paramenthatriene (**49**) p-methylacetophenone (**50**) piperitone (**51**) *o*-cymene (**52**) carvone (**53**) *p*-menth-8-en-2-one (**54**) *α*-thujene (**55**) *α*-terpineol (**56**) *p*-cymen-8-ol (**57**) *p*-meth-8-en-2-one (**58**) piperitone epoxide (**59**) sylvestrene (**60**) menthofuran (**61**) *β, β*-dimethylstyrene (**62**) camphor (**63**) teresantalol (**64**) benzene (**65**) 1-methyl-4-(1-methylpropyl) (**66**) 2-norpinanone (**67**) borneol (**68**) bornyl acetate (**69**) (*E*)-chrysanthenyl acetate (**70**) (*Z*)-cinerone (**71**) (*Z*)-sabinol (**72**) 2-(2,5-dihydroxy-4-methylcyclohex-3-enyl)propanoic acid (**73**) camphene (**74**) 3-carene (**75**) 2-carene (**76**) ascaridole (**77**) *α*-pinene (**78**) *β*-pinene (**79**) cineole (**80**) *cis*-ocimene (**81**) citronellal (**82**) geranial (**83**) neral (**84**) myrcene (**85**) *R*-citronellene (**86**) citronellyl pentanoate (**87**) nerol (**88**) geraniol (**89**) iso-artemisia ketone (**90**) *trans*-ocimene (**91**) linalool (**92**) neryl acetate (**93**) geranic acid (**94**) geranyl acetate (**95**) 3-bornanone (**96**) 4,8-dimethyl-3,7-nonadien-2-ol (**97**) 3,4,5,6-tetramethyl-2,5-octadiene (**98**) 3,7-dimethyl-6-nonenal (**99**) 2,6-dimethyl-2,6-octadiene-1,8-diol (**100**) 4,5-dimethyl-2,6-octadiene (**101**) *ar*-turmerone (**102**) *α*-turmerone (**103**) *β*-turmerone (**104**) 2-methyl-6-(4-hydroxyphenyl)-2-hepten-4-one (**105**) 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one (**106**) 2-methoxy-5-hydroxybisabola-3,10-diene-9-one (**107**) 2-methyl-6-(4-formylphenyl)-2-hepten-4-one (**108**) 5-hydroxyl-ar-turmerone (**109**) 4-methylene-5-hydroxybisabola-2,10-diene-9-one (**110**) *ar*-curcumene (**111**) *ar*-turmerol (**112**) bisabola-3,10-diene-2-one (**113**) bisabolone (**114**) 4, 5-dihydroxybisabola-2,10-diene (**115**) 4-hydroxybisabola-2,10-diene-9-one (**116**) 4-methoxy-5-hydroxy-bisabola-2,10-diene-9-one (**117**) bisacurone (**118**) bisabolone-9-one (**119**) bisacumol (**120**) turmeronol A (**121**) turmeronol B (**122**) *α*-oxobisabolene (**123**) *α*-zingiberene (**124**) xanthorrhizol (**125**) zingerone (**126**) dehydrozingerone (**127**) *(Z)-α*-atlantone (**128**) *(E)-α*-

atlantone (**129**) *β*-bisabolene (**130**) (6*S*,7*R*)-bisabolene (**131**) *γ*-bisabolene (**132**) *γ*-curcumene (**133**) *β*-curcumene (**134**) *α*-curcumene (**135**) *β*-sesquiphellandrene (**136**) *(Z)*-*γ*-atlantone (**137**) *(E)*-*γ*-atlantone (**138**) (6*S*)-2-methyl-6-[(1*R*,5*S*)-(4-methene-5-hydroxyl-2-cyclohexen)-2-hepten-4-one (**139**) curcuphenol (**140**) curlone (**141**) (**142**)curculonone C (**143**) curculonone D (**144**) curculonone B (**145**) curculonone A (**146**) 2, 5-dihydroxybisabola-3, 10-diene (**147**) (6*R*)-[(1*R*)-1,5-dimethylhex-4-enyl]-3-methylcyclohex-2-en-1-one (**148**) *β*-atlantone (**149**) 2,8-epoxy-5-hydroxybisabola-3,10-diene-9-one (**150**) α-bisabolol (**151**) dihydro-ar-turmerone (**152**) dehydrocurcumene (**153**) (4*S*,5*S*)-germacrone-4,5-epoxide (**154**) dehydrocurdione (**155**) germacrene D (**156**) germacrone (**157**) germacrone-13-al (**158**)*β*-germacene (**159**) 1,10-dehydro-10-deoxy-9-oxozedoarondiol (**160**) curcumenol (**161**)epiprocurcumenol (**162**) isoprocurcumenol (**163**) zedoaronediol (**164**) procurcumadiol (**165**)procurcumenol (**166**) naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene) (**167**) *α*-selinene (**168**) juniper camphor (**169**) corymbolone (**170**) *α*-santalol (**171**) *β*-santalene (**172**) (*E*)-caryophyllene (**173**) caryophyllene oxide (**174**) *β*-elemene (**175**) γ-elemene (**176**) acoradiene (**177**) aristolene (**178**) (*Z*)-*α*-bergamotene (**179**) curcumenone (**180**) di-epi-cedrene (**181**) himachalene (**182**) (*E*)-sesquisabinene hydrate (**183**) bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene) (**184**) γ-gurjunen epoxide (**185**) 1-epi-cubenol (**186**) cubebene (**187**) 7-epi-sesquithujene (**188**) caryophyllene (**189**) 6*α*-hydroxycurcumanolide A (**190**) curcumanolide A (**191**) curcumanolide B (**192**) curcumin L (**193**) *α*-humulene (**194**) 12-oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-,adoxal, (**195**)2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (**196**) (*E*,*E*)-*α*-farnesene (**197**) 5,9-undecadien-2-one, 6,10-dimethyl-, (Z)-, hxadecane-1,2-diol (**198**) nerolidal (**199**) (*Z*)-*β*-farnesene (**200**) nerolidyl propionate (**201**) phytol (**202**) (*E,E,E*)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene (**203**) 2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene (**204**)1,6,10,14-hexadecatetraen-3-ol,3,7,11,15-tetramethyl-, (E,E)- (**205**) hopenone I (**206**) hop-17(21)-en-3β-ol (**207**) hop-17(21)-en-3β-yl acetate (**208**) *β*-sitosterol (**209**) stigmasterol (**210**) gitoxigenin (**211**) 20-oxopregn-16-en-12-yl acetate (**212**) linoleic acid (**213**) 8,11-Octadecadienoic acid (**214**) methyl ester (**215**) palmitic acid (n-hexadecanoic acid) (**216**) oleic acid (**217**) stearic acid (**218**) curcuma-J (**219**) 2-(2'-methyl-1'-propenyl)-4, 6-dimethyl-7-hydroxyquinoline (**220**) 2,3,5-trimethylfuran (**221**) (1,2,3-trimethyl-cyclopent-2-enyl)-methanol (**222**) dicumyl peroxide (**223**) 1-(3-cyclopentylpropyl)-2,4-dimethy-benzene (**224**) 1,4-dimethyl-2-(2-methylpropyl)-benzene (**225**) 2,2'-oxybis[octahydro-7,8,8-trimethyl-4,7-methanobenzofuran (**226**) cyclohexyl formate (**227**) methyleugenol, 3,3,5-trimethyl-cyclohexanol acetate (**228**) 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one (**229**) 2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-cyclohexene-1-carboxaldehyde (**230**) bicyclo[3.3.1]nonan-9-one, 2,4-dimethyl-3-nitro-(exo)- (**231**) 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol (**232**) pyrazolo[1,5-a]pyridine, 3,3a,4,7-tetrahydro-3,3-dimethyl-, (3aS) (**233**) β-vatirenene (**234**) (–)-isolongifolol (**235**) 2,4,6-triethylcyclohexyl)methanol (**236**) 2-ethenyl-1,1-dimenthyl-3-methylene-cyclohexane (**237**) 2-isopropylidene-3-methylhexa-3,5-dienal, 2-methoxy-4-vinyl phenol (**238**) 5-isopropenyl-1,2-dimethylcyclohexan-2-enol7-epi-*cis*-sesquisabinene hydrate, agarospirol (**239**) benzene-2-methyl-1,4-bis(1-methylethyl, *cis*-*p*-menth-2,8-dienol (**240**) *cis*-sabinol (**241**) *cis*-*Z*-α-

bisabolene epoxide (**242**) *cis*-β-elemenone (243) curdione, (**243**) curzerene, (**244**)dehydrosaussurea lactone dihydrocostunolide (**245**) *DL*-2,3-butanediol (**246**) furanodiene (**247**) geranyl-*p*-cumene, (**248**) hemellitol (**249**) isocurcumenol (**250**) isolongifolol (**251**) isoshyobunone (**252**) *L-trans*-chrysanthenyl acetate (**253**) *m*-eugenol (**254**) α-cedrene (**255**) α-cubebene (**256**) α-thujone (**257**) β-cedrene

The literature-based 3D or 2D structure of phytochemicals of target proteins histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) and the modelled protein were retrieved in .sdf format from NCBI PubChem. For the conversion of 2D to 3D conformation open Babel molecule format converter were used, Marvin Sketch software (version 15.10.0) performed conversion from .sdf to .pdb (for docking) and mol (for molecular properties prediction) file. For the structure of the ligand that was not available in NCBI PubChem, chemical structures were prepared using Marvin Sketch. Ligands energy was minimized by applying mmff94 force field and conjugate gradients optimization algorithm using PyRx-Python prescription 0.8. for 200 steps (Dallakyan *et al.,* 2015).

3.2.4. Molecular docking

Crystal structure of target enzyme histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) were obtained from the RCSB protein data bank along with the structure of a protein that have been modeled. The preparation of the target enzyme with the Auto Dock Tools involved in the addition of hydrogen atoms to the target enzyme, which is a necessary step for the computation of partial atomic charges. Gasteiger charges will be considered for each atom present in the target in Auto Dock 4.2.

To dock small-molecule libraries to a macromolecule virtual molecular screening is used in order to hit upon lead compounds with desired biological function. In PyRx software, we perform docking of the ligands and proteins. PyRx software is open version software with an intuitive user interface that runs on all major operating systems (Linux, Windows, and Mac OS) (Dallakyan *et al.,* 2015). Receptor-based

molecular docking was conducted using GLIDE software from PyRx-Python prescription 0.8. Each of these compounds was docked into target protein accordingly with positions, orientations, and conformations of the ligand in the receptor binding site, and the docking structure possessing the lowest energy was preferred (Singh *et al.,* 2015). The proteins and the ligands were loaded into Auto Dock Tools 4.2 (ADT) for docking experiments. After merging non-polar hydrogen and torsions applied to the ligands by rotating all rotatable bonds gasteiger partial charges are assigned Docking calculations carried out on the protein models. With the aid of Auto Dock tools polar hydrogen atoms and solvation parameters were added. AutoDock 4.2 offers the option of three search algorithms to explore the space of active binding with different efficacy. Docking was performed with the targeted proteins interface by keeping the number of points 25.0000, 25.0000 and 25.0000, in X, Y and Z dimension and centre grid box values were kept. The grid box includes the entire binding site of the proteins interface and provides enough space for the ligands translational and rotational walk. After that, PyRx-Python prescription 0.8 is used for visualization of the interaction pattern in the protein-ligand complex (Kumar *et al.,* 2016).

3.2.5 Protein-Ligand Interaction and LigPlot

The protein-ligand complexes in .pdb format are displayed, edited and run via the software LigPlot+ (version v.1.4.5) for generation of LigPlot schematic diagrams. The protein-ligand interaction along with the hydrogen bonding and hydrophobic interactions with the complex binding residues are given for the lead phytochemicals. The LigPlot shows the amino acid of the target protein which is involved in the interaction with the ligand. The interaction profile of lead phytochemicals and the interface between heterodimers of a protein can be determined. The amino acids involved in hydrogen bonding and hydrophobic interaction with the ligands can also be easily determined.

3.2.5 Protein-Ligand Binding Surface-Structure Determination

The binding of the modified ligands with active sites of the targeted protein is determined and visualized by surface structure determination of protein and ligand

binding. It is a molecular modelling technique whereby the interaction between the protein and ligands is determined by the position and orientation of the ligand when bound to the protein. The surface structure of the protein and ligand is determined through PyMOL Molecular Graphics System software (version 1.1). The modified pdbqt files of proteins and ligands are prepared and displayed in PyMOL which gives a commendable visualization of the protein-ligand interaction. The best docking score of proteins are chosen with the ligands having the best binding affinity and the surface structure was prepared.

3.3 *In vitro* validation

3.3.1 Histamine detection in fermented curd

Fresh samples of curd were taken in different test tubes such that each test tube has 4ml samples each. These samples were incubated for about 10-14 days at 37ºC. After 14days the test tubes were retained and sought for histamine detection by certain biochemical tests utilizing colour forming ability.

3.3.2 Histamine detection by biochemical tests for colour formation

The incubated curd samples were obtained on day 14 and tested for the presence of histamine. Each sample was added with 1N NaOH (sodium hydroxide) in the ratio 7:3. Next, a 10ml sulfanilic acid solution of concentration 0.05mg/ml is added along with 2 drops of 2M HCl(hydrochloric acid) and 2drops of $NaNo_2$ (sodium nitrite). Formation of a deep red colour gives a positive result to this biochemical identification test (Nguyen and Nguyen, 2015).

3.3.3 Semi-Qualitative Analysis and Histamine detection

Based on the rudimentary detection test we have prepared an experimental setup for qualitative analysis of histamine detection and its inhibition using turmeric. A fresh sample of curd was taken in a set of 11 test tubes containing 3.5ml of curd in each test tube. Next powdered turmeric was taken in different concentrations which were initially dissolved in milk as a vehicle in the experiment. Turmeric was measured as 10, 20, 25, 50, 100, 150, 200, 250 and 300mg per 0.5ml of the vehicle and was added

to 9 of the tubes as a sample. A vehicle control was taken which comprised of the 0.5ml vehicle in the absence of turmeric. Also, a negative control containing 4ml of curd was taken and incubated for about two weeks at 37ºC along with the other tubes. These test samples were then tested for the presence or formation of histamine as well as the interference of turmeric for its inhibition.

3.4 *In vitro* anticancer activity by MTT assay

In vitro cell proliferation assay were perform using MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]. The compound 002 was tested for the antiproliferative activity against HT29 (colon cancer) cell lines at different concentrations (0.5, 0.25, 0.125, 0.08, and 0.04 mg/ml). Cells were seeded at a density of 1×10^4cells/well in a 96-well plate with media containing 10% FBS and 1% penicillin/streptomycin. The cells were allowed to attach and grow for 48 hrs. To test the growth inhibitory effects of the compound, cells were treated with various concentrations and incubated for 3 days at 37°C and 5% CO2 humidified atmosphere. After incubation 100µL of 5mg/ml, MTT solution was added to cells and further incubated for 4 hrs at 37°C. After incubation, the medium was removed and 200µL DMSO was added to each well to dissolve the formazan crystals. The absorbance of formazan dye was read using an ELISA plate reader at 595nm and the optical density (OD) was recorded. The following formula was used to calculate the inhibitory rate of cell growth. Controls and samples were assayed in triplicate. The results were shown as mean ± SD (Rahamoz Haghighi *et al.,* 2016).

Growth inhibition% = $[(A_C - A_S)/A_C] \times 100$

Where, *I* represented inhibition and A_C and A_S are the absorbance values of the control and the sample, respectively. Three replicates were made for each sample and results were expressed as mean ± SD (Kumar *et al.,* 2014).

3.5 *In vitro* antioxidant activity assay by DPPH assay

The free radical scavenging activity of the compound A was measured *in vitro* by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Appropriate DPPH solution concentrations were prepared in methanol followed by addition of 1 mL of the test sample at different

concentration. The content was mixed and allowed to stand at room temperature for 30 minutes and absorbance was measured at 517 nm. The lower absorbance of the reaction mixture indicated higher free radical activity (Tailor *et al.,* 2014). The percentage scavenging activities (%Inhibition) at different concentrations of the extracts were calculated using the following formula:

$$(\%)\ I = [(A_C - A_S)/A_C] \times 100$$

Where, *I* represented inhibition and A_C and A_S are the absorbance values of the control and the sample, respectively. Three replicates were made for each sample and results were expressed as mean ± SD (Kumar *et al.,* 2014).

3.6 In vitro antibacterial activity assay by disk diffusion method

Antimicrobial activity of the test compound A will be determined by using Kirby-Bauer disc diffusion method against Gram-negative *Escherichia coli*. The inoculum suspension of bacterial strains will be swabbed on the entire surface of the prepared Luria-Bertani (LB) agar plates. Sterile 6 mm diameter paper discs (Himedia) saturated with 20 µL of phytochemicals prepared in DMSO (containing 2 mg extract/disc) will be aseptically placed on the upper layer of the inoculated agar surfaces and the plates will be incubated at 37°C for 24 hours. The antibacterial activity will be determined by measuring the diameter of the zone of inhibition (ZOI) surrounding discs. Standard antibiotic discs of penicillin (10 µg/disc) and norfloxacin (10 µg/disc) would be used as positive control. Discs containing 20 µL DMSO will be used as a negative control. The antimicrobial assay will be performed in triplicate and the results will be reported as the average of three replicates (Bauer *et al*., 1966)

4. Results

4.1 Protein Preparation and modelling

The protein structures were retrieved from PDB and the remaining protein structure of histamine H_4 receptor that could not be retrieved from PDB was generated by using SWISS-MODEL. After generation of protein structure by protein modelling techniques, the modelled protein was checked for its efficacy by analysing several parameters. Structure analysis of the modelled protein was done along with the determination of Ramachandran Plot. Figure 4.1 shows the quality factor or efficacy of the protein structure. The structural resolution of the protein of the protein must exceed 95% to be considered as competent and acceptable. The quality factor of the modelled protein was found to be 97.112 which show that it qualifies the 95% rejection limit.

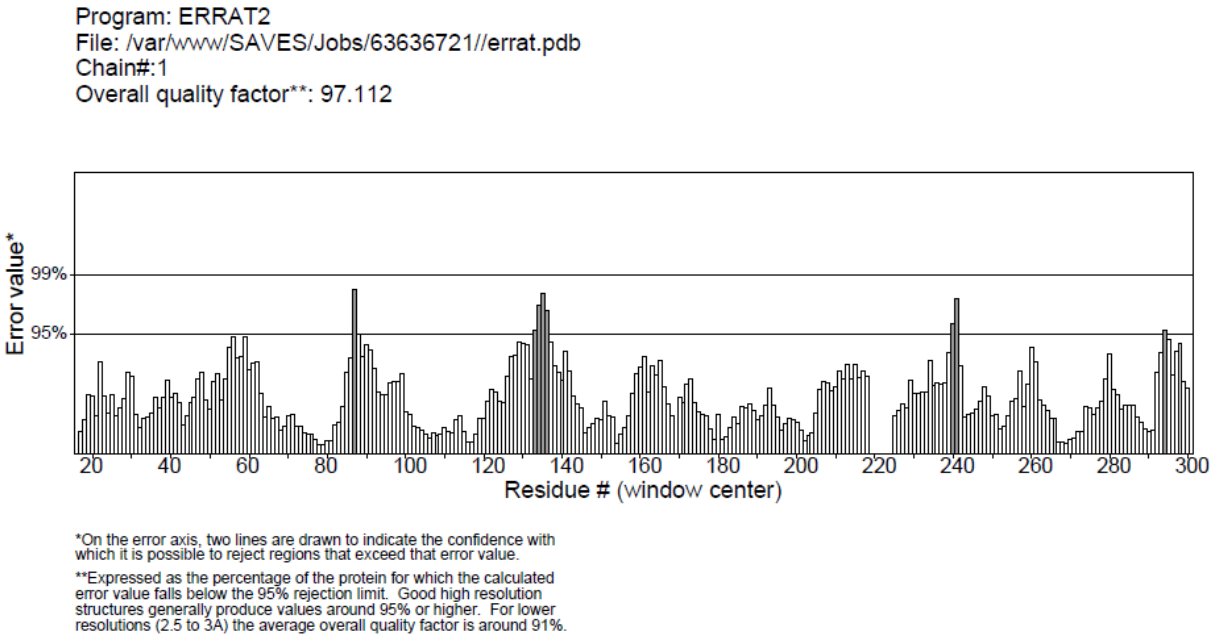


Figure 4.1- The ERRAT graph for determining protein model quality factor for the modelled protein histamine H_4 receptor

The Ramachandran Plot of the modelled histamine H_4 receptor protein retrieved from PROCHECK is shown in figure 4.2. An ideally accepted model must have about 90% of the values of ϕ and ψ dihedral angles of the amino acid residues lying in the most

favoured region (red). The modelled protein shows 89% in terms of residues in the most favoured region. The rest of the dihedral angles of the remaining amino acid residues lying in the additional or generously allowed regions are minimally distributed for about 9.4% and 0.6%.

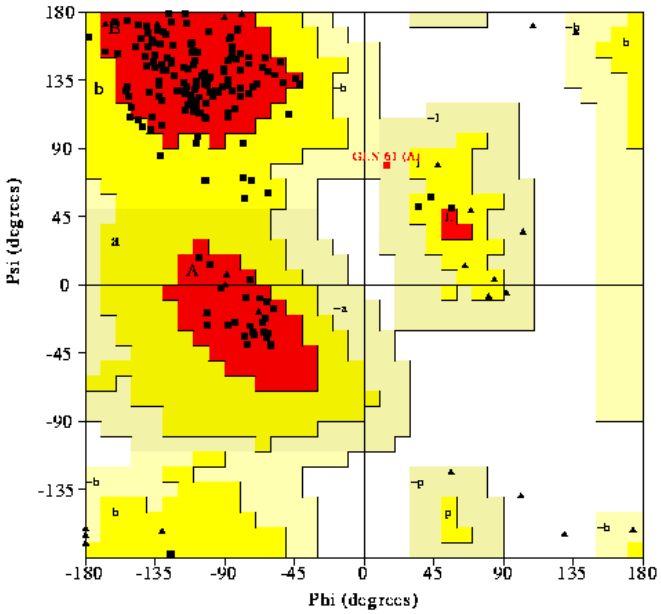


Figure 4.2- Ramachandran plot showing values in phi and psi angles of the modelled protein histamine H_4 receptor.

The PROVE Plot helps us determine and calculate an average statistical Z-score deviation for the modelled protein. Figure 4.3 shows the estimated average Z-score and Z-score RMS.

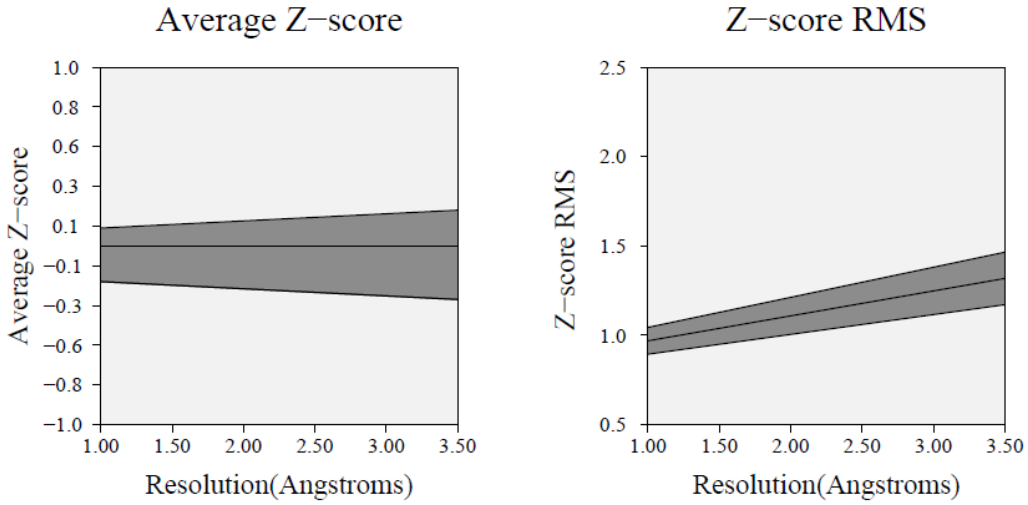


Figure 4.3- Graphical representation of Z-score of the modelled

4.2 Ligand- Protein Binding Analysis

Different literature database has been searched for the presence of phytochemicals in curcumin powder. At the end of literature survey we found the presence of 265 phytochemicals in curcumin powder of different origin and brand (Li *et al*., 2011). Over all literature showed that all the phytochemicals were present in every test sample but the quantity of some of the phytochemicals varied in some of the test samples. The literature based ligands of *C. longa* like curcumin (curcumin I), demethoxycurcumin (curcumin II), 1-(4-hydroxy-3-methoxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione, 1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione, bisdemethoxycurcumin (curcumin III), tetrahydroxycurcumin, 5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one, 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one, 1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione, 5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one. 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione, 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one, 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one, 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one, 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one, 1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene, Cyclocurcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one, 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, 1,5-bis(4-hydroxyphenyl)-penta-(1E,4E)-1,4-dien-3-one, 1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1, 4-pentadiene-3-one, 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one, 4''-(4'''-hydroxyphenyl)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate, 4''-(4'''-hydroxyphenyl-3-methoxy)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl)-propenoate, calebin-A, (*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one, (*E*)-ferulic acid, (*Z*)-ferulic acid, vanillic acid, vanillin, *p*-cymene, *m-cymene*, *α*-terpinene, *γ*-terpinene α-cedrene, *β*-phellandrene, *p*-mentha-1,4(8)-diene, terpinen-4-ol, 4-terpinol, limonene, terpinolene, thymol, phellandrol, carvacrol, (*E*)-carveol, *γ*-terpineol, menthol, 1,3,8-paramenthatriene, p-methylacetophenone, piperitone, *o*-cymene, carvone, *p*-menth-

8-en-2-one, *α*-thujene, *α*-terpineol, *p*-cymen-8-ol, *p*-meth-8-en-2-one, piperitone epoxide, sylvestrene, menthofuran, *β, β*-dimethylstyrene, camphor, teresantalol, benzene, 1-methyl-4-(1-methylpropyl), 2-norpinanone, borneol, bornyl acetate, (*E*)-chrysanthenyl acetate, (*Z*)-cinerone, (*Z*)-sabinol, 2-(2,5-dihydroxy-4-methylcyclohex-3-enyl)propanoic acid, camphene, 3-carene, 2-carene, ascaridole, *α*-pinene, *β*-pinene, cineole, *cis*-ocimene, citronellal, geranial, neral, myrcene, *R*-citronellene, citronellyl pentanoate, nerol, geraniol, iso-artemisia ketone, *trans*-ocimene, linalool, neryl acetate, geranic acid, geranyl acetate, 3-bornanone, 4,8-dimethyl-3,7-nonadien-2-ol, 3,4,5,6-tetramethyl-2,5-octadiene, 3,7-dimethyl-6-nonenal, 2,6-dimethyl-2,6-octadiene-1,8-diol, 4,5-dimethyl-2,6-octadiene, *ar*-turmerone, *α*-turmerone, *β*-turmerone, 2-methyl-6-(4-hydroxyphenyl)-2-hepten-4-one, 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one, 2-methoxy-5-hydroxybisabola-3,10-diene-9-one, 2-methyl-6-(4-formylphenyl)-2-hepten-4-one, 5-hydroxyl-ar-turmerone, 4-methylene-5-hydroxybisabola-2,10-diene-9-one, *ar*-curcumene, *ar*-turmerol, bisabola-3,10-diene-2-one, bisabolone, 4, 5-dihydroxybisabola-2,10-diene, 4-hydroxybisabola-2,10-diene-9-one, 4-methoxy-5-hydroxy-bisabola-2,10-diene-9-one, bisacurone, bisacurone A, bisabolone-9-one, bisacumol, turmeronol A, turmeronol B, *α*-oxobisabolene, *α*-zingiberene, xanthorrhizol, zingerone, dehydrozingerone, *(Z)-α*-atlantone, *(E)-α*-atlantone, *β*-bisabolene, (6*S*,7*R*)-bisabolene, *γ*-bisabolene, *γ*-curcumene, *β*-curcumene, *α*-curcumene, *β*-sesquiphellandrene, *(Z)-γ*-atlantone, *(E)-γ*-atlantone, (6*S*)-2-methyl-6-[(1*R*,5*S*)-(4-methene-5-hydroxyl-2-cyclohexen)-2-hepten-4-one, curcuphenol, curlone, curculonone C, curculonone D, curculonone B, curculonone A, 2, 5-dihydroxybisabola-3, 10-diene, (6*R*)-[(1*R*)-1,5-dimethylhex-4-enyl]-3-methylcyclohex-2-en-1-one, *β*-atlantone, 2,8-epoxy-5-hydroxybisabola-3,10-diene-9-one, α-bisabolol, dihydro-ar-turmerone, dehydrocurcumene, (4*S*,5*S*)-germacrone-4,5-epoxide, dehydrocurdione, germacrene D, germacrone, germacrone-13-al, *β*-germacene, 1,10-dehydro-10-deoxy-9-oxozedoarondiol, curcumenol, epiprocurcumenol, isoprocurcumenol, zedoaronediol, procurcumadiol, procurcumenol, naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene), *α*-selinene, juniper camphor, corymbolone, *α*-santalol, *α*-santalene, *β*-santalene, (*E*)-caryophyllene, caryophyllene oxide, *β*-elemene, γ-

elemene, acoradiene, aristolene, (*Z*)-*α*-bergamotene, curcumenone, di-epi-cedrene, himachalene, (*E*)-sesquisabinene hydrate, bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene), γ-gurjunen epoxide, 1-epi-cubenol, cubebene, 7-epi-sesquithujene, caryophyllene, 6*α*-hydroxycurcumanolide A, curcumanolide A, curcumanolide B, curcumin L, *α*-humulene, 12-oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-,adoxal, 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (*E*,*E*)-*α*-farnesene, 5,9-undecadien-2-one, 6,10-dimethyl-, (Z)-, hxadecane-1,2-diol, nerolidal, (*Z*)-*β*-farnesene, nerolidyl propionate, phytol, (*E,E,E*)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene, 2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene, 1,6,10,14-hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-, hopenone I, hop-17(21)-en-3β-ol, hop-17(21)-en-3β-yl acetate, *β*-sitosterol, stigmasterol, gitoxigenin, 20-oxopregn-16-en-12-yl acetate, linoleic acid, 8,11-Octadecadienoic acid, methyl ester, palmitic acid (n-hexadecanoic acid), oleic acid, stearic acid, curcuma-J, 2-(2'-methyl-1'-propenyl)-4, 6-dimethyl-7-hydroxyquinoline, 2,3,5-trimethylfuran, (1,2,3-trimethyl-cyclopent-2-enyl)-methanol, dicumyl peroxide, 1-(3-cyclopentylpropyl)-2,4-dimethy-benzene, 1,4-dimethyl-2-(2-methylpropyl)-benzene, 2,2'-oxybis[octahydro-7,8,8-trimethyl-4,7-methanobenzofuran, cyclohexyl formate, methyleugenol, 3,3,5-trimethyl-cyclohexanol acetate, 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-cyclohexene-1-carboxaldehyde,bicyclo [3.3.1] nonan-9-one, 2,4-dimethyl-3-nitro- (exo)-, 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol, pyrazolo[1,5-a]pyridine, 3,3a,4,7-tetrahydro-3,3-dimethyl-, (3aS), β-vatirenene, (–)-isolongifolol, 2,4,6-triethylcyclohexyl)methanol, 2-ethenyl-1,1-dimenthyl-3-methylene-cyclohexane, 2-isopropylidene-3-methylhexa-3,5-dienal, 2-methoxy-4-vinyl phenol, 5-isopropenyl-1,2-dimethylcyclohexan-2-enol, 7-epi-*cis*-sesquisabinene hydrate, agarospirol, benzene-2-methyl-1,4-bis(1-methylethyl, *cis*-*p*-menth-2,8-dienol, *cis*-sabinol, *cis*-*Z*-α-bisabolene epoxide, *cis*-β-elemenone, curdione, curzerene, dehydrosaussurea lactone, dihydrocostunolide, *DL*-2,3-butanediol, furanodiene, geranyl-*p*-cumene, hemellitol, isocurcumenol, isolongifolol , isoshyobunone, *L*-*trans*-chrysanthenyl acetate, *m*-eugenol, α-cedrene, α-cubebene, α-thujone, β-cedrene were chosen and their structures were either downloaded from Pubchem (of those which are available)

and the rest were prepared in Marvin Sketch (Version 5.10.0). These ligands were docked against the targeted proteins for prediction of the binding score. The table 4.1 summarizes the results of molecular d docking studies comprising lowest binding energy and proteins residues involved in hydrogen bonding (265 docking runs by library preparation) with screened ligands (selected standard inhibitors and phytochemicals). Further, prediction of ligands with lowest binding energy is done.

Table 4.1: Comparative table of binding affinity between ligands with targeted proteins is given. Here A(3rze), B(4e1o), C(2c9y), D(3hkk), E(3o8y), F(4qj4), G(modeled protein).

PCID	Standard	A	B	C	D	E	F	G
667477	Doxepin	-12.02	-	-	-	-	-	-
92893	Histidine methyl ester	-	-5.23	-	-	-	-	-
5437	2,4-thiazolidinedione	-	-	-9.43	-	-	-	-
4441104	Glutathione sulfonic acid	-	-	-	-7.42	-	-	-

PCID	Name	A	B	C	D	E	F	G
5280450	linoleic acid	-10.21	-2.59	-3.56	-0.77	-4.21	-1.21	-2.02
445639	Oleic acid	-9.42	-2-.83	-2.99	-1.39	-1.61	-1.21	-2.21
5281	Stearic acid	-9.35	-2.01	-2.27	-0.43	-3.64	-0.98	-2.21
5312487	8,11-Octadecadienoic acid	-9.02	-2.86	-3.55	-0.76	-3.79	-0.93	-2.63
15858385	Tumeronol-A	-8.97	-4.43	-4.59	-3.95	-6.12	-4.19	-5.07
-	Zingerone	-8.91	-3.41	-4.55	-2.87	-5.8	-3.6	-4.52
-	Limonene	-8.88	-2.18	-4.41	-3.92	-4.78	-3.7	-3.92
-	*α*-selinene	-8.9	-	-5.61	-5.66	-6.02	-4.95	-4.45
360253	Curcuphenol	-8.72	-4.22	-4.83	-4.47	-5.54	-4.11	-4.45
-	Menthol	-8.7	-	-3.81	-4.33	-4.48	-1.86	-2.63
5315469	2-Methyl-6-(4-methylphenyl)hept-2-en-4-ol	-8.69	-2.76	-4.23	-3.87	-5.48	-3.52	-4.07
93135	Xanthorrhizol	-8.57	-4.8	-4.42	-4.36	-5.35	-3.96	-4.42

167812	(+)-Curcumenol	-8.55	-2.65	-3.58	-4.36	-2.77	-3.2	-2.45
-	naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene)	-8 .51	-	-5.09	-4.47	-6.25	-4.86	-5.93
53464495	Curcumin-d6	-8.8	-6.18	-5.38	-4.73	-6.67	-4.49	-4.08
10955433	Turmeronol-B	-8.36	-4.76	-4.95	-4.7	-5.98	-3.82	-3.99
92281781	Curcumenol	-8.31	-3.82	-4.07	-3.74	-3.71	-3.6	-3.68
10921984	dihydro-ar-turmerone	-8.28	-2.47	-4.16	-3.51	-5.09	-2.93	-4.12
2889	Curcumin	-8.62	-5.84	-5.9	-6.52	-6.77	-5.07	-5.43
11241433	(4R,6S)-2-Methyl-6-(4-methylphenyl)hept-2-en-4-ol	-8.25	-3.94	-4.04	-4.49	-5.34	-2.89	-3.75
11063457	Dehydrocurcumene	-8.23	-1.71	-3.38	-4.44	-4.47	-1.29	-3.12
10399139	Isocurcumenol	-8.16	-2.68	-4.67	-	-4.18	-2.25	-1.23
102165199	Curculonone-C	-8.14	-3.08	-4.14	-3.23	-5.37	-2.98	-2.94
643779	Neral	-8.11	-2.17	-4.07	-	-3.84	-3.88	-2.6
20055539	(*E*)-sesquisabinene hydrate	-8.08	-	-	-	-	-	-
14191392	dehydrocurdione	-8.08	-3.03	-3.23	-1.53	-2.46	-2.65	-2.43
91753574	cis-(Z)-.alpha.-Bisabolene epoxide	-8.08	-2.51	-4.03	-3.65	4.75	2.48	-3.5
15095	Himachalene	-8.05	-2.19	-3.82	-3.09	-3.49	-1.12	-2.3
12299867	*(Z)-α*-atlantone	-8.03	-2.34	-3.43	-4.78	-5.37	-2.43	-3.63
-	bisabolone-9-one	-8.03		-2.87	-2.13	-3	-2.79	-2.12
519857	1-epi-cubenol	-7.94	-2.12	-3.97	-3.15	-4.29	-2.69	-2.13
92139	*ar*-curcumene	-7.93	-2.23	-3.47	-4.03	-4.32	-1.79	-3.36
13967857	*β*-atlantone	-7.92	-3.06	-3.51	-4.17	-5.35	-1.54	-3.47
442360	*α*-curcumene	--7.92	-1.96	-3.41	-4.29	-4.03	-1.56	-2.68
6436348	Germacrone	-7.89	-2.3	-3.03	-3.32	-3.08	-2.34	-1.52

14543198	Isoprocurcumenol	-7.88	-2.75	-5.19	-3.2	-4.48	-3.85	-2.59
14633002	Germacrone-13-al	-7.85	-2.85	-3.4	-2.95	-3.64	-2.36	-2.59
91747196	Cubebene	-7.8	-2.75	-3.61	-3.76	-4.26	-2.28	-2.19
91750423	gamma.-Gurjunene epoxide	-7.8	-2.72	-3.27	-	-2.73	-1.58	-0.5
86609	Alpha-Cubebene	-7.77	-2.31	-3.44	-3.72	-3.46	-2.2	-2.06
12304273	Gamma-Curcumene	-7.76	-2.3	-3.18	-4.16	-4.05	-1.51	-3.41
10421034	1-Bisabolone	-7.74	-2.41	-3.32	-3.42	-5.45	-2.35	-3.25
519762	*cis*-β-elemenone	-7.73	-2.74	-3.54	-2.86	-2.97	-1.64	-2.95
6432312	Gamma-Elemene	-7.72	-1.82	-3.61	-3.5	-3.09	-1.02	-2.31
1742210	Caryophyllene oxide	-7.69	-2.06	-3.44	-3.15	-3.69	-2.4	-1.09
10104370	Beta-Bisabolene	-7.67	-2.04	-3.7	-4.03	-4.52	-1.76	-2.86
196216	Curlone	-7.65	-3.68	-3,7	-3.4	-5.09	-2.1	-2.94
56927990	7-epi-sesquithujene	-7.64	-2.06	-3.11	-3.93	-2.7	-1.54	-2.66
10857025	Bisabolone	-7.61	-3.78	-3.99	-3.6	-5.11	-1.55	-2.85
101967134	Dihydrocostunolide	-7.6	-3.97	-3.47	-3.45	-2.37	-2.24	-1.61
17750987	Alpha-cedrene	-7.59	-1.49	-2.99	-3.16	-3,86	-1.64	-
-	2,8-epoxy-5-hydroxybisabola-3,10-diene-9-one	-7.56	-4.37	-3.64	-3.25	-5.3	-2.07	-2.98
1548883	(*Z*)-ferulic acid	-7.56	-6	-3.97	-3.61	-3.74	-2.59	-4.11
189061	Procurcumenol	-7.55	-3.2	-4.82	-	-4.44	-3.4	-2.63
10586	Bisabolol	-7.55	-1.87	-4.31	-3.15	-5.72	-2.45	-3.27
5368797	*α*-santalol	-7.55	-3.49	-4.41	-3.31	-4.02	-2.72	-3.6
10703	o-cymene	-7.9	-1.93	-4.95	-4.84	-6.24	-3.74	-3.54
91698329	(Z)-gamma-Atlantone	-7.48	-3.77	-3.72	-3.35	-5.17	-2.32	-3.47
14014430	Beta-Curcumene	-7.47	-2.27	-3.21	-4.1	-4.09	-1.66	-3.18

12305301	Curzerene	-7.46	-2.64	-4.01	-3.01	-2.87	-1.84	-2.63
5280435	Phytol	-7.44	-1.46	-2.49	-2.92	-4.96	-1.88	-1.88
90475698	Beta-Germacrene C	-7.44	-2.12	-3.53	-3.26	-3.3	-2.08	-1.69
-	(*E*)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one	-7.43	-5.82	-4.07	-4.09	-4.22	-2.03	-3.42
5373727	Germacrene D	-7.43	-2.2	-3.58	-3.6	-3.62	-1.88	-2.26
31211	Zingerone	-7.43	-3.33	-5.45	-4.21	-4.72	-3.31	-5.12
-	Curcumenol	-7.4	-	-3.39	-4.37	-3.57	-1.32	-2.01
92776	Alpha-Zingiberene	-7.38	-2.11	-3.24	-3.81	-4.7	-1.51	-2.83
-	*γ*-curcumene	-7.37	-	-3.45	-3.88	-4.8	-2.05	-3.71
-	4-hydroxybisabola-2,10-diene-9-one	-7.33	-4.3	-4.04	-0.92	-3.41	-3.63	-2.93
91751355	Geranyl-p-cymene	-7.32	-2.27	-4.11	-4.13	-5.27	-1.73	-3.65
10263440	Epiprocurcumenol	-7.32	-3.96	-3.49	-3	-	-3.46	-2.7
-	*β*-pinene	-7.3		-3.45	-2.73	-3.13	-2.3	-1.48
14287397	Bisacurone	-7.28	-4.1	-5.43	-4.08	-6.98	-3.52	-5.91
24834047	1,10-dehydro-10-deoxy-9-oxozedoarondiol	-7.24	-2.62	-4.65	-	-	-4	-2.67
12315492	Beta-Sesquiphellandrene	-7.24	-1.98	-3.55	-3.89	-4.54	-1.67	-2.64
14633011	Procurcumadiol	-7.23	-3.3	-4.27	-	-4.59	-3.1	-3.02
102165198	curculonone B	-7.23	-3.15	-4.17	-3.68	-5.89	-2.88	-3.05
90351	Acoradiene	-7.23	-2.28	-3.35	-3.73	-2.97	-2.32	-1.77
14287395	Bisacurone-A	-7.2	-3.24	-5.05	-4.13	-6.15	-3.23	-4.23
21675005	Agarospirol	-7.18	-2.79	-3.96	-3.14	-4.86	-2.77	-2.56
-	*γ*-terpineol	-7.18	-	-3.09	-3.6	-3.16	-1.68	-2.77

-	3-carene	-7.17	-4	-4.3	-3.12	-3.84	-2.28	-3.93
-	vanillic acid	-7.17	-3.76	-4	-3.37	-3.99	-3.15	-3.87
196216	Curlone	-7.16	-3.68	-3.7	-3.4	-5.09	-2.1	-2.94
6641	dicumyl peroxide	-7.15	-3.18	-3.45	-4.13	-4.53	-1.79	-1.97
985	Palmitic acid	-7.12	-2.35	-2.12	-0.61	-3.95	-0.76	-1.77
-	*α*-oxobisabolene	-7.08	-	-4.29	-3.28	-4.98	-3.54	-3.76
6918391	Beta-elemene	-7.08	-2.22	-2.83	-3.33	-2.78	-1.38	-2.49
5281522	Caryophyllene	-7.08	-1.98	-3.26	--	-3.31	-1.55	-1.5
3033866	Gamma-Bisabolene	-7.07	-2.92	-3.52	-3.75	-5.03	-1.97	-2.75
94164	Alpha-Santalene	-7.04	-1.6	-2.91	-3.84	-3.82	-0.98	-1.89
12315160	Cis-Sabinol	-7.03	-3.96	-3.76	-3.52	-3.5	-2.98	-2.98
14543198	Isoprocurcumenol	-7.02	-2.75	-5.19	-3.2	-4.48	-3.85	-2.59
-	2-methyl-6-(4-formylphenyl)-2-hepten-4-one	-7.01	-3.77	-3.16	-2.94	-3.16	-1.58	-2.31
5318673	Isoshyobunone	-7.01	-2.54	-3.12	-3.07	-3.67	-	-2.72
5362828	Curdione	-6.99	-2.54	-3.15	-3.56	-2.68	-2.53	-1.63
10856614	Alpha-selinene	-6.95	-2.32	-3.7	-3.51	-3.31	-1.64	-2.35
153845	Curcumenone	-6.95	-3.24	-4.6	-3.89	-4.24	-2.63	-2.57
6365122	(E)-Atlantone	-6.92	-3.03	-3.83	-4.45	-4.96	-2.2	-2.88
10545	Ascaridole	-6.89	-	-3.07	-3.01	-4.31	-1.45	-2.56
-	2-norpinanone	-6.89	-	-2.36	-3.49	-3.36	-2.37	-3.11
-	iso-artemisia ketone	-6.86	-	-3.47	-3.37	-3.25	-1.78	-1.12
608753	β-vatirenene	-6.81	-2.72	-3.17	-3.67	-3.83	-2.11	-2.08
102165200	Curculonone-D	-6.81	-3.87	-4.43	-3.74	-5.33	-5.6	-5.74
6989	Thymol	-6.77	-2.47	-3.53	-3.15	-3.64	-1.87	-2
5318101	Alpha-Humulene	-6.76	-1.88	-2.95	-3.34	-3.42	-1.97	-1.85

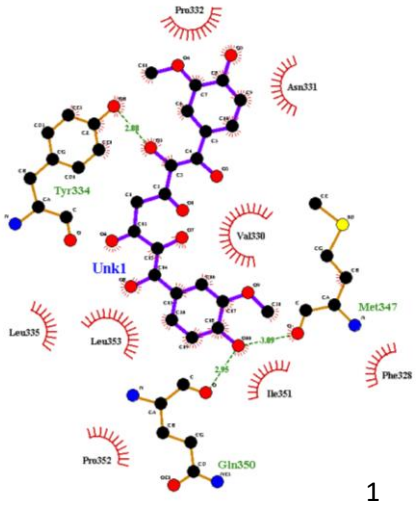
12311096	Isolongifolol	-6.73	-3.2	-4.02	-2.44	-3.53	-2.15	-0.88
44409528	dehydrosaussurea lactone	-6.72	-3.14	-4.53	-3.49	-3.69	-1.96	-2.29
14191394	Curcumanolide B	-6.72	-4.3	-3.75	-2.62	-3.38	-2.02	-2.32
-	4-terpinol	-6.69	-	-3.25	-2.42	-2.39	-2.25	-1.75
636458	Furanodiene	-6.68	-2.16	-3.56	-3.96	-3.09	-2.56	-2.79
7462	*α*-terpinene	-6.66	-1.91	-3.69	-3.5	-1.89	-1.81	-2.72
10812	10812	-6.65	-	-2.98	-2.6	-2.82	-1.9	-1.39
102165197	Curculonone-A	-6.61	-2.91	-5.06	-3.78	-5.4	-2.81	-3.49
16217350	(–)-isolongifolol	-6.6	-3.03	-3.27	-2.87	-3.18	-2.42	-2.31
90475698	*β*-germacene	-6.6	-2.12	-3.53	-3.26	-3.3	-2.08	-1.69
1183	Vanillin	-6.58	-3.68	-4.15	-4.26	-4.67	-2.64	-3.75
596375	*m*-eugenol	-6.58	-4.62	-4.1	-3.46	-4.14	-2.8	-3.32
91698330	(E)-gamma-Atlantone	-6.57	-2.51	-4.35	-3.41	-4.7	-2.65	-3.61
98037	1,2-Hexadecanediol	-6.54	-2.14	-2.78	-3.73	-4.86	-2.68	-1.56
6429303	(*Z*)-*α*-bergamotene	-6.54	-2.47	-3.44	-3.83	-2.63	-1.8	-1.93
261491	Alpha-Thujone	-6.53	-3.44	-2.98	-3.31	-3	-1.9	-2.58
64685	Borneol	-6.47	-3.44	-2.07	-2.13	-3.56	-1.47	-1.86
-	1,10-dehydro-10-deoxy-9-oxozedoarondiol	-6.42	-	-4.23	-4	-4.58	-3.07	-3.82
637429	calebin-A	-6.39	-6.65	-2.5	-3.6	-3.73	-1.37	-1.86
5354238	dehydrozingerone	-6.37	-3.71	-5.65	-3.89	-4.76	-3.21	-4.45
10686	Hemellitol	-6.36	-1.79	-3.07	-2.89	-3.51	-1.68	-2.12
530421	Aristolene	-6.34	-1.8	-2.99	-3.08	-2.07	-1.22	-0.52
5281516	(E,E)-alpha-farnesene	-6.33	-0.56	-2.31	-2.66	-3.35	-0.16	-1.84
6448	bornyl acetate	-6.29	-4.02	-1.81	-4.47	-2.67	-1.71	-1.67
14191393	Curcumanolide A	-6.28	-2.48	-3.24	-	-2.71	-2.99	-2.52

7463	p-cymene	-6.27	-1.79	-3.34	-3.12	-4.72	-2.98	-3.43
8500	p-methylacetophenone	-6.27	-2.93	-3.53	-	-2.74	-3.1	-3.06
10889018	*β*-santalene	-6.23	-2.41	-3.24	-3.73	-3.31	-1.56	-2.21
98403	Farenal	-6.19	-1.31	-2.17	-1.91	-2.56	-0.4	-1.98
5317319	(Z)-beta-Farnesene	-6.02	-0.44	-1.91	-2.47	-3.18	-0.1	-1.68
-	5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one	-6.02	-7.09	-4.07	-3.01	-2.89	-3.88	-1.48
-	*p*-mentha-1,4(8)-diene	-5.94	-	-3.11	-	-	-	-1.17
7127	Methyleugenol	-5.92	-3.23	-3.76	-3.25	-4.25	-1.65	-3.5
11463	Terpinolene	-5.75	-	-3.26	-1.38	-2.94	-3.4	-2.81
129762283	Tetrahydroxycurcumin	-	-8.48	-	-	-	-	-
-	1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	-	-8.14	-	-	-	-	-
-	1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	-	-7.93	-	-	-	-	-
-	1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one	-	-7.39	-	-	-	-	-

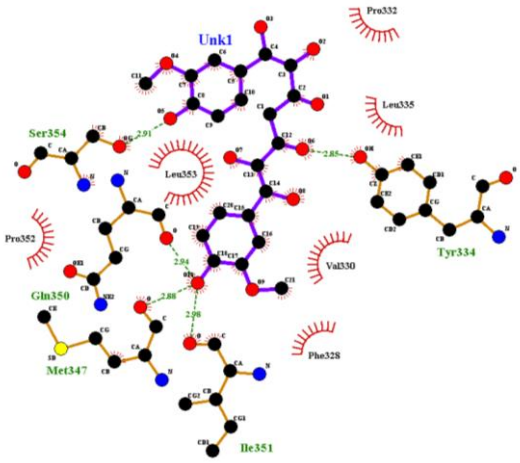
-	3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione	-	-6.8	-	-	-	-	-
637429	Calebin-A	-	-6.65	-	-	-	-	-
-	2-methyl-6-(4-formylphenyl)-2-hepten-4-one	-	-6.51	-	-	-	-	-
1548883	Cis-ferulic acid	-	-6	-	-	-	-	-

4.3 Protein-Ligand interaction and Lig-Plot

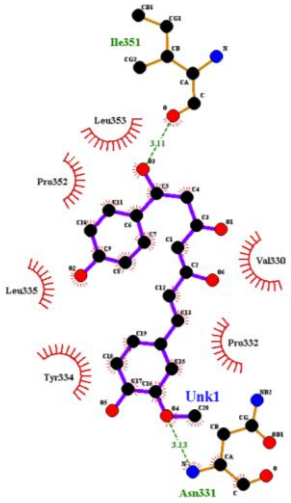
The protein-ligand complexes in .pdb format are displayed, edited and run via the software LigPlot+ (version v.1.4.5) for generation of LigPlot schematic diagrams. The protein-ligand interaction along with the hydrogen bonding and hydrophobic interactions with the complex binding residues are given for the lead phytochemicals. The table-2 shows the hydrogen bonding and hydrophobic interactions of the ligands with the protein histidine decarboxylase. The LigPlot structures are also given below (Figure 4.4).



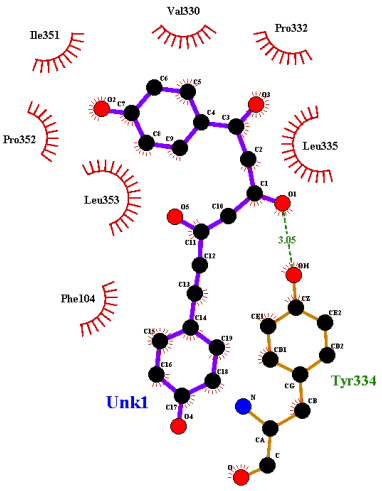
1



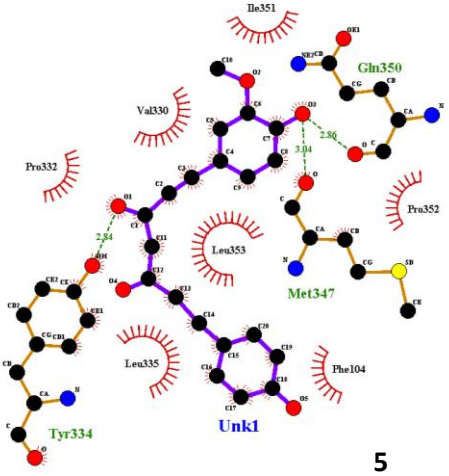
2


Ile351
Leu353
Pro352
Val330
Leu335
Pro332
Tyr334
Unk1
Asn331

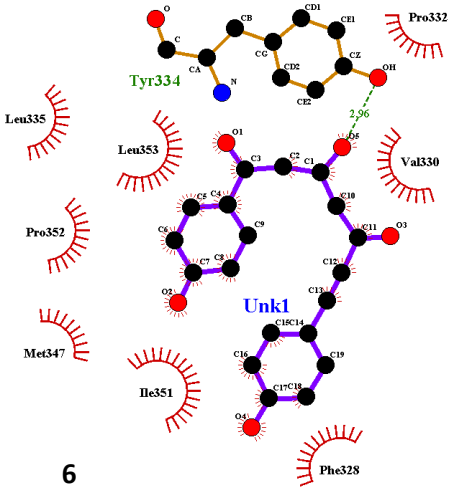
3


Val330
Ile351
Pro332
Pro352
Leu335
Leu353
Phe104
Unk1
Tyr334

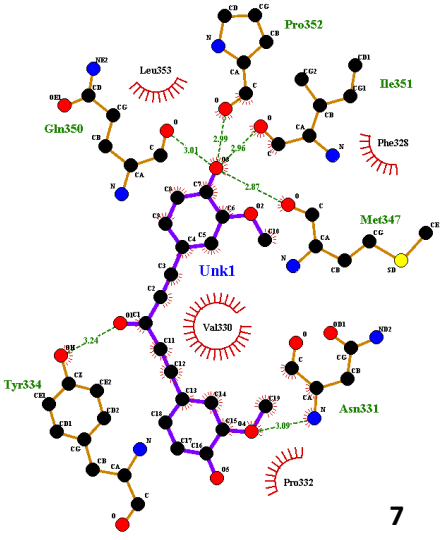
4


Ile351
Gln350
Val330
Pro332
Pro352
Leu353
Met347
Leu335
Phe104
Tyr334
Unk1

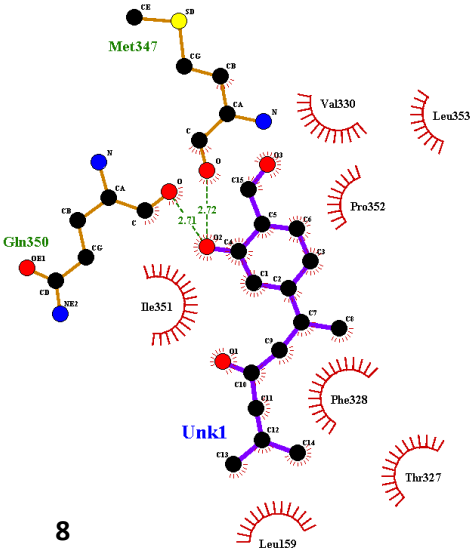
5


Pro332
Tyr334
Leu335
Leu353
Val330
Pro352
Unk1
Met347
Ile351
Phe328

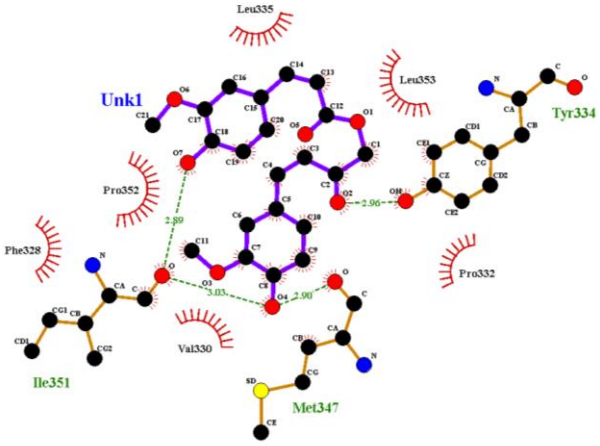
6


Pro352
Leu353
Ile351
Gln350
Phe328
Met347
Unk1
Val330
Tyr334
Asn331
Pro332

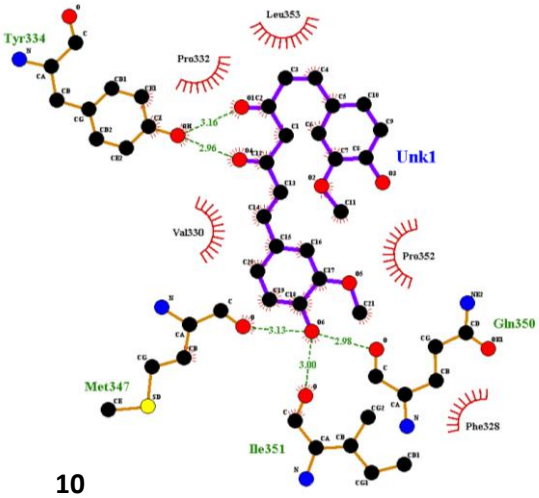
7


Met347
Val330
Leu353
Pro352
Gln350
Ile351
Phe328
Unk1
Thr327
Leu159

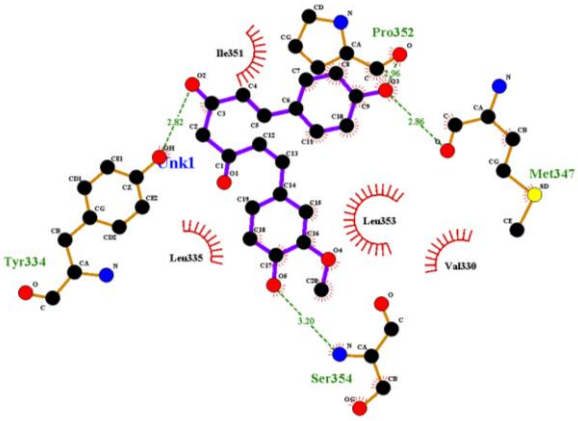
8



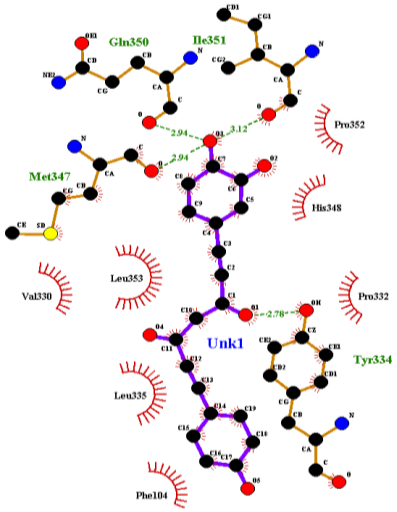
9



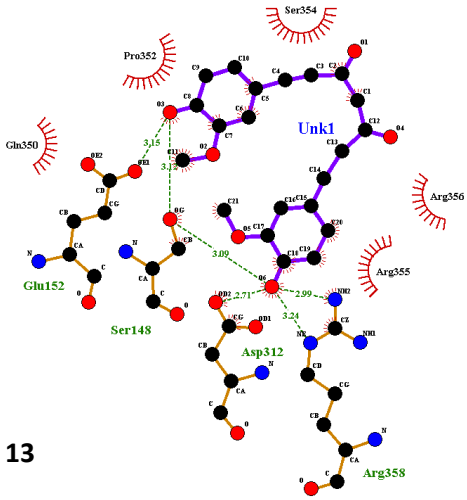
10



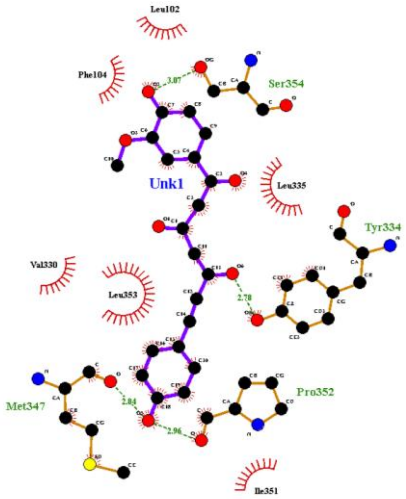
11



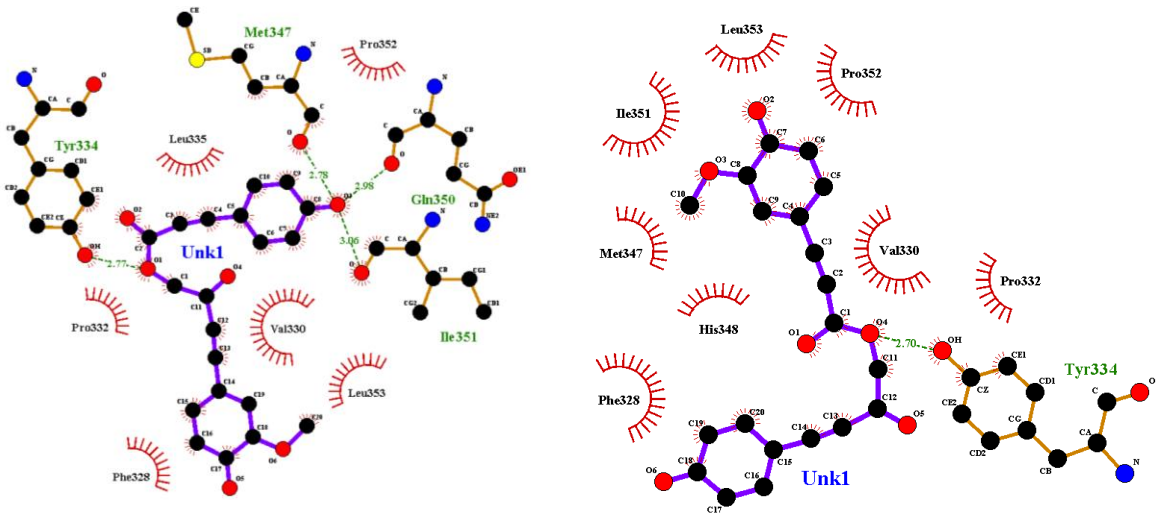
12



13



14



15 16

Figure 4.4- Lig Plots of Histidine decarboxylase protein interactions with the ligands or lead phytochemicals. The interaction of histidine decarboxlylase enzyme and ligands including- (**1**) tetrahydroxycurcumin (**2**) 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (**3**) 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (**4**) 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one (**5**) 5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one (**6**) 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione (**7**) 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one (**8**) 2-methyl-6-(4-formylphenyl)-2-hepten-4-one (**9**) calebin-A (**10**) curcumin I (**11**) curcumin II (**12**) 1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione (**13**) 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (**14**) 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one (**15**) 4''-(4'''-hydroxyphenyl)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate (**16**) 4''-(4'''-hydroxyphenyl-3-methoxy)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl)-propenoate

The LigPlot shows the amino acid of the target protein which is involved in the interaction with the ligand. The interaction profile of lead phytochemicals and the interface between heterodimers of the protein histidine decarboxylase with lowest binding energy to its known standard inhibitor is shown. The amino acids involved in hydrogen bonding and hydrophobic interaction with the lead phytochemicals are given in table 4.2.

Table 4.2- The amino acid residues involved in the Hydrogen bonding and hydrophobic interaction with the ligands

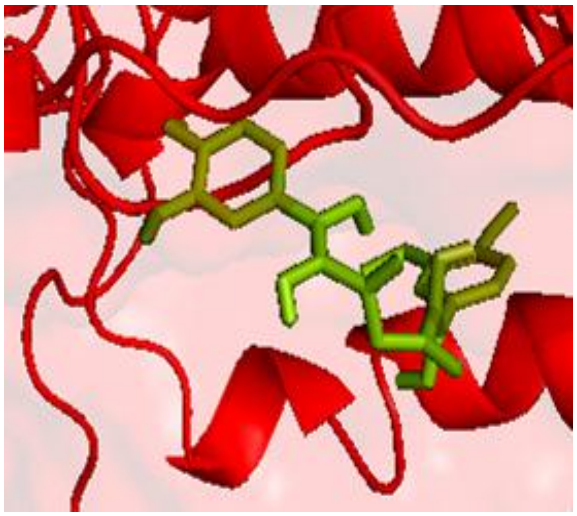
Sl. No.	Protein-Ligand interaction	Hydrogen Bonding	Hydrophobic Interaction
1	Histidine decarboxylase and tetrahydroxycurcumin	Gln347, Gln350, Tyr334	Pro332, Asn331, Val330, Leu335, Leu353, Pro352, Ile351, Phe328
2	Histidine decarboxylase and 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Ser354, Gln350, Met347, Ile351, Tyr334	Pro352, Leu353, Pro328, Val330, Leu335, Pro332
3	Histidine decarboxylase and 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Ile351, Asn331	Leu353, Pro352, Leu335, Tyr334, Pro332, Val330
4	Histidine decarboxylase and 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one	Tyr334	Ile351, Val330, Pro332, Pro352, Leu353, Leu335, Phe104
5	Histidine decarboxylase and 5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one	Met347,Tyr334, Gln350	Pro332, Val330, Ile351, Leu353, Pro352, Leu335, Phe304
6	Histidine decarboxylase and 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione	Tyr334	Leu335, Leu353, Pro352, Met347, Ile351, Phe328, Val330, Pro332

7	Histidine decarboxylase and 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1*E*,4*E*)-1,4-dien-3-one	Gln350, Pro352, Ile351, Met347, Asn331, Tyr334	Leu353, Phe328, Val330, Pro332
8	Histidine decarboxylase and 2-methyl-6-(4-formylphenyl)-2-hepten-4-one	Gln350, Met347	Val330, Leu353, Pro352, Ile351, Phe328, Leu159, Thr327
9	Histidine decarboxylase and calebin-A	Ile351, Met347, Tyr334	Phe328, Pro352, Leu335, Leu353, Val330, Pro332
10	Histidine decarboxylase and curcumin I	Tyr334, Met347, Ile351, Gln350	Pro332, Leu353, Val330, Pro352, Phe328
11	Histidine decarboxylase and curcumin II	Tyr334, Met347, Pro352, Ser354	Ile351, Leu335, Leu353, Val330
12	Histidine decarboxylase and 1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione	Gln350, Ile351, Met347, Tyr334	Val330, Leu353, Leu335, Phe104, His348, Pro332, Pro352
13	Histidine decarboxylase and 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one	Gln152, Ser148, Asp312, Arg358	Gln350, Pro352, , Ser354, Arg356, Arg355
14	Histidine decarboxylase and 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one	Pro352, Met347, Tyr334, Ser354	Leu102, Phe104, Val330, Leu353, Ile351, Leu335
15	Histidine decarboxylase and 4"-(4"'-	Tyr334, Met347,	Leu335, Pr332, Phe328,

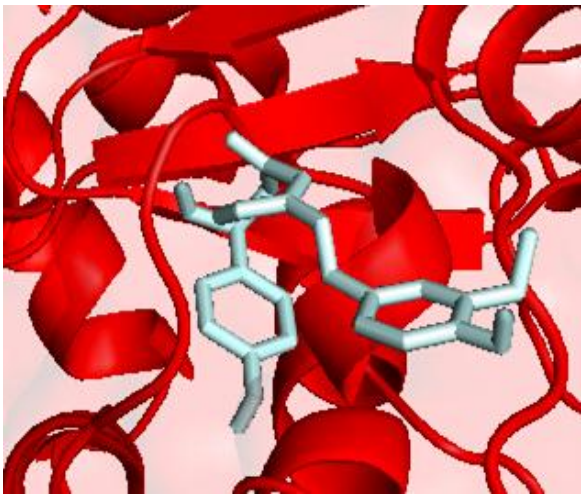
	hydroxyphenyl)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate	Gln350, Ile351	Val330, Leu355, Pro352
16	Histidine decarboxylase and 4''-(4'''-hydroxyphenyl-3-methoxy)-2''-oxo-3''-butenyl-3-(4'-hydroxyphenyl)-propenoate	Tyr334	Leu353, Ile351, Met347, Phe328, His348, Pro352, Val330, Pro332

4.4 Protein-Ligand Binding Surface-Structure Determination

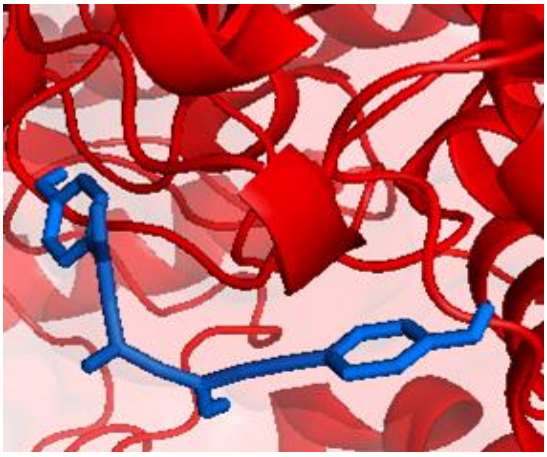
The binding of the modified ligands with active sites of the targeted protein was determined and visualized by surface structure determination of protein and ligand binding. The surface structure of the protein and ligand is determined through PyMOL Molecular Graphics System software (version 1.1). The best docking score of proteins are chosen with the ligands having the best binding affinity and the surface structure was prepared. Proteins like Histamine H_1 receptor (3rze) and histidine decarboxylase (4e1o) showed better interaction with the ligands. These phytochemicals have a higher affinity of binding to the targeted protein which resulted into a change in the conformation of the targeted protein. The high binding affinity of the interaction between protein-ligand complexes increased the greater intermolecular force between the ligands and its protein. Also, the high-affinity binding resulted in a higher degree of occupancy for the ligand at its protein binding sites. The following are the surface structure of the target proteins and best lead compounds in figure 4.5.



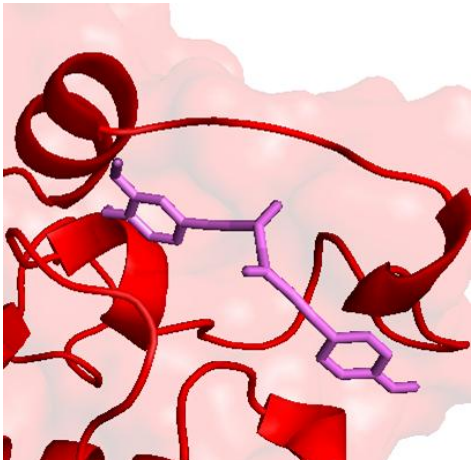
1



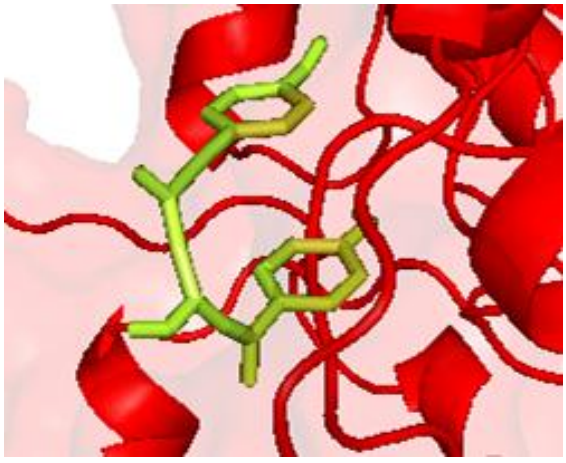
2



3



4



5

Figure 4.5- The surface structures with molecular interaction of the protein and ligands. The surface structures of the lead phytochemicals with highest dock score are- (**1**) Histidine decarboxylase and tetrahydroxycurcumin (**2**) Histidine decarboxylase and 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (**3**) Histidine decarboxylase and 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (**4**) Histidine decarboxylase and 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one (**5**) Histidine decarboxylase and 5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one

4.5 *In vitro* validation

4.5.1 Histamine detection in fermented curd by biochemical tests for colour formation

In accordance with United States Pharmacopeia 23, the red colour formation in the final solution detects the presence of histamine. The results of the experiment are shown below in the following images. Figure 4.6b shows the red colouration in Day 14 which confirms the presence of histamine in comparison to Day 0 (Figure 4.6b).

Figure 4.6a- Detection of histamine on Day 0

Figure 4.6b- Detection of histamine on Day 14

4.5.2 Semi-Qualitative analysis and detection of histamine

After performing the qualitative analysis in different samples along with the presence of turmeric we performed the tests for histamine detection. The control samples showed the presence of histamine by the formation of red colour although in very small amount. But the test samples turned red after addition of NaOH which might be due to degradation of curcumin in basic pH medium thus giving us false positive results. Figure 4.7 and 4.8 shows the various test samples before and after incubation period and subsequent biochemical analysis respectively. The negative and vehicle control samples are shown in Figure 4.9.

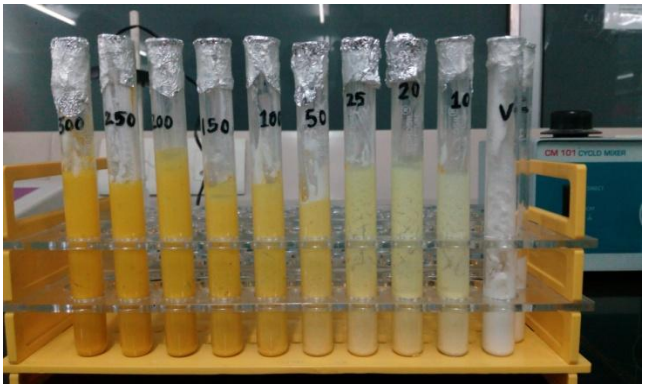


Figure 4.7 The samples before incubation

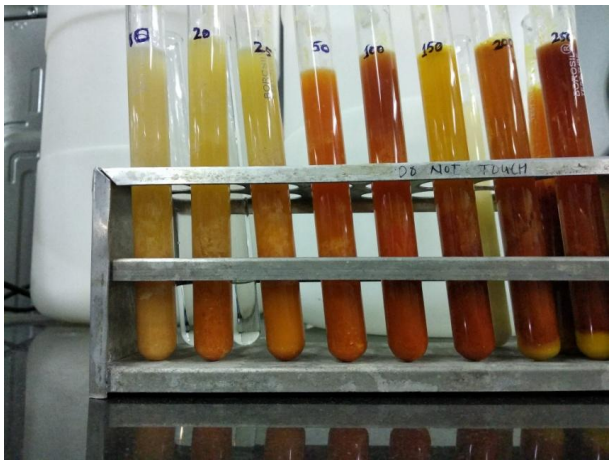


Figure 4.8 The samples after biochemical analysis

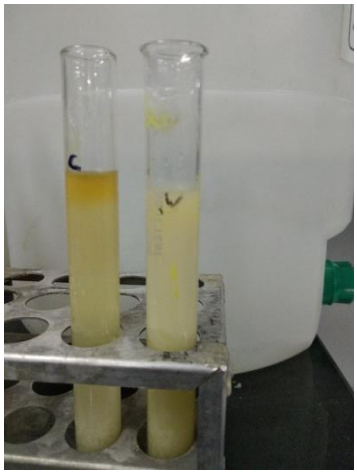


Figure 4.9- Biochemical analysis in negative control (c) and vehicle control (V)

The expected result was the red colour formation in the controls and not in the test samples. But as we know, turmeric is a natural pH indicator it gave false positive results in our experiment. Practically, turmeric is insoluble in water under neutral or acidic conditions but it becomes soluble in the alkaline condition being an oil-soluble pigment. Thus it gives a discolouration from yellow to red in the alkaline pH as it gains instability in its structure due to the conversion of stable predominant keto-form to enol form of its several phytoconstituents. As a result, when NaOH was added to the samples, red colour formation took place which concurred with the actual result of our experiment (Priyadarsini, 2014).

The expected result was the inhibition or reduction of histamine formation in the samples by lesser or no colour formation as observed in the negative and vehicle control. Thus we planned for HPLC based detection of histamine in the samples.

4.6 In vitro anticancer activity (MTT assay)

To access the antiproliferative activity of compound 002, the colon adenocarcinoma cell lines (HT-29) were used using MTT assay and the results are shown in figure 4.10. About 50% growth inhibition activity was observed at lower test concentration (500nmolar) against colon cancer cell line. The log(x) values were plotted in terms of molar concentrations. The IC_{50} values can be determined from the graph which distinctively shows the % inhibition with respect to the concentrations at 0.39µm in 48hours.

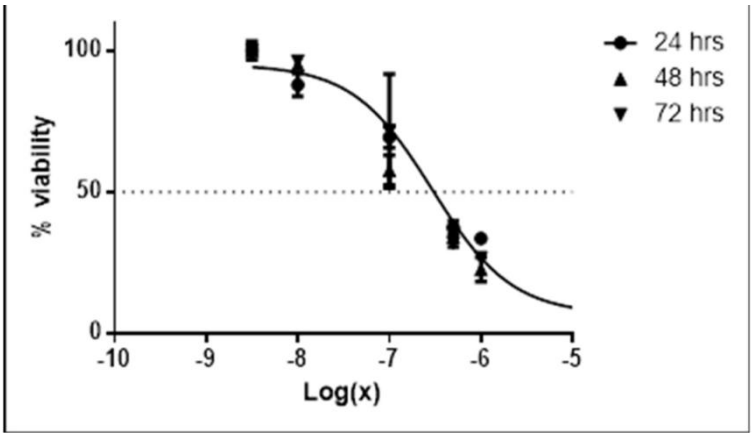


Figure 4.10- Graphical representation of compound 002 treatment on MTT assay in Log(x) v/s % viability of cells

4.7 DPPH radical scavenging activity

The antioxidant activity of compound A was measured at different concentrations(1, 2, 5, 10, 20, 30 and 100 μl/mL) by using DPPH assay. Result showed that the antioxidant activity increased with increasing concentration of the sample (Figure 4.11). The radical scavenging activity of compound A was found to be 75-80% at different test concentration (1–20 μl/mL). It was found at higher concentrations to increase with increase in the concentration.

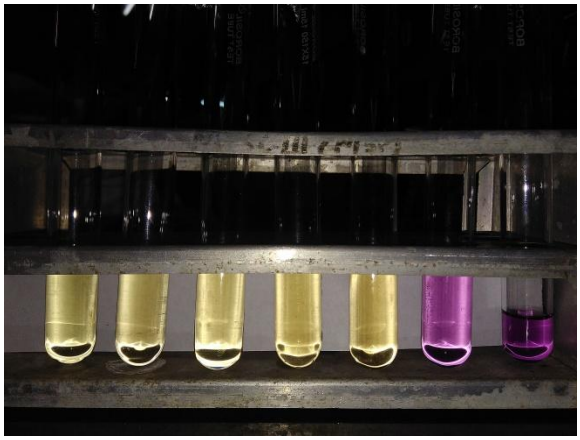


Figure 4.11**-** Percentage scavenging activity of compound A

4.8 Antibacterial activity by Disk Diffusion Method

Antimicrobial activity of the drug as test compound A was determined by using Kirby-Bauer disc diffusion method against Gram-negative *Escherichia coli*. The LB agar plates containing *Escherichia coli* bacterial strains were retained and the paper disks saturated with the phytochemicals were placed in the media. After 24hour incubation, the zone of inhibition (ZOI) was observed in the plates with standard antibiotic disks of penicillin (0 µg/disc) and norfloxacin (10 µg/disc). However, the efficient zone of inhibition could not be seen in the agar plates with the test samples (Bauer *et al*.,1966). The figures 4.12 and 4.13 show zone of inhibition in the bacterial colonies with disks of penicillin and norfloxacin respectively. Norfloxacin showed a greater zone of inhibition than penicillin. There was no ZOI observed in the control i.e DMSO as shown in figure 4.14 alongwith the test compound which did not show antibacterial effiency.

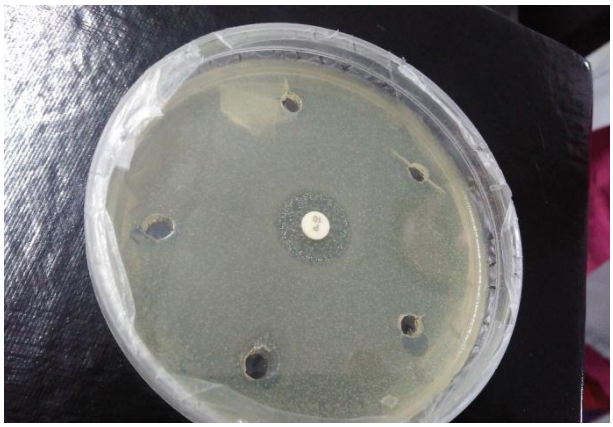


Figure 4.12 Culture containing disk of Penicillin

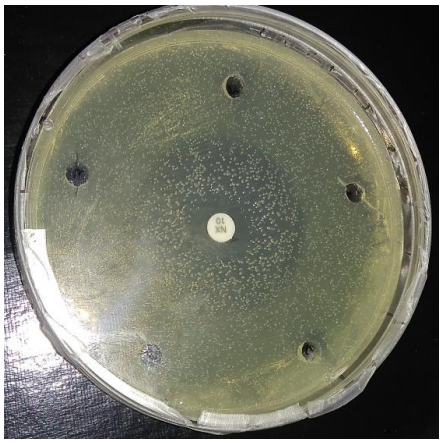


Figure 4.13 Culture containing disk of Norfloxacin

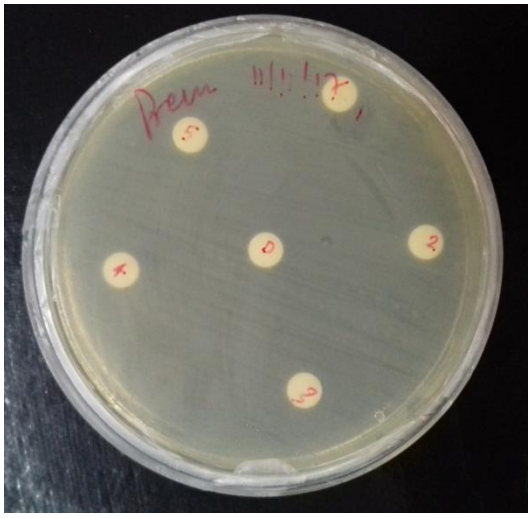


Figure 4.14- Culture containing disks of DMSO (D) and test compound A.

4.9 Experimental Setup for production of histamine in the presence of curcumin: Future Perspective

Histidine decarboxylase is the enzyme responsible for histamine formation from histamine in the granules, the release of which results in the generation of allergic symptoms commonly in allergic rhinitis. The targeted enzyme histidine decarboxylase is also mainly involved mainly in the conversion of histamine from histidine resulting in accumulation of histamine or its increased levels leading to histamine intolerance. It is a toxic histamine response by the body because of accumulation of excessive endogenous or exogenous histamine in the body. Histamine is a biogenic amine and its accumulation may also result from the intake of various foods exogenously. Various foods resulting in increased histamine levels include fish, meat or dairy products, fermented foods, beer, wine etc. The enzyme HDC is responsible for the conversion of the amino acid histidine present in many fermented foods including curd by the action of lactic acid bacteria. Most of the species of lactic acid bacteria present in curd possess the *hdc* gene which is responsible for the formation of the enzyme histidine decarboxylase. The activity of the enzyme is mainly to convert histidine to histamine with the help of certain cofactors like vitamin B6, Mg and Zn.

Based on *in silico* validation along with in vitro detection tests we confirmed the presence of histamine. The tests performed are rudimentary detection and confirmation tests. Based on these tests we have prepared a qualitative experimental setup. The gradation can be visually detected but due to the reaction of NaOH with curcumin, we have obtained a false positive result. This can be quantitatively analyzed by means of the experimental setup mentioned below.

4.9.1 Histamine production and detection

Isolation of *Lactobacillus* from curd is carried out and must be inoculated in MRS media for bacterial growth and it is incubated for 48hours. Pure culture colonies will be obtained from which a pure *Lactobacilli* strain must be inoculated in MRS broth along with additional components like 1%histidine, 0.6%vitamin B_6 and 0.003% bromocresol (for detecting the presence of histamine). The bacterial culture is then incubated for around 1-2 weeks (Pham and Nguyen, 2016).

4.9.2 Extraction of histamine and its quantitative analysis by HPLC

The bacterial culture is retained and centrifuged separating the supernatant. Addition of chloroform and ethanol further separates and subsequent heating leads to extraction of histamine. Quantitative analysis of histamine is done by HPLC against standard histamine for comparison (Pham and Nguyen, 2016).

4.10 Discussion

From the time beginning it has been recorded that *C. longa* plant plays an important role in various diseases/ailments. Anti-inflammatory properties of *C. longa* phytochemicals are well documented in Ayurvedic literature and in another medicinal system of the world. The present study was designed to assess the potential of *C. longa* phytochemicals against allergic rhinitis. The literature showed the involvement of different proteins in the pathophysiology of disease (Figure 1.1, 1.2 and 1.3). Based on these we have targeted those proteins in the present study.

The present study determines the *in silico* potential screening of the literature based phytocomponents of *C. longa* and their molecular docking with significant proteins involved in the biosynthetic pathway of histamine formation and subsequent generation of allergic symptoms. The proteins involved in this pathway including histamine H_1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C_4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) were targeted and molecular docking was performed. However, only the protein histidine decarboxylase showed efficient binding with the ligands in comparison to its standard inhibitor HME.

The commonly prescribed treatment options for allergic rhinitis mainly include drug therapies like H1-antihistamines, LTRAs and intranasal glucocorticoids. Apart from these, there are alternatives like oral decongestants, newly inhaled steroids, intranasal mast cell stabilizers, intranasal anticholinergics, monoclonal anti- Ig E antibody treatment and immunotherapy are also opted for but less frequently. Although these are ideal for treatment of allergic rhinitis, they sometimes can lead to side effects along with longer duration for a cure. For instance, the prescribed drugs are carried out for a longer period of time yet there is no sign of recovery and the symptoms still prevail, there may be other conditions related to it like histamine intolerance. Histamine intolerance or pseudo-allergy, unlike allergies with IgE mediated histamine response to allergens, is a toxic histamine response by the body because of accumulation of excessive endogenous or exogenous histamine in the body. It is mainly caused due to defected or mutated enzymes involved in its biosynthesis or degradation (Jernigan, 2015). Histamine is a biogenic amine and its accumulation may also result from the intake of various foods exogenously. Various foods resulting in increased histamine levels include fish, meat or dairy products, fermented foods, beer, wine etc (Leech, 2018). The symptoms of histamine intolerance mainly coincide with those of allergic rhinitis which is why it is often confused or misinterpreted as allergic rhinitis whereas on the other hand it is barely allergy and is involved in the increased level of histamine or its accumulation. Because of this, the treatments opted for show less or no improvement failing to target the enzymes involved in histamine intolerance. The enzyme histidine

decarboxylase plays a crucial role as it leads to histamine biosynthesis from histidine. Thus targeting this enzyme along with other enzymes like diamine oxidases and HMTs is significant in treating histamine intolerance.

Histidine decarboxylase is the enzyme responsible for histamine formation from histidine in the granules, the release of which results in the generation of allergic symptoms commonly in allergic rhinitis (Komori *et al*., 2012). The lead phytochemicals including tetrahydroxycurcumin, cyclocurcumin, 1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene etc showed binding affinity percentage of 162%, 155% and 151% respectively as compared to its standard inhibitor HME. Although the remaining proteins showed efficient binding their G-scores are comparatively lesser in comparison to the scores of their respective standard inhibitors. For the protein histamine H_1 receptor the binding affinity percent was found to be 84.9% for linoleic acid, 78.3% for oleic acid and 77.7% for stearic acid against standard doxepin. Similarly, the lead compounds of adenylate kinase showed 82% for curcumin-d6, 70.4% for curcumin.

The targeted enzymes involving allergic rhinitis could not be potentially inhibited by the ligands based on their Dock score. But, the enzyme HDC showed efficient binding and percentage of inhibition by the ligands. Thus, this enzyme was targeted for further *in vitro* validation. Since the synthesis of histamine is dependent on the HDC enzyme, so it was targeted for *in vitro* studies and its potential inhibition by phytochemicals present in turmeric.

The enzyme histidine decarboxylase (HDC) is responsible for the conversion of the amino acid histidine present in curd by the action of lactic acid bacteria. Most of the species of lactic acid bacteria present in curd possess the *hdc* gene which is responsible for the formation of the enzyme histidine decarboxylase. The activity of the enzyme is mainly to convert histidine to histamine with the help of certain cofactors like vitamin B6, Mg and Zn (Jernigan, 2015). *In silico* validation of the phytoconstituents of turmeric show that the enzyme histidine decarboxylase is potentially inhibited by ligands like tetrahydroxycurcumin, cyclocurcumin, 1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene etc based on which the expected

result was the inhibition or reduction of histamine formation in the samples showing lesser or no color formation as observed in the negative and vehicle control.

Turmeric is a natural pH indicator which directed false positive results in the in vitro experiment performed. Turmeric consists of several active compounds including curcumin which exhibits an extended conjugation and keto-enol tautomerism besides having a two-ringed aromatic phenolic structure. These properties in combination give turmeric its yellow colouration. Being chemically acidic in nature, curcumin can lose its proton easily in an alkaline environment. A sudden change in pH alters the stable predominated keto-form of their structure alters to unstable enol-form. Due to the loss of proton in the alkaline environment especially from its phenolic site, structural changes occur from benzenoid to quinonoid due to which alterations in extended conjugation, as well as tautomerism, occurs. Due to this structural alteration, a bathochromic shift occurs i.e., the quinonoid form emerges with a longer wavelength resulting in red colour rather than yellow. Practically, turmeric is an oil-soluble pigment and insoluble in water under neutral or acidic conditions. It becomes soluble in alkaline condition and changes its colour from yellow to red. This disco.louration occurs mainly due to the alterations in pH mainly from the range of 7.4 and above. As a result, the colour of turmeric in neutral or acidic pH i.e yellow or deep yellow is changed to dark pink or red colour (Priyadarsini, 2014). Thus when we added NaOH, which is a strong base to the samples; there was an instant irreversible change in colour to red which directed false positive results. The degradation of curcumin is slower in acidic medium and rapid in physiological pH or basic medium which makes it an intricate drawback in case of therapeutic utility.

5. Conclusion

Natural products are the popular remedies against a number of diseases and allergic rhinitis is no exception. *Curcuma longa* is a common medicinal herb with numerous medicinal properties. The anti-inflammatory potential is another commonly known property of turmeric that has been reported in various traditional medicinal systems including Ayurveda. The current study explores the anti-inflammatory potential of turmeric for its therapeutic usage against allergic disorders or histamine intolerance by *in silico* and *in vitro* validation. Various *in vitro* and *in vivo* studies are going on and a lot must be carried out in order to explore its absolute efficacy or medicinal potential. Moreover, the phytochemicals present in the herb is yet to be considered in modern medicine and pharmacology which can unwrap numerous utilities and potential in modern pharmacological drug therapies with lesser side effects and more efficacies.

List of Publications

Swagata Das, Prareeta Mahapatra, Priyanka Kumari, Prem Prakash Kushwaha, and Shashank Kumar. (2018) Phytochemicals as Hope for the Treatment of Hepatic and Neuronal Disorders Phytochemistry, *In* Volume 2: Pharmacognosy, Nanomedicine, and Phytochemicals as foes, Eds: Egbuna Chukwuebuka, Shashank Kumar, Ifemeje Jonathan Chinenye, Jaya Vikas Kurhekar, CRC press, USA. *(Communicated)*

Swagata Das, Rebati Malik, Prem Prakash Kushwaha, Pushpendra Singh, Santosh Maurya and Shashank Kumar. (2018) Environmental toxicology and allergic rhinitis, *In* Environmental toxicology and Associated Health Concern, Eds: Abbas Ali Mahdi, Y. K. Sharma, Murtaza Abid & M.M Abid Ali Khan, Springer Nature, New Delhi, India. *(Communicated)*

Swastika Dash, **Swagata Das**, Prem Prakash Kushwaha and Shashank Kumar. (2018) Environmental toxicology and diabetes, *In* Environmental toxicology and Associated Health Concern, Eds:Abbas Ali Mahdi, Y. K. Sharma, Murtaza Abid & M.M Abid Ali Khan, Springer Nature, New Delhi, India. *(Communicated)*

Prem Prakash Kushwaha, **Swagata Das**, Atul Kumar Singh and Shashank Kumar. (2018) Drug Development Drug delivery carriers and Clinical Trial, *In* Clinical Biochemistry, Cell Culture, and Drug Development, Ed: Shashank Kumar Cambridge Scholars Publishing, U.K. *(Communicated)*

References

Aaronson, D.W. (1998). Side effects of rhinitis medications. *Journal of Allergy and Clinical Immunology*. **101**: 379-382.

Barnes, P.J. (2011). Pathophysiology of allergic inflammation. *Immunological Reviews.* **242**:31–50.

Bauer, W., Kirby, W.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*. **45**: 493-496.

Bousquet, J., Vignola, A.M., Campbell, A.M., Michel, F.B. (1996). Pathophysiology of allergic rhinitis. *International Archives of Allergy and Immunology*. **110**: 207-218.

Braido, F., Lagasio, C., Piroddi, I.M.G., Baiardini, I., Canonica, G.W. (2008). New treatment options in allergic rhinitis: patient considerations and the role of ciclesonide. *Therapeutics and Clinical Risk Management*. **4**: 353-61.

Brozek, J.L., Bousquet, J. *et al.* (2017). Allergic rhinitis and its impact on asthma (ARIA) guidelines 2016 revision. *Journal of Allergy and Clinical Immunology*. **140**: 950-958.

Busse, W.W. and Lemanske, K.F. (2001). Asthma. *New England Journal of Medicine*. **344**: 350-362

Chazot, P.L. (2009). Advances in histamine pharmacology reveal new drug targets. *British Journal of Pharmacology*. **157**:1-3

Church, M.K. (2011). Safety and efficacy of bilastine: a new H(1)- antihistamine and utricaria. *Expert Opinion on Drug Safety*. **10**: 779-93.

Dallakyan, S., and Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. *Chemical Biology: Methods and Protocols*. **1263**: 243-250.

Dempsey, O.J. (2000). Leukotriene receptor antagonist therapy. *Postgraduate Medical Journal.* **76**: 767–773.

Druce, H.M. and Kaliner, M.A. (1988). Allergic rhinitis. *Journal of the American Medical Association*. **259**:260-3

He, Y., Yue, Y., Zheng, X., Zhang, K., Chen, S., Du, Z. (2015). Curcumin, inflammation, and chronic diseases: how are they linked? *Molecules.* **20**: 9183-9213.

Islam, F., Gopalan, V., Lam, A.K. (2017). RETREG1 (FAM134B): A new player in human diseases: 15 years after the discovery in cancer. *Journal of Cellular Physiology*. **233**: 4479-89.

Javed, S. and Michael, A.K. (2003). Decongestants. Medscape. <https://emedicine.medscape.com/article/134825-medication#5>

Jernigan, D. (2015). Histamine intolerance; The cause of Hundreds of Symptoms. Advanced Alternative Healthcare for Chronic Illness. Changing Lives, Not Just Bodies. <http:// davidjernigan.blogspot.in/2015/07/histamine-intolerance-not-allergies-may.html.>

Leech, J. (2018). Histamine Intolerance: Everything You Need To Know Explained in Plain English. Diet vs Disease. < https://www.dietvsdisease.org/histamine-intolerance/ >

Jurenka, J.S. (2009). Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa : a review of preclinical and clinical research. *Alternative Medicine Review*. **14**:141-153.

Kalpaklioglu, F., Baccioglu, A. (2012). Efficacy and safety of H1-antihistamines: an update.*Antiinflammatory & Anti-allergy Agents in Medicinal Chemistry*. **11**: 230-7.

Komori, H., Nitta, Y., Ueno, H., Higuchi, Y. (2012). Structural study reveals that Ser-354 determines substrate specificity on human histidine decarboxylase. *The Journal of Biological Chemistry*. **287**: 29175-83

Kumar, S., Chashoo, G., Saxena, A.K., Pandey, A.K. (2013). *Parthenium hysterophorus*: a probable source of anticancer, antioxidant and anti-hiv agents. *BioMed Research International.* **2013:** 810734-45

Kumar, S., Kumar, R., Dwivedi, A., & Pandey, A. K. (2014). In vitro antioxidant, antibacterial, and cytotoxic activity and in vivo effect of Syngonium podophyllum and Eichhornia crassipes leaf extracts on isoniazid induced oxidative stress and hepatic markers. *BioMed Research International*. **2014**: 1- 11.

Kumar, S.; Kumar, R.; Dwivedi, A.; Pandey, A.K. (2014). *In vitro* antioxidant, antibacterial, and cytotoxic activity and *in vivo* effect of *Syngonium podophyllum* and *Eichhornia crassipes* leaf extracts on isoniazid induced oxidative stress and hepatic markers. *BioMed Research International.* **2014**: 459452.

Lahlou, M. (2013). The success of natural products in drug discovery. *Pharmacology & Pharmacy* **4**: 17.

Lee, B.J., Kim, Y.J., Kim, J.H., Shin, H.S., Chung, Y.S. (2001). A comparative study of intranasal budesonide and oral terfenadine in perennial allergic rhinitis: effect on the symptom score and nasal secretion eosinophils. *Journal of Asthma Allergy and Clinical Immunology*. **21**:216-22.

Lieberman, P. (1988). Rhinitis: allergic and nonallergic. *Hospital Practice*. **23**:117-45.

Li, S., Yuan, W., Deng, G., Wang, P., Yang, P., Aggarwal, B. (2011). Chemical composition and product quality control of turmeric (*Curcuma longa L*.). *Pharmaceutical Crops*. **2**: 28-54.

Matsuse, H., Kohno, S. (2014). Leukotriene receptor antagonists pranlukast and montelukast fortreating asthma. *Expert Opinion on Pharmacotherapy*. **15**: 353-63.

Min, Y.G. (2010). The pathophysiology, diagnosis and treatment of allergic rhinitis. *Allergy Asthma Immunology Research.* **2**: 65-76.

Mygind, N., Dahl, R., Bisgaard, H. (1999). Leukotrienes, leukotriene receptor antagonists and rhinitis. *Allergy*. **55**: 421-424.

Nguyen, L.P.T., Nguyen T.H.K. (2015). Histamine Production by Lactobacillus rhamnosus. Global Journal of Biology, Agriculture & Health Science. **4**: 70-74.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. *Journal of Cheminformatics*. **3**:1.

Owen, J.A., Punt, J. (2013). Overview of the immune system. In: Freeman WH (ed) Kuby Immunology, 7th edn. Macmillan, New York.

Parnes, S.M. (2002). The role of leukotriene inhibitors in allergic rhinitis and paranasal sinusitis. *Expert Opinion on Pharmacotherapy*. **3**: 33-8.

Patel, S. and Goyal, A. (2012). Recent developments in mushrooms as anti-cancer therapeutics: a review. *3 Biotech.* **2**:1–15.

Pawankar, R., Mori, S., Ozu, C., Kimura, S. (2011). Overview on the pathomechanisms of allergic rhinitis. *Asia Pacific Allergy*. **1**: 157-167

Pham, l. and Nguyen, Tu. (2016). Detection of histamine production by *Lactobacillus*. *Journal of Chemical and Pharamceutical Research*. **8**: 1207-1209.

Priyadarsini, K.I. (2014). The Chemistry of Curcumin: From extraction to therapeutic agent. *Molecules*. **19**: 20091-20112.

Rachelefsky, G.S. (1998). Pharmacologic management of allergic rhinitis. *Journal of Allergy and Clinical Immunology.* **101**:367-369.

RahamozHaghigi, S., Asaadi, M.H. Akrami, H., Baghizade, A. (2016). Anticarcinogenic and antiangiogenic properties of the extract of *Acorus calamus* on gastric cancer cells. *Avicenna Journal of Phytomedicine*. **7**: 145-156.

Sin, B. and Togias, A. (2011) Pathophysiology of allergic and non allergic rhinitis. *Proceedings of the American Thoracic Society*. **8**: 106-114.

Slater, J.W., Zechnich, A.D., Haxby, D.G. (1999). Second-generation antihistamines: a comparativereview. *Drugs.* **57**: 31-47

Spector, S. (1999). Ideal pharmacotherapy for allergic rhinitis. *Journal of Allergy and Clinical Immunology.* **103**: 386-7.

Stella, R. and Donnell, O. (1999). Leukotriene-biosynthesis and mechanisms of action. *Australian Prescriber.* **22**: 55-7.

Thornhill, S.M. and Kelly, A.M. (2000). Natural Treatment of Perennial Allergic Rhinitis. *Alternative Medicine Reviews*. **5**: 448-454.

Walekar, A., Chodankar, D., Naqvi, M., Trivedi, C. (2016). Assessment of bioequivalence of fexofenadine and montelukast fixed dose combination tablet versus separate formulations of the individual components at the same dose levels. *Indian Journal of Pharmaceutical Sciences.***78**:651-656

Wheatley, L.M. and Togias, A. (2015). Allergic rhinitis. *New England Journal of Medicine*. **372**:456-463.

White, M.V. (1990). Role of histamine in allergic diseases. *Journal of Allergy and Clinical Immunology*. **86**: 599-605

URKUND

Urkund Analysis Result

Analysed Document: Introduction to conclusion.docx (D38762157)
Submitted: 5/16/2018 9:49:00 AM
Submitted By: kpramodk82@gmail.com
Significance: 1 %

Sources included in the report:

https://emedicine.staging.medscape.com/article/134825-overview
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3434398/
https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf
https://biblio.ugent.be/publication/8538875
http://recipp.ipp.pt/bitstream/10400.22/7861/1/DM_H%C3%A9lioGuedes_2015.pdf

Instances where selected sources appear:

7

1. Introduction Ayurveda science is one of the oldest medical systems available in the world. As per the study, approximately more than 25% of modern medicines are obtained from natural sources. Natural products are molecules produced by plants or microorganisms. These are organic substances having small molecular weight. The past has been evident for the fact that natural products are pharmacologically important compounds that are usually taken as home remedies in treating various types of diseases ranging from common cold to life-threatening cancer (Patel, 2012). In this view, herbal products may do a lot. A number of traditional herbal medical practices have been accepted for the prevention, diagnosis, and treatment of numerous diseases such as diabetes, cancer, neurological disorders and cardiac dysfunction, etc. The advantage of these medicinal plants is hundred percent natural.

Plant-derived products were majorly used as foods or botanical potions and extracted powders which have been used successfully in cure and prevention of diseases throughout history. There has been an exponential growth in the field of herbal medicine in the last few years because of their fewer side effects and natural origin. The World Health Organization (WHO) has listed about 21,000 plants, which are used for medicinal purposes around the world (Kumar et al., 2014). Natural products were screened as templates for structure optimization programs for designing novel drugs. In spite of the present day concern with synthetic chemistry for drug designing and manufacture, the role of plants in the treatment of disease and prevention is still enormous. Phytochemicals are bioactive metabolites present naturally in plants showing biological significance in plants, playing an essential role in the defence mechanism of plants by inhibiting or killing the interacting pathogen. A number of plants are known for their anti-inflammatory property including Urtica dioica or stinging nettle, pineapple plant that contains bromelain (a proteolytic enzyme) and many herbs and vegetables having the natural phytochemical quercitin (flavonoid). Moreover, certain other naturally derived components like N-acetylcysteine (a natural sulfur-containing amino acid derivative) and vitamin C had potential antihistamine and anti-inflammatory activities (Thornhill and Kelly, 2000). The concept of natural products has its roots back in the 19th century. Common reported examples of drugs discovered initially this way is morphine (the active agent present in Opium) and digoxin, which is a heart stimulant originating from Digitalis lanata (Lahlou, 2013). Historically natural products have been a rich source of compounds that have a great importance in medicine, pharmacy and biology. A number of important new commercial drugs have been obtained from natural sources. Drugs of natural origin can be classified as original natural products, products derived semi-synthetically from natural products or synthetic products based on natural product model.

Allergic rhinitis (AR) commonly known as hay fever is a symptomatic disorder inducing an allergic response to specific allergens. It is a nose related disorder due to exposure to different types of allergens which may be seasonal or perennial. Seasonal allergens include pollens and moulds whereas perennial allergens include dust, mites, pests etc. It is a type of inflammation caused due to Ig-E mediated hypersensitivity reactions resulting in its four cardinal symptoms watery rhinorrhea (running nose), nasal obstruction, nasal itching and sneezing. Its prevalence is ever increasing throughout the globe. In the United States alone it is estimated to affect about 60 million people and among which 10-30% being adults and 40% being children (Min, 2010). In India itself, one out of six people suffers from AR.

The adequate treatment of allergic rhinitis is important due to its impact and perturbance in quality of life and its increased risk of asthma. The mast cells respond to the offending allergens which lead to inflammation which in turn is responsible for the release of chemical mediators including histamine, prostaglandins and leukotrienes. As a result, targeting these events of the hypersensitivity reactions became significant in treating allergic rhinitis. Many studies in AR and its possible therapeutics have been reported and ther0efore allergic rhinitis and its impact on asthma (ARIA) guidelines have been published (2001) and revised (2008). According to the revised ARIA guidelines, second generation H1- antihistamines were preferred over the first generation H1-antihistamines due to safety concern. Leukotriene receptor antagonists (LTRA) also became commonly recommended drugs for the treatment of allergic rhinitis (Min, 2010). Histamines are responsible for symptoms like rhinorrhea, nasal itching and sneezing while leukotrienes increase resistance in nasal airway or nose blockade and vascular permeability. Among the most common drugs for the treatment of AR, fexofenadine, a second generation non-sedating H1-antihistamine and montelukast, a leukotriene antagonist are popularly recommended (Walekar et al., 2016). Although these drugs have higher effectiveness and lesser side effects, these seem to further decrease the quality of life. Due to this, it is important to search for natural or plant-derived alternatives for treatment of AR which are more effective and have lesser or no side effects. Although very less is known about the potent natural inhibitors of AR, some studies show that Urtica dioica or stinging nettle, bromelain (a proteolytic enzyme derived from the stem of pineapple plant), quercetin (a flavonoid found in many herbs and vegetables), N-acetylcysteine (a natural sulfur-containing amino acid derivative) and vitamin C had potential antihistamine and anti-inflammatory activities (Thornhill and Kelly, 2000). Similarly, Curcuma longa or turmeric also showed potential anti-inflammatory activity but its target sites of inhibition and the mechanism of action are still unknown and uncertain. In this study, we will emphasize on the anti-inflammatory and anti-allergic activities of Curcuma longa and its possible target sites for the treatment of allergic rhinitis.

Curcuma longa or turmeric is a commonly used curry spice as well as a traditional Chinese medicinal herb with a long history of anti-inflammatory, antioxidant, anti-carcinogenic, anticoagulant and antidiabetic activities. Its constituents mainly include three curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), volatile oils (natlantone, tumerone and zingiberone), proteins, sugar and resins (He et al., 2015). Among these, the curcuminoids including curcumin and its derivatives are the major bioactive compounds. Curcumin, a pleiotropic molecule has the capacity of interacting with numerous molecular targets involved in inflammation. Thus curcumin plays a major role in the anti-inflammatory action of Curcuma longa (Jurenka, 2009). Thus, it is important to relate the role of curcumin and its anti-inflammatory activity for the possible treatment of common allergic diseases like allergic rhinitis.

1.1 Hypothesis The present study has been designed to identify literature based active compounds in C. longa as well as its scientific validation as a potential drug against allergic rhinitis through in silico and in vitro studies.

2. Review of literature 2.1 Allergic, non-allergic and mixed rhinitis

Allergic rhinitis (AR) which is widely recognized as hay fever is a symptomatic disorder inducing an allergic response to specific allergens. It was demarcated by symptoms including nasal blockage or congestion, sneezing, itching of the conjunctiva, rhinorrhea, lacrimation or teary eyes, pruritis of oropharynx and nasal mucosa, allergic shiners and fatigue. A person with an ancestry of the disease or the similar symptoms or with a personal history of diseases like asthma or eczematous dermatitis is most likely of having this disease. Although it may also have a genetic cause as recent researches and studies identified the gene FAM134B encoding a reticulophagy receptor protein having a role-play in the disease (Islam et al., 2017). Based on the types of allergens, AR can be classified as seasonal, occupational or perennial. Seasonal allergens mainly include pollens and moulds whereas perennial allergens include dust, mites, pests etc. Environmental pollution and diverse pollutants may also be a serious concern for the incidence of allergic rhinitis. Classification of AR is also based on whether the symptoms are intermittent or persistent, with categories of mild, and moderate to severe based on the guidelines of allergic rhinitis and its impact on asthma (ARIA). Its prevalence is ever increasing throughout the globe. About 60 million people are affected by it, of which 1-40% was adults and 2-25% was children (Brozek et al., 2017). On the other hand, non-allergic rhinitis (NAR) is another symptomatic disorder but without a cause. It mainly involves sneezing, drippy nose, congestion and other symptoms similar to allergic rhinitis but without any apparent cause as any such allergic reaction is prominently observed or evident. But certain factors are known to trigger non-allergic rhinitis proving chronic or transient symptoms. These factors mainly include environmental, weather, occupational, certain medications or food and beverages and infections. Similarly, mixed rhinitis (MR) is a condition with combinable symptoms of allergic plus non-allergic rhinitis. Sometimes mixed rhinitis may be more common than allergic and non-allergic rhinitis. Sometimes MR is considered to have occurred when people having allergic rhinitis show its different symptoms of being exposed to some environmental or other related irritants, strong odours and even smokes (Sin and Togias, 2011). 2.2 Allergic rhinitis is a type I hypersensitivity

Our body’s incitive response for the offending allergen results in inflammation which depends upon a series of events for its initiation. These complex sequences of events include the release of certain chemical mediators which play a major role in the initiation of inflammatory responses in our body. Inflammation due to allergic reactions is mainly characterized by the mast cell activation which is Ig-E dependent along with the activation of CD4+ Th2 lymphocytes and the simultaneous influx of eosinophils. Allergic rhinitis also triggers an immune response particularly a type I hypersensitivity reaction (Min, 2010). Type I reactions of hypersensitivity are mainly characterized by the release of chemical mediators during the mast cell or basophil degranulation which in turn acts on secondary effector immune cells like eosinophils, monocytes, T lymphocytes, neutrophils and platelets. These chemical mediators are classified as primary and secondary mediators. The primary mediators which are stored in the granules located in the cytoplasm are synthesized before the mast cell or basophil degranulation. These mediators mainly include histamine, neutrophil chemotactic factor, eosinophils chemotactic factor and heparin. On the other hand, secondary mediators like leukotrienes, cytokines, prostaglandins, platelet-activating factor and bradykinins are produced during degranulation process with their release on significant phospholipid breakdown of the biological membranes or after the activation of the target cell (White, 1990).

Histamine, which is a primary chemical mediator, released by a number of cells primarily acts in response to tissue damage by binding to nearby receptors inducing permeability of vessels and vasodilation. The presence of histamine in the mast cell granules along with other mediators which are pharmacologically active are generally noted. Several cytokines including GM-CSF, IL-6 and IL-2 along with others like IL-4, IL-5, IL-9, IL-13 etc are involved in the degranulation of basophil and proliferation of eosinophil along with the significant release of histamine (Bousquet et al., 1996). The receptor subfamilies of these cytokines have structural similarity with an identical signal transducing subunit. Certain chemokines are also involved in inflammatory reactions which when bind to its particular receptor initiates a series of signal transduction cascade which in turn stimulates the release of contents of the cytoplasmic granules, histamine from basophils, proteases and certain cytotoxic proteins (Barnes, 2011). On random exposure to an allergen, mainly the responses are categorized in mainly three ways: sensitization, early phase reaction, late phase reaction. The initial step or sensitization of allergen consists of events like the random exposure of antigen (allergen), it's subsequent processing by dendritic cells as well as its presentation to certain immune cells like T lymphocytes. This induces interaction of B lymphocytes with the T lymphocytes along with the activity of certain cytokines and co-stimulatory molecules. This interaction, in turn, triggers the B cells to synthesize specific IgE molecules. Further the exposure or vulnerability of the specific allergen by the sensitized individual triggers the second phase i.e. early phase reaction (Busse and Lemanske, 2001). The immediate or early phase reaction occurs immediately within a few minutes and can last for about 2-3 hour in a sensitized person on cross-linking of the allergen to mast cell surface bound Ig E. Next, the degranulation of mast cells upon its activation takes place which results in the release pre-formed primary mediators specifically histamine. The early phase reaction is succeeded by the late phase reaction about 4-6 hours later with a number of predominant symptoms including rhinorrhea, sneezing and specifically nasal congestion. A number of mediators including leukotrienes, prostaglandins, bradykinins, histamine released in the late phase are predominantly related to inflammation due to the activity of basophils, T lymphocytes and eosinophils. Additionally, the release of several cytokines and chemokines acts as chemoattractants for other chemokines like eotaxin, RANTES, MCP-4 as well as causes the influx of eosinophils, basophils and T lymphocytes towards the nasal mucosa. As a result of this infiltration of cells, late reaction phase has a continuation of symptoms due to their prolonged survival (Pawankar et al., 2011). Activation of mast cell and its subsequent degranulation are important events in the initiation of allergic reaction in allergic rhinitis. The cross-linking of allergen is the initiation step which further stimulates a cascade of reactions leading to degranulation of the cytoplasmic granules in mast cell releasing histamine and other mediators. The offending allergen crosslinks with the IgE antibody attached to the mast cell in the Fc receptor leading to aggregation of FcεRI which in turn activates a membrane attached protein tyrosine kinase. This enzyme helps in the phosphorylation of phospholipase C, which is involved in the conversion of phosphatidylinositol-4,5 bisphosphate or PIP2 to two compounds namely- inositol triphosphate (IP3) and diacylglycerol (DAG). IP3 helps in the mobilization of intracellular calcium ions whereas DAG helps in the microtubule assembly and fusion of the granule to the membrane by activating the protein kinase C which works in conjugation with calcium ions for the assembly and fusion as shown in figure 2.1.

Figure 2.1- The events occurring post receptor ligand interaction in the FcεRI. PTK-Protein tyrosine kinase, PC-phosphatidylcholine, PIP2-phosphatidylinositol-4,5 bisphosphate IP3-inositol triphosphate, DAG-diacylglycerol, PKC-protein kinase C

The crosslinking also helps in conversion of phosphatidylserine (PS) to phosphatidylethanolamine (PE) after which PE gets methylated and converts into phosphatidylcholine or PC with the help of two enzymes namely- phospholipid methyltransferase I (PMTI) and phospholipid methyltransferase II (PMTII). Next, this PC gets accumulated on the outer side of the membrane developing fluidity of plasma membrane which leads to the formation of calcium ion channels for calcium influx. This leads to the disintegration of PC into arachidonic acid and lysophosphatidylcholine by the activation of phospholipase A2, of which, arachidonic acid breaks down to form leukotrienes and prostaglandins by the lipooxygenase(LOX) and the cyclooxygenase(COX) pathway respectively (shown in figure 1.2).

Fig 2.2- The events occurring post receptor ligand interaction. PS- phosphatidyl serine, PE-phosphatidylethanolamine, PC-phosphatidylcholine, LysoPC-lysophosphatidylcholine

Another simultaneous event that occurs due to the FcεRI cross-linkage is the activation of adenylate cyclase located in the membrane. The activated adenylate cyclase increases the cAMP (cyclic AMP) level by conversion of ATP to cAMP. Later the cAMP-dependent protein kinase reduces the cAMP level and also phosphorylates the granule membrane protein leading to change in membrane fluidity and permeability which helps the calcium ions and water molecules to enter. This influx of molecules results in subsequent swelling of the granules along with its fusion with the plasma membrane and ultimately releasing the potent mediators like histamine. On the other hand, the increase in the level of calcium ions mediates the conversion of arachidonic acid to leukotrienes and prostaglandins along with its function in the fusion of granules to the membrane which is described diagrammatically in figure 1.3 (Owen and Punt, 2013).

Fig 2.3- The biochemical events taking place simultaneously after the receptor ligand interaction. Activation of adenylate kinase converts ATP to cAMP which activates Protein kinase A(PKA) to ultimately release histamine from the granules.

2.3 Histamine Intolerance: another aspect of allergies Histamine intolerance or pseudo-allergy, unlike allergies with IgE mediated histamine response to allergens, is a toxic histamine response by the body because of accumulation of excessive endogenous or exogenous histamine in the body. It is caused mainly due to the impaired breakdown of histamine or its overproduction. Defects in the enzymes involved in its synthesis and breakdown are responsible for its occurrence. It is a condition whereby the level of histamine is raised in the body either due to defective or mutated enzyme histidine decarboxylase responsible for its synthesis from the amino acid histidine or due to deficiency of enzyme diamine oxidase (DAO) responsible for the further breakdown of histamine. However, DAO is responsible mainly for the breakdown of exogenous or ingested histamine while another enzyme Histamine methyltransferase (HMT) is responsible for the degradation of endogenic histamine into its constituent components.

Histamine intolerance can be caused due to many factors that may lead to a rise in the levels of histamine. Sometimes, certain food chemicals are responsible for this condition to occur as a result of food allergy that causes inflammation. Fermented foods are primarily responsible for the production of these biogenic amines in excess due to activation of mast cells. Histamine is a biogenic amine and its accumulation may also result from the intake of various foods exogenously. Various foods resulting in increased histamine levels include fish, meat or dairy products, fermented foods, beer, wine etc. The symptoms of histamine intolerance mainly coincide with those of allergic rhinitis which is why it is often confused or misinterpreted as allergic rhinitis whereas on the other hand it is barely allergy and is involved in the increased level of histamine or its accumulation. Because of this, the treatments opted for show less or no improvement failing to target the enzymes involved in histamine intolerance.

2.4 Allergic rhinitis and its impact on asthma (ARIA) guidelines

Extensive studies in allergic rhinitis and its possible therapeutics have been reported and therefore during a

0: https://emedicine.staging.medscape.com/article/134825-overview 87%

workshop by

World Health Organization, allergic rhinitis and its impact on asthma (

ARIA) guidelines have been initiated in 1999. It was then published (2001) and updated (2008) and again revised in 2010 for following the GRADE (Grading of Recommendation, Assessment, Development and Evaluation) approach which was the first ever guideline in allergy based on scientific evidence. It mainly consisted of a detailed summary of the highlights and challenges related to the recommendations along with the potential and preferred recommendations for the well being and up-gradation of the lives of the common mass in relation to the decreased quality of life. Another revision of ARIA guidelines (2014) was considered significant in the development and management of AR. It is a global health initiative with a sturdy purpose of educating, implementing and proper execution of allergic rhinitis disease management guidelines based on evidence in correlation to asthma. It classifies the diseases and categorizes it into four stages based on the severity- (1) Intermittent (mild, moderate and severe) (2) Persistent (mild, moderate and severe) Based on these guidelines and severity of the disease, common drug treatments like oral H1- antihistamine, intranasal H1-antihistamine, leukotriene receptor antagonists and intranasal corticosteroids are recommended for treating allergic rhinitis either alone or in combination. According to the revised ARIA guidelines, second generation H1- antihistamines were preferred over the first generation H1-antihistamines due to safety concern. Leukotriene receptor antagonists (LTRA) also became another class of commonly recommended drugs for allergic rhinitis (Min, 2010). Antihistamine comprises the most widely used and largest class of drugs for AR which mainly targets the blockade of H1 histamine receptors which are present in the nerve endings and the nasal vascular system. The presence of ethylamine moieties in the first generation

antihistamine provides them easy access to the blood-brain being highly lipophilic (Rachelefsky, 1998). Due to this a number of its negative effects may be noticed including euphoria, blurred vision, dizziness, upset stomach, tremors etc. The apparent side effects commonly drowsiness makes it less potent and therefore were not recommended after the revised ARIA guidelines were published. In contrast, second generation antihistamines had a higher H1 receptor selectivity and so they are commonly recommended under the ARIA guidelines (Walekar et al., 2016). Similarly, other classes of drugs against AR are recommended either alone or in combination.

2.5 Pharmacological treatment options for allergic rhinitis

Although not a serious disorder, AR has mild or moderate symptoms initially. However, it may also turn to chronic and even worse are the risk of other serious issues like asthma or anaphylactic shock. It also decreases the quality of life as it interferes with the sleep pattern, school or work due to its prolonged symptoms. Thus it is necessary to undergo proper treatment. Antihistamines and leukotriene receptor antagonists are the most common and widely used pharmacological drug against allergic rhinitis. Many standard drug treatments have been proposed against allergic rhinitis, of which H1-antihistamines, intranasal glucocorticoids, and leukotriene-receptor antagonists are most common. Fexofenadine is a common second-generation non-sedating H1-antihistamine with greater selectivity for the H1 receptor (Walekar et al., 2016). Montelukast and zafirlukast are two of the most commonly prescribed LTRA available worldwide (Dempsey, 2017). Recently, budesonide, triamcinolone acetonide, fluticasone propionate, mometasone furoate and fluticasone furoate are widely used intranasal glucocorticoids (Min, 2010). All these drugs are USFDA approved and are commonly used for the treatment of allergic rhinitis In addition to these, several other pharmacological treatment options like oral decongestants, newly inhaled steroids, intranasal mast cell stabilizers, intranasal anticholinergics, monoclonal anti- Ig E antibody treatment and immunotherapy. It is to be noted that different pharmacological treatment options are to be opted based on its symptoms and severity. Allergic testing, RAST testing or skin testing is another option for allergic rhinitis through which direct avoidance of allergen can be made. Intranasal corticosteroids are common allergic drugs that provide inhibition of early phase response and late phase response by blocking cytokine secretion (IL-5, IL-13 and IL-4) as well as decreasing the level of eosinophils and Ig-E production in our body (Lee et al., 2001). It can be used for all symptoms but is especially effective in case of nasal obstruction and eye symptoms. Common corticosteroids include fluticasone furoate, mometasone furoate, fluticasone propionate, budesonide, beclomethasone dipropionate etc are commonly prescribed intranasal corticosteroids mainly used for nasal congestion (Min, 2010). Common oral decongestants like pseudoephedrine act mainly on the α- adrenergic receptors of the respiratory or nasal mucosa for the stimulation of vasoconstriction. It stimulates α- adrenergic receptors which increase the blood pressure as well as stimulate β-adrenergic receptors which enhances our heart pulse rate and its contractility. They are recommended to be used either alone or with antihistamines as a combination therapy for treatment of nasal congestion (Medscape). New inhaled steroids is a recent approach to the treatment of AR. Ciclesonide, a common example of this class is inhaled in an inactive state which later modifies to be pharmacologically active by certain esterases present in the upper as well as a lower

respiratory tract and functions effectively. It is currently under clinical development for more efficient results in the treatment of AR (Braido et al., 2008). Cromolyn sodium, a common mast cell stabilizer is used against seasonal allergic rhinitis. It is generally considered to block the histamine release along with the release of other mediators mainly by stabilizing mast cells during inflammation (Druce and Kaliner, 1998). It blocks the calcium ion influx during degranulation. But its biggest disadvantage is that it must be used continuously for weeks until the required efficacy is obtained (Lieberman, 1988). Intranasal anticholinergic drugs mainly function by inhibiting the interaction of acetylcholine to its specific receptors located on the mucous glandular tissue. This specifically reduces the nasal secretion of mucus and efficiently reduces rhinorrhea (Spector, 1999). Ipratropium bromide, an anticholinergic drug is particularly used for the treatment of rhinorrhea.

There are certain immunotherapeutic approaches discovered which work efficiently by enhancing the quality of life by reducing the symptoms overall by modification of the progression of the disease. Monoclonal anti- Ig E antibody treatment and immunotherapy are two efficient treatment options for allergic rhinitis. Monoclonal anti- Ig E antibody treatment uses humanized monoclonal anti-IgE antibody modified through recombinant DNA technology to block the interaction of IgE antibodies with the mast cells or basophils. It mainly works by binding itself to the IgE antibodies (ARIA, 2007). This approach efficiently reduces IgE level in the blood. Omalizumab is a monoclonal anti-IgE antibody which is used to decrease nasal symptoms in addition to reducing inflammation. It is especially helpful in case of seasonal allergic rhinitis with allergens like ragweed pollen, birch and other outdoor allergens (Braido et al., 2008). Immunotherapy is another immunological therapeutic approach which involves desensitization of allergens such that the basic allergic mechanism is altered. Allergen extract doses are either injected subcutaneously or administered orally, sublingually or through nasal route. It is used for allergic treatment against seasonal allergens (pollens), animal dander, dust, hymenoptera etc. The advantage of using immunotherapy is the prolonged effect even after its subsequent discontinuation (Min, 2010).

2.6 Adverse effects of these drugs

Although pharmacotherapeutic drugs have higher effectiveness with instant or immediate results, the incidence of their adverse effects can also be equally observed and encountered. Pharmacological drugs for allergic rhinitis have certain mild or severe side effects. These side effects may also be dependent on the dose, delivery or coordination of the drug administration or simply the attributes of the drug itself (Braido et al., 2008). According to revised guidelines of ARIA in 2007, the first generation antihistamines were not considered safe due to certain health issues faced by the patients as side effects. The ability of a drug to cross the blood-brain barrier has a significant effect on its action. First generation antihistamines are mainly small chemical components that circulate in the bloodstream until it easily crosses the blood-brain barrier having a greater impact on the CNS. Due to this a number of its adverse effects were visible including insomnia, blurred vision, anxiety, nervousness, hallucinations, tremors and depression (Aaronson, 1998). Even some of the commonly used drug therapies have many disadvantages and the effects of which including gastrointestinal, nasopharyngeal, CNS, sensory or anticholinergic effects. There may also be

certain general side effects including a headache, rash, gastric dyspepsia, dental pain, respiratory tract infections or dream abnormalities due to administration drugs of leukotriene receptor inhibitors. Oral antihistamines may have side effects like a headache, drowsiness, stomach upset or myalgias. Certain intranasal therapies including corticosteroids, antihistamines and mast cell stabilizers may also lead to a range of nasopharyngeal or sensory side effects like epistaxis, nasal irritation, cough, pharyngitis, loss of sense of taste or smell and headache. Similarly, certain oral decongestants may also cause adverse symptoms like dizziness, weakness, headache, insomnia, nervousness, palpitations and urinary retention (UMHS AR Guideline, 2013). Moreover efficient pharmacological as well as immunological therapies may also be cost intensive. 2.7 Knowledge gap Natural products are used as remedies for various diseases and ailments. Curcuma longa, having a wide history of anti-inflammatory activity is a common medicinal herb being used as traditional medicine for a number of chronic diseases. Presence of active components like curcumin renders it anti-inflammatory property which is important for treatment of allergic diseases like allergic rhinitis. The mechanism of action of curcumin and its potential target sites of inhibition are not distinct and clear. Thus there is an immense necessity for the study of curcumin and its role in the possible treatment of allergic rhinitis as an alternative therapy which is cost effective and with lesser or no side effects. Furthermore, the in silico scientific validation of C. longa as a potential source of treatment of AR is yet to be studied.

2.8 History of anti-inflammatory natural products against Allergic Rhinitis The concept of natural products has its roots back in the 19th century. Classical examples of drug compounds discovered this way is morphine, the active agent in Opium, and digoxin, a heart stimulant originating from flower Digitalis lanata (Lahlou, 2013). Historically natural products have been a rich source of compounds that have a great importance in medicine, pharmacy and biology. A number of important new commercial drugs have been obtained from natural sources. Drugs of natural origin can be classified as original natural products, products derived semi-synthetically from natural products or synthetic products based on natural product model. Many standard drug treatments have been proposed against allergic rhinitis, of which H1-antihistamines, intranasal glucocorticoids, and leukotriene-receptor antagonists are most common. Fexofenadine is a common second-generation non-sedating H1-antihistamine with greater selectivity for the H1 receptor (Walekar et al., 2016). Montelukast and zafirlukast are two of the most commonly prescribed LTRA available worldwide (Dempsey, 2017). Recently, budesonide, triamcinolone acetonide, fluticasone propionate, mometasone furoate and fluticasone furoate are widely used intranasal glucocorticoids (Min, 2010). All these drugs are USFDA approved and are commonly used for the treatment of allergic rhinitis.

2.9 Importance and advantages of natural anti-inflammatory products

The pharmaceutical drugs come with many pros and cons and most importantly its cons could be highly felt in comparison to the naturally derived products. The benefits of modern drugs could only be utilized primarily in developed countries, being highly cost intensive. Not being able to access the modern healthcare products, the developing countries still relied on ethnobotanical remedies as primary medicines. Moreover, some of the significant pharmaceutical drugs come with many side effects (Ratini, 2017). Natural products considered

to be the best source of drugs for past many years as these having no or very poor side-effects and easy availability in addition to their cost-effectiveness. Moreover, natural products have structural and chemical diversity. Natural products are a good source of active therapeutic agents.

2.10 Currently used natural anti-inflammatory products Several plants are known for their anti-inflammatory activity due to the presence of many important phytochemicals. But most of them are not used clinically despite their potent activity. Although very less is known about the potent natural inhibitors of AR, some studies show that Urtica dioica or stinging nettle, bromelain (a proteolytic enzyme derived from the stem of pineapple plant), quercetin (a flavonoid found in many herbs and vegetables), N-acetylcysteine (a natural sulfur-containing amino acid derivative) and vitamin C had potential antihistamine and anti-inflammatory activities. Moreover, some plants like Curcuma longa are shown efficient anti-inflammatory property but are not yet identified as a cure for AR. Thus the importance of natural compounds that can be effectively used for its treatment without any side effects is highly felt. There is a number of phytochemicals showing high efficiency against allergic rhinitis due to their anti-inflammatory property which is yet to be accepted as therapeutic or diagnostic agents. Potential identification and screening are utterly important in order of these phytochemicals to be recognized as therapeutic standards.

3. Material and methods 3.1 In silico potential screening 3.1.1 Protein preparation and modelling The protein structures of target proteins histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) were downloaded from RCSB Protein Data Bank in .pdb format. The structure of another protein histamine H4 receptor was not available in PDB so the protein structure modelling was done using online servers like SWISS-MODEL. The protein sequence was initially obtained from NCBI FASTA format and searched for homology protein in BLAST for sequence recognition and alignment. A detailed 3d structure modelling was done by online server SWISS-MODEL and a QMEAN Z-score was checked for its checking its efficiency and % similarity. Next, structure analysis was carried out using model analysis tools like PDBSUM and SAVESERVER. The model analysis was checked using PROCHECK which analyses residue-by-residue geometry along with the general or comprehensive geometry to check the stereochemical protein quality and the modelled structure. PROVE gives us a calculated statistical Z-score deviation for the modelled protein. The Ramachandran plot is also calculated which is also available in the tools.

3.1.2 Retrieval of receptors The crystal structure proteins histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) were retrieved from RCSB Protein Data Bank. Standard inhibitors such as doxepin, histidine methyl ester (HME), glutathione sulfonate, 2,4-thiazolidinedione and Galphaq binds with the targeted proteins histamine H1 receptor, histidine decarboxylase, leukotriene C4 synthase, adenylate kinase and phospholipase C respectively on the heterodimer interface. For the remaining proteins that were not complexed or inhibitor-bound structures originally as well as for the modelled protein structure, active site prediction was done using SiteMap in Maestro (Version 11.2.013). All the

heteroatoms were removed leaving only the residues of the receptor. Preparation of the target protein with ADT involved the addition of polar hydrogen to the macromolecule, an essential step to correct the calculation of partial charge. Finally, Gasteiger charges will be calculated for each atom of the macromolecule.

3.1.3 Ligand preparation Different literature database has been searched for the presence of phytochemicals in curcumin powder. At the end of literature survey, we found the presence of 265 phytochemicals in curcumin powder of different origin and brand (Abdel-Lateef et al., 2016). The structures of the phytochemicals are given below.

1 2 3

4 5 6

7 8 9

10

11 12

13 14 15

16 17 18

19 20

21

22 23

24

25 26 27

28 29 30

31 32

33

34 35

36

37 38 39

40

41

42

43 44 45

46 47 48

49 50 51

52 53 54

55 56 57

58 59 60

61 62 63

64 65 66

67 68 69

70 71 72

73 75 74

76 77 78

79 80 81

82 83

84 85 86

87 88 89

90 91 93

94 95 96

97 98 99

100 101 102

103 104 105

106 107 108

109 110 111

112 113 114

115 116 117

118 119 120

121 122 123

124 125 126

127 128 129

130 131 132

133 134 135

136 137 138

139 140 141

142 143 144

145 146 147

148 149 150

151 152 153

154 155 156

157 158 159

160

161 162

163 164 165

166 167 168

169 170 171

172 173 174

175 176 177

178 179 180

181 182 183

184 185 186

187 188 189

190 191 192

193 194 195

196 197 198

199 200

202 201

203

204

205

206 207 208

209 210 211

212 213 214

215 216

217 218 219

220 221 222

223 224 225

226 227 228

229 230 231

232 233 234

235 236 237

238 239 240

241 242 243

244 246 245

247 248 249

250 251 252

253 254 255

256 257

Figure3.1- The structures shown above are phytochemicals present in turmeric namely- (1) curcumin (curcumin I) (2) demethoxycurcumin (curcumin II) (3) 1-(4-hydroxy-3-methoxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione (4)1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione (5) bisdemethoxycurcumin (curcumin III) (6) tetrahydroxycurcumin (7)5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one (8) 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (9) 1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione (10) 5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one (11) 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione (12) 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one (13) 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (14) 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (15) 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one (16) 1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene (17) Cyclocurcumin (18) 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one (19) 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one (20) 1,5-bis(4-hydroxyphenyl)-penta-(1E,4E)-1,4-dien-3-one (21) 1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1, 4-pentadiene-3-one (22) 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1E,4E)-1,4-dien-3-one (23) 4"-(4"'-hydroxyphenyl)-2"-oxo-3"-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate (24) 4"-(4"'-hydroxyphenyl-3-methoxy)-2"-oxo-3"-butenyl-3-(4'-hydroxyphenyl)-propenoate (25) calebin-A (26) (E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one (27) (E)-ferulic acid (28) (Z)-ferulic acid (29) vanillic acid (30) vanillin (31) p-cymene (32) m-cymene (33) α-terpinene (34) γ-terpinene (35)α-cedrene (36) β-phellandrene (37) p-mentha-1,4(8)-diene (38) terpinen-4-ol (39) 4-terpinol (40) limonene (41) terpinolene (42) thymol (43)phellandrol (44) carvacrol (45) (E)-carveol (46) γ-terpineol (47) menthol (48) 1,3,8-paramenthatriene (49) p-methylacetophenone (50) piperitone (51) o-cymene (52) carvone (53) p-menth-8-en-2-one (54) α-thujene (55) α-terpineol (56) p-cymen-8-ol (57) p-meth-8-en-2-one (58) piperitone epoxide (59) sylvestrene (60) menthofuran (61) β, β-dimethylstyrene (62) camphor (63) teresantalol (64) benzene (65) 1-methyl-4-(1-methylpropyl) (66) 2-norpinanone (67) borneol (68) bornyl acetate (69) (E)-chrysanthenyl acetate (70) (Z)-cinerone (71) (Z)-sabinol (72) 2-(2,5-dihydroxy-4-methylcyclohex-3-enyl)propanoic acid (73) camphene (74) 3-carene (75) 2-carene (76) ascaridole (77) α-pinene (78) β-pinene (79) cineole (80) cis-ocimene (81) citronellal (82) geranial (83) neral (84) myrcene (85) R-citronellene (86) citronellyl pentanoate (87) nerol (88) geraniol (89) iso-artemisia ketone (90) trans-ocimene (91) linalool (92) neryl acetate (93) geranic acid (94) geranyl acetate (95) 3-bornanone (96) 4,8-dimethyl-3,7-nonadien-2-ol (97) 3,4,5,6-tetramethyl-2,5-octadiene (98) 3,7-dimethyl-6-nonenal (99) 2,6-dimethyl-2,6-octadiene-1,8-diol (100) 4,5-dimethyl-2,6-octadiene (101) ar-turmerone (102) α-turmerone (103) β-turmerone (104) 2-methyl-6-(4-hydroxyphenyl)-2-hepten-4-one (105) 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one (106) 2-methoxy-5-hydroxybisabola-3,10-diene-9-one (107) 2-methyl-6-(4-formylphenyl)-2-hepten-4-one (108) 5-hydroxyl-ar-turmerone (109) 4-methylene-5-hydroxybisabola-2,10-diene-9-one (110) ar-curcumene (111) ar-turmerol (112) bisabola-3,10-diene-2-one (113) bisabolone (114) 4, 5-dihydroxybisabola-2,10-diene (115) 4-hydroxybisabola-2,10-diene-9-one (116) 4-methoxy-5-hydroxy-bisabola-2,10-diene-9-one (117) bisacurone (118) bisabolone-9-one (119) bisacumol (120) turmeronol A (121) turmeronol B (122) α-oxobisabolene (123) α-zingiberene (124) xanthorrhizol (125) zingerone (126) dehydrozingerone (127) (Z)-α-atlantone (128) (E)-α-

atlantone (129) β-bisabolene (130) (6S,7R)-bisabolene (131) γ-bisabolene (132) γ-curcumene (133) β-curcumene (134) α-curcumene (135) β-sesquiphellandrene (136) (Z)-γ-atlantone (137) (E)-γ-atlantone (138) (6S)-2-methyl-6-[(1R,5S)-(4-methene-5-hydroxyl-2-cyclohexen)-2-hepten-4-one (139) curcuphenol (140) curlone (141) (142)curculonone C (143) curculonone D (144) curculonone B (145) curculonone A (146) 2, 5-dihydroxybisabola-3, 10-diene (147) (6R)-[(1R)-1,5-dimethylhex-4-enyl]-3-methylcyclohex-2-en-1-one (148) β-atlantone (149) 2,8-epoxy-5-hydroxybisabola-3,10-diene-9-one (150) α-bisabolol (151) dihydro-ar-turmerone (152) dehydrocurcumene (153) (4S,5S)-germacrone-4,5-epoxide (154) dehydrocurdione (155) germacrene D (156) germacrone (157) germacrone-13-al (158)β-germacene (159) 1,10-dehydro-10-deoxy-9-oxozedoarondiol (160) curcumenol (161)epiprocurcumenol (162) isoprocurcumenol (163) zedoaronediol (164) procurcumadiol (165)procurcumenol (166) naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene) (167) α-selinene (168) juniper camphor (169) corymbolone (170) α-santalol (171) β-santalene (172) (E)-caryophyllene (173) caryophyllene oxide (174) β-elemene (175) γ-elemene (176) acoradiene (177) aristolene (178) (Z)-α-bergamotene (179) curcumenone (180) di-epi-cedrene (181) himachalene (182) (E)-sesquisabinene hydrate (183) bicyclo[7.2.0]undecane, 10,10-dimethyl-2,6-bis(methylene) (184) γ-gurjunen epoxide (185) 1-epi-cubenol (186) cubebene (187) 7-epi-sesquithujene (188) caryophyllene (189) 6α-hydroxycurcumanolide A (190) curcumanolide A (191) curcumanolide B (192) curcumin L (193) α-humulene (194) 12-oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-,adoxal, (195)2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (196) (E,E)-α-farnesene (197) 5,9-undecadien-2-one, 6,10-dimethyl-, (Z)-, hxadecane-1,2-diol (198) nerolidal (199) (Z)-β-farnesene (200) nerolidyl propionate (201) phytol (202) (E,E,E)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene (203) 2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene (204)1,6,10,14-hexadecatetraen-3-ol,3,7,11,15-tetramethyl-, (E,E)- (205) hopenone I (206) hop-17(21)-en-3β-ol (207) hop-17(21)-en-3β-yl acetate (208) β-sitosterol (209) stigmasterol (210) gitoxigenin (211) 20-oxopregn-16-en-12-yl acetate (212) linoleic acid (213) 8,11-Octadecadienoic acid (214) methyl ester (215) palmitic acid (n-hexadecanoic acid) (216) oleic acid (217) stearic acid (218) curcuma-J (219) 2-(2'-methyl-1'-propenyl)-4, 6-dimethyl-7-hydroxyquinoline (220) 2,3,5-trimethylfuran (221) (1,2,3-trimethyl-cyclopent-2-enyl)-methanol (222) dicumyl peroxide (223) 1-(3-cyclopentylpropyl)-2,4-dimethy-benzene (224) 1,4-dimethyl-2-(2-methylpropyl)-benzene (225) 2,2'-oxybis[octahydro-7,8,8-trimethyl-4,7-methanobenzofuran (226) cyclohexyl formate (227) methyleugenol, 3,3,5-trimethyl-cyclohexanol acetate (228) 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one (229) 2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-cyclohexene-1-carboxaldehyde (230) bicyclo[3.3.1]nonan-9-one, 2,4-dimethyl-3-nitro- (exo)- (231) 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol (232) pyrazolo[1,5-a]pyridine, 3,3a,4,7-tetrahydro-3,3-dimethyl-, (3aS) (233) β-vatirenene (234) (–)-isolongifolol (235) 2,4,6-triethylcyclohexyl)methanol (236) 2-ethenyl-1,1-dimenthyl-3-methylene-cyclohexane (237) 2-isopropylidene-3-methylhexa-3,5-dienal, 2-methoxy-4-vinyl phenol (238) 5-isopropenyl-1,2-dimethylcyclohexan-2-enol7-epi-cis-sesquisabinene hydrate, agarospirol (239) benzene-2-methyl-1,4-bis(1-methylethyl, cis-p-menth-2,8-dienol (240) cis-sabinol (241) cis-Z-α-bisabolene epoxide (242) cis-β-elemenone (243) curdione, (243) curzerene, (244)dehydrosaussurea lactone dihydrocostunolide (245) DL-2,3-butanediol (246) furanodiene (247) geranyl-p-cumene, (248) hemellitol (249) isocurcumenol (250) isolongifolol (251) isoshyobunone (252) L-trans-

chrysanthenyl acetate (253) m-eugenol (254) α-cedrene (255) α-cubebene (256) α-thujone (257) β-cedrene

The literature-based 3D or 2D structure of phytochemicals of target proteins histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) and the modelled protein were retrieved in .sdf format from NCBI PubChem. For the conversion of 2D to 3D conformation open Babel molecule format converter were used, Marvin Sketch software (version 15.10.0) performed conversion from .sdf to .pdb (for docking) and mol (for molecular properties prediction) file. For the structure of the ligand that was not available in NCBI PubChem, chemical structures were prepared using Marvin Sketch. Ligands energy was minimized by applying mmff94 force field and conjugate gradients optimization algorithm using PyRx-Python prescription 0.8. for 200 steps (Dallakyan et al., 2015).

3.2.4. Molecular docking Crystal structure of target enzyme histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) were obtained from the RCSB protein data bank along with the structure of a protein that have been modeled. The preparation of the target enzyme with the Auto Dock Tools involved in the addition of hydrogen atoms to the target enzyme, which is a necessary step for the computation of partial atomic charges. Gasteiger charges will be considered for each atom present in the target in Auto Dock 4.2. To dock small-molecule libraries to a macromolecule virtual molecular screening is used in order to hit upon lead compounds with desired biological function. In PyRx software, we perform docking of the ligands and proteins. PyRx software is open version software with an intuitive user interface that runs on all major operating systems (Linux, Windows, and Mac OS) (Dallakyan et al., 2015). Receptor-based molecular docking was conducted using GLIDE software from PyRx-Python prescription 0.8. Each of these compounds was docked into target protein accordingly with positions, orientations, and conformations of the ligand in the receptor binding site, and the docking structure possessing the lowest energy was preferred (Singh et al., 2015). The proteins and the ligands were loaded into Auto Dock Tools 4.2 (ADT) for docking experiments. After merging non-polar hydrogen and torsions applied to the ligands by rotating all rotatable bonds gasteiger partial charges are assigned Docking calculations carried out on the protein models. With the aid of Auto Dock tools polar hydrogen atoms and solvation parameters were added. AutoDock 4.2 offers the option of three search algorithms to explore the space of active binding with different efficacy. Docking was performed with the targeted proteins interface by keeping the number of points 25.0000, 25.0000 and 25.0000, in X, Y and Z dimension and centre grid box values were kept. The grid box includes the entire binding site of the proteins interface and provides enough space for the ligands translational and rotational walk. After that, PyRx-Python prescription 0.8 is used for visualization of the interaction pattern in the protein-ligand complex (Kumar et al., 2016).

3.2.5 Protein-Ligand Interaction and LigPlot The protein-ligand complexes in .pdb format are displayed, edited and run via the software LigPlot+ (version v.1.4.5) for generation of LigPlot schematic diagrams. The protein-ligand interaction along with the hydrogen bonding and hydrophobic interactions with the complex binding residues are given for the lead

phytochemicals. The LigPlot shows the amino acid of the target protein which is involved in the interaction with the ligand. The interaction profile of lead phytochemicals and the interface between heterodimers of a protein can be determined. The amino acids involved in hydrogen bonding and hydrophobic interaction with the ligands can also be easily determined.

3.2.5 Protein-Ligand Binding Surface-Structure Determination The binding of the modified ligands with active sites of the targeted protein is determined and visualized by surface structure determination of protein and ligand binding. It is a molecular modelling technique whereby the interaction between the protein and ligands is determined by the position and orientation of the ligand when bound to the protein. The surface structure of the protein and ligand is determined through PyMOL Molecular Graphics System software (version 1.1). The modified pdbqt files of proteins and ligands are prepared and displayed in PyMOL which gives a commendable visualization of the protein-ligand interaction. The best docking score of proteins are chosen with the ligands having the best binding affinity and the surface structure was prepared.

3.3 In vitro validation 3.3.1 Histamine detection in fermented curd Fresh samples of curd were taken in different test tubes such that each test tube has 4ml samples each. These samples were incubated for about 10-14 days at 37°C. After 14days the test tubes were retained and sought for histamine detection by certain biochemical tests utilizing colour forming ability.

3.3.2 Histamine detection by biochemical tests for colour formation The incubated curd samples were obtained on day 14 and tested for the presence of histamine. Each sample was added with 1N NaOH (sodium hydroxide) in the ratio 7:3. Next, a 10ml sulfanilic acid solution of concentration 0.05mg/ml is added along with 2 drops of 2M HCl(hydrochloric acid) and 2drops of NaNo2 (sodium nitrite). Formation of a deep red colour gives a positive result to this biochemical identification test (Nguyen and Nguyen, 2015).

3.3.3 Qualitative Analysis and Histamine detection Based on the rudimentary detection test we have prepared an experimental setup for qualitative analysis of histamine detection and its inhibition using turmeric. A fresh sample of curd was taken in a set of 11 test tubes containing 3.5ml of curd in each test tube. Next powdered turmeric was taken in different concentrations which were initially dissolved in milk as a vehicle in the experiment. Turmeric was measured as 10, 20, 25, 50, 100, 150, 200, 250 and 300mg per 0.5ml of the vehicle and was added to 9 of the tubes as a sample. A vehicle control was taken which comprised of the 0.5ml vehicle in the absence of turmeric. Also, a negative control containing 4ml of curd was taken and incubated for about two weeks at 37°C along with the other tubes. These test samples were then tested for the presence or formation of histamine as well as the interference of turmeric for its inhibition.

3.4 In vitro anticancer activity by MTT assay In vitro cell proliferation assay were perform using MTT [3-(4, 5- dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide]. The compounds were tested for the antiproliferative activity against ht29 (colon cancer) cell lines at different concentrations (0.5, 0.25, 0.125, 0.08, and 0.04 mg/ml). Cells were seeded at a density of 1×104cells/well in a 96-well plate with media containing 10% FBS and 1% penicillin/

streptomycin. The cells were allowed to attach and grow for 48 hrs. To test the growth inhibitory effects of the compound, cells were treated with various concentrations and incubated for 3 days at 37°C and 5% CO2 humidified atmosphere. After incubation 100µL of 5mg/ml, MTT solution was added to cells and further incubated for 4 hrs at 37°C. After incubation, the medium was removed and 200µL DMSO was added to each well to dissolve the formazan crystals. The absorbance of formazan dye was read using an ELISA plate reader at 595nm and the optical density (OD) was recorded. The following formula was used to calculate the inhibitory rate of cell growth. Controls and samples were assayed in triplicate. The results were shown as mean ± SD (Rahamoz Haghighi et al., 2016).

Growth inhibition% = [(AC−AS)/AC] ×100

Where, I represented inhibition and AC and AS are the absorbance values of the control and the sample, respectively. Three replicates were made for each sample and results were expressed as mean ± SD (Kumar et al., 2014). 3.5 In vitro antioxidant activity assay

The free radical scavenging activity of the compound was measured in vitro by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay. Appropriate DPPH solution concentrations were prepared in methanol followed by addition of 1 mL of the test sample at different concentration. The content was mixed and allowed to stand at room temperature for 30 minutes and absorbance was measured at 517 nm. The lower absorbance of the reaction mixture indicated higher free radical activity (Tailor et al., 2014). The percentage scavenging activities (%Inhibition) at different concentrations of the extracts were calculated using the following formula: (%) I= [(AC−AS)/AC] ×100

Where, I represented inhibition and AC and AS are the absorbance values of the control and the sample, respectively. Three replicates were made for each sample and results were expressed as mean ± SD (Kumar et al., 2014). 3.6 In vitro antibacterial activity assay by disk diffusion method

Antimicrobial activity of phytochemicals will be determined by using Kirby-Bauer disc diffusion method against Gram-negative Escherichia coli. The inoculum suspension of bacterial strains will be swabbed on the entire surface of the prepared Luria-Bertani (LB) agar plates. Sterile 6 mm diameter paper discs (Himedia) saturated with 20 µL of phytochemicals prepared in DMSO (containing 2 mg extract/disc) will be aseptically placed on the upper layer of the inoculated agar surfaces and the plates will be incubated at 37°C for 24 hours. The antibacterial activity will be determined by measuring the diameter of the zone of inhibition (ZOI) surrounding discs. Standard antibiotic discs of penicillin (10 µg/disc) and norfloxacin (10 µg/disc) would be used as positive control. Discs containing 20 µL DMSO will be used as a negative control. The antimicrobial assay will be performed in triplicate and the results will be reported as the average of three replicates (Bauer et al., 1966)

4. Results 4.1 Protein Preparation

The protein structures were retrieved from PDB and the remaining protein structure of histamine H4 receptor that could not be retrieved from PDB was generated by using SWISS-

MODEL. After generation of protein structure by protein modelling techniques, the modelled protein was checked for its efficacy by analysing several parameters. Structure analysis of the modelled protein was done along with the determination of Ramachandran Plot. Figure 4.1 shows the quality factor or efficacy of the protein structure. The structural resolution of the protein of the protein must exceed 95% to be considered as competent and acceptable. The quality factor of the modelled protein was found to be 97.112 which show that it qualifies the 95% rejection limit.

Figure 4.1- The ERRAT graph for determining protein model quality factor

The Ramachandran Plot of the modelled histamine H4 receptor protein retrieved from PROCHECK is shown in figure 4.2. An ideally accepted model must have about 90% of the values of ϕ and ψ dihedral angles of the amino acid residues lying in the most favoured region (red). The modelled protein shows 89% in terms of residues in the most favoured region. The rest of the dihedral angles of the remaining amino acid residues lying in the additional or generously allowed regions are minimally distributed for about 9.4% and 0.6%.

Fig 4.2- Ramachandran plot showing values in phi and psi angles.

The PROVE Plot helps us determine and calculate an average statistical Z-score deviation for the modelled protein. Figure 4.3 shows the estimated average Z-score and Z-score RMS.

Fig 4.3- Graphical representation of Z-score 4.2 Ligand- Protein Binding Analysis

Different literature database has been searched for the presence of phytochemicals in curcumin powder. At the end of literature survey we found the presence of 265 phytochemicals in curcumin powder of different origin and brand (Abdel-Lateef et al., 2016). Over all literature showed that all the phytochemicals were present in every test sample but the quantity of some of the phytochemicals varied in some of the test samples. The literature based ligands of C. longa like curcumin (curcumin I), demethoxycurcumin (curcumin II), 1-(4-hydroxy-3-methoxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione, 1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione, bisdemethoxycurcumin (curcumin III), tetrahydroxycurcumin, 5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one, 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one, 1,7-bis(4-hydroxyphenyl)-1-heptene-3,5-dione, 5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one. 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione, 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one, 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one, 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one, 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one, 1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene, Cyclocurcumin, 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,4,6-heptatrien-3-one, 1,7-bis-(4-hydroxyphenyl)-1,4,6-heptatrien-3-one, 1,5-bis(4-hydroxyphenyl)-penta-(1E,4E)-1,4-dien-3-one, 1-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)-1, 4-pentadiene-3-one, 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1E,4E)-1,4-dien-3-one, 4"-(4"'-hydroxyphenyl)-2"-oxo-3"-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate, 4"-(4"'-hydroxyphenyl-3-methoxy)-2"-oxo-3"-butenyl-3-(4'-hydroxyphenyl)-

propenoate, calebin-A, (E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one, (E)-ferulic acid, (Z)-ferulic acid, vanillic acid, vanillin, p-cymene, m-cymene, α-terpinene, γ-terpinene α-cedrene, β-phellandrene, p-mentha-1,4(8)-diene, terpinen-4-ol, 4-terpinol, limonene, terpinolene, thymol, phellandrol, carvacrol, (E)-carveol, γ-terpineol, menthol, 1,3,8-paramenthatriene, p-methylacetophenone, piperitone, o-cymene, carvone, p-menth-8-en-2-one, α-thujene, α-terpineol, p-cymen-8-ol, p-meth-8-en-2-one, piperitone epoxide, sylvestrene, menthofuran, β, β-dimethylstyrene, camphor, teresantalol, benzene, 1-methyl-4-(1-methylpropyl), 2-norpinanone, borneol, bornyl acetate, (E)-chrysanthenyl acetate, (Z)-cinerone, (Z)-sabinol, 2-(2,5-dihydroxy-4-methylcyclohex-3-enyl)propanoic acid, camphene, 3-carene, 2-carene, ascaridole, α-pinene, β-pinene, cineole, cis-ocimene, citronellal, geranial, neral, myrcene, R-citronellene, citronellyl pentanoate, nerol, geraniol, iso-artemisia ketone, trans-ocimene, linalool, neryl acetate, geranic acid, geranyl acetate, 3-bornanone, 4,8-dimethyl-3,7-nonadien-2-ol, 3,4,5,6-tetramethyl-2,5-octadiene, 3,7-dimethyl-6-nonenal, 2,6-dimethyl-2,6-octadiene-1,8-diol, 4,5-dimethyl-2,6-octadiene, ar-turmerone, α-turmerone, β-turmerone, 2-methyl-6-(4-hydroxyphenyl)-2-hepten-4-one, 2-methyl-6-(4-hydroxy-3-methylphenyl)-2-hepten-4-one, 2-methoxy-5-hydroxybisabola-3,10-diene-9-one, 2-methyl-6-(4-formylphenyl)-2-hepten-4-one, 5-hydroxyl-ar-turmerone, 4-methylene-5-hydroxybisabola-2,10-diene-9-one, ar-curcumene, ar-turmerol, bisabola-3,10-diene-2-one, bisabolone, 4, 5-dihydroxybisabola-2,10-diene, 4-hydroxybisabola-2,10-diene-9-one, 4-methoxy-5-hydroxy-bisabola-2,10-diene-9-one, bisacurone, bisacurone A, bisabolone-9-one, bisacumol, turmeronol A, turmeronol B, α-oxobisabolene, α-zingiberene, xanthorrhizol, zingerone, dehydrozingerone, (Z)-α-atlantone, (E)-α-atlantone, β-bisabolene, (6S,7R)-bisabolene, γ-bisabolene, γ-curcumene, β-curcumene, α-curcumene, β-sesquiphellandrene, (Z)-γ-atlantone, (E)-γ-atlantone, (6S)-2-methyl-6-[(1R,5S)-(4-methene-5-hydroxyl-2-cyclohexen)-2-hepten-4-one, curcuphenol, curlone, curculonone C, curculonone D, curculonone B, curculonone A, 2, 5-dihydroxybisabola-3, 10-diene, (6R)-[(1R)-1,5-dimethylhex-4-enyl]-3-methylcyclohex-2-en-1-one, β-atlantone, 2,8-epoxy-5-hydroxybisabola-3,10-diene-9-one, α-bisabolol, dihydro-ar-turmerone, dehydrocurcumene, (4S,5S)-germacrone-4,5-epoxide, dehydrocurdione, germacrene D, germacrone, germacrone-13-al, β-germacene, 1,10-dehydro-10-deoxy-9-oxozedoarondiol, curcumenol, epiprocurcumenol, isoprocurcumenol, zedoaronediol, procurcumadiol, procurcumenol, naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene), α-selinene, juniper camphor, corymbolone, α-santalol, α-santalene, β-santalene, (E)-caryophyllene, caryophyllene oxide, β-elemene, γ-elemene, acoradiene, aristolene, (Z)-α-bergamotene, curcumenone, di-epi-cedrene, himachalene, (E)-sesquisabinene hydrate, bicyclo[7.2.0] undecane, 10,10-dimethyl-2,6-bis(methylene), γ-gurjunen epoxide, 1-epi-cubenol, cubebene, 7-epi-sesquithujene, caryophyllene, 6α-hydroxycurcumanolide A, curcumanolide A, curcumanolide B, curcumin L, α-humulene, 12-oxabicyclo[9.1.0]dodeca-3,7-diene, 1,5,5,8-tetramethyl-,adoxal, 2,6,10-dodecatrien-1-ol, 3,7,11-trimethyl-, (E,E)-α-farnesene, 5,9-undecadien-2-one, 6,10-dimethyl-, (Z)-, hxadecane-1,2-diol, nerolidal, (Z)-β-farnesene, nerolidyl propionate, phytol, (E,E,E)-3,7,11,15-tetramethylhexadeca-1,3,6,10,14-pentaene, 2,6,11,15-tetramethyl-hexadeca-2,6,8,10,14-pentaene, 1,6,10,14-hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)-, hopenone I, hop-17(21)-en-3β-ol, hop-17(21)-en-3β-yl acetate, β-sitosterol, stigmasterol, gitoxigenin, 20-oxopregn-16-en-12-yl acetate, linoleic acid, 8,11-Octadecadienoic acid, methyl ester, palmitic acid (n-hexadecanoic acid), oleic acid, stearic acid, curcuma-J, 2-(2'-

methyl-1'-propenyl)-4, 6-dimethyl-7-hydroxyquinoline, 2,3,5-trimethylfuran, (1,2,3-trimethyl-cyclopent-2-enyl)-methanol, dicumyl peroxide, 1-(3-cyclopentylpropyl)-2,4-dimethy-benzene, 1,4-dimethyl-2-(2-methylpropyl)-benzene, 2,2'-oxybis[octahydro-7,8,8-trimethyl-4,7-methanobenzofuran, cyclohexyl formate, methyleugenol, 3,3,5-trimethyl-cyclohexanol acetate, 2,4-dimethyl-8-oxabicyclo[3.2.1]oct-6-en-3-one, 2,6-dimethyl-6-(4-methyl-3-pentenyl)-2-cyclohexene-1-carboxaldehyde, bicyclo[3.3.1]nonan-9-one, 2,4-dimethyl-3-nitro-(exo)-, 2,2,4-trimethyl-3-(3,8,12,16-tetramethyl-heptadeca-3,7,11,15-tetraenyl)-cyclohexanol, pyrazolo[1,5-a]pyridine, 3,3a,4,7-tetrahydro-3,3-dimethyl-, (3aS), β-vatirenene, (–)-isolongifolol, 2,4,6-triethylcyclohexyl)methanol, 2-ethenyl-1,1-dimenthyl-3-methylene-cyclohexane, 2-isopropylidene-3-methylhexa-3,5-dienal, 2-methoxy-4-vinyl phenol, 5-isopropenyl-1,2-dimethylcyclohexan-2-enol, 7-epi-cis-sesquisabinene hydrate, agarospirol, benzene-2-methyl-1,4-bis(1-methylethyl, cis-p-menth-2,8-dienol, cis-sabinol, cis-Z-α-bisabolene epoxide, cis-β-elemenone, curdione, curzerene, dehydrosaussurea lactone, dihydrocostunolide, DL-2,3-butanediol, furanodiene, geranyl-p-cumene, hemellitol, isocurcumenol, isolongifolol , isoshyobunone, L-trans-chrysanthenyl acetate, m-eugenol, α-cedrene, α-cubebene, α-thujone, β-cedrene were chosen and their structures were either downloaded from Pubchem (of those which are available) and the rest were prepared in Marvin Sketch (Version 5.10.0). These ligands were docked against the targeted proteins for prediction of the binding score. The table 4.1 summarizes the results of molecular d docking studies comprising lowest binding energy and proteins residues involved in hydrogen bonding (265 docking runs by library preparation) with screened ligands (selected standard inhibitors and phytochemicals). Further, prediction of ligands with lowest binding energy is done.

Table 4.1: Comparative table of binding affinity between ligands with targeted proteins is given. Here A(3rze), B(4e1o), C(3hkk), D(), E(), F(), G() PCID Standard A B C D E F G

Doxepin -12.02

Histidine methyl ester -5.23

2,4-thiazolidinedione -9.43

Glutathione sulfonate

PCID Name A B C D E F G 5280450 linoleic acid -10.21

-2.59 -3.56 -0.77 -4.21 -1.21 -2.02 445639 Oleic acid -9.42 -2-.83 -2.99 -1.39 -1.61 -1.21 -2.21 5281 Stearic acid -9.35 -2.01 -2.27 -0.43 -3.64 -0.98 -2.21 5312487 8,11-Octadecadienoic acid -9.02 -2.86 -3.55 -0.76 -3.79 -0.93 -2.63 15858385 Tumeronol-A -8.97 -4.43 -4.59 -3.95 -6.12 -4.19 -5.07 - Zingerone -8.91 -3.41 -4.55 -2.87 -5.8 -3.6 -4.52 - Limonene -8.88

-2.18 -4.41 -3.92 -4.78 -3.7 -3.92 - α-selinene -8.9 - -5.61 -5.66 -6.02 -4.95 -4.45 360253 Curcuphenol -8.72 -4.22 -4.83 -4.47 -5.54 -4.11 -4.45 - Menthol -8.7 - -3.81 -4.33 -4.48 -1.86 -2.63 5315469 2-Methyl-6-(4-methylphenyl)hept-2-en-4-ol -8.69 -2.76 -4.23 -3.87 -5.48 -3.52 -4.07 93135 Xanthorrhizol -8.57 -4.8 -4.42 -4.36 -5.35 -3.96 -4.42 167812 (+)-Curcumenol -8.55 -2.65 -3.58 -4.36 -2.77 -3.2 -2.45 - naphthalene,1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethylidene) -8 .51 - -5.09 -4.47 -6.25 -4.86 -5.93 53464495 Curcumin-d6 -8.8 -6.18 -5.38

-4.73 -6.67 -4.49 -4.08 10955433 Turmeronol-B -8.36 -4.76 -4.95 -4.7 -5.98 -3.82 -3.99 92281781 Curcumenol -8.31 -3.82 -4.07 -3.74 -3.71 -3.6 -3.68 10921984 dihydro-ar-turmerone -8.28 -2.47 -4.16 -3.51 -5.09 -2.93 -4.12 2889 Curcumin -8.62 -5.84 -5.9 -6.52 -6.77 -5.07 -5.43 11241433 (4R,6S)-2-Methyl-6-(4-methylphenyl)hept-2-en-4-ol -8.25 -3.94 -4.04 -4.49 -5.34 -2.89 -3.75 11063457 Dehydrocurcumene -8.23 -1.71 -3.38 -4.44 -4.47 -1.29 -3.12 10399139 Isocurcumenol -8.16 -2.68 -4.67 - -4.18 -2.25 -1.23 102165199 Curculonone-C -8.14 -3.08 -4.14 -3.23 -5.37 -2.98 -2.94 643779 Neral -8.11 -2.17 -4.07 - -3.84 -3.88 -2.6 20055539 (E)-sesquisabinene hydrate

-8.08

- - - - - - 14191392 dehydrocurdione -8.08 -3.03 -3.23 -1.53 -2.46 -2.65 -2.43 91753574 1. cis-(Z)-.alpha.-Bisabolene epoxide -8.08 -2.51 -4.03 -3.65 4.75 2.48 -3.5 15095 Himachalene -8.05 -2.19 -3.82 -3.09 -3.49 -1.12 -2.3 12299867 (Z)-α-atlantone -8.03

-2.34 -3.43 -4.78 -5.37 -2.43 -3.63 - bisabolone-9-one -8.03

-2.87 -2.13 -3 -2.79 -2.12 519857 1-epi-cubenol -7.94

-2.12 -3.97 -3.15 -4.29 -2.69 -2.13 92139 ar-curcumene -7.93

-2.23 -3.47 -4.03 -4.32 -1.79 -3.36 13967857 β-atlantone -7.92

-3.06 -3.51 -4.17 -5.35 -1.54 -3.47 442360 α-curcumene --7.92

-1.96 -3.41 -4.29 -4.03 -1.56 -2.68 6436348 Germacrone -7.89 -2.3 -3.03 -3.32 -3.08 -2.34 -1.52 14543198 Isoprocurcumenol -7.88 -2.75 -5.19 -3.2 -4.48 -3.85 -2.59 14633002 Germacrone-13-al -7.85 -2.85 -3.4 -2.95 -3.64 -2.36 -2.59 91747196 Cubebene -7.8

-2.75 -3.61 -3.76 -4.26 -2.28 -2.19 91750423 gamma.-Gurjunene epoxide -7.8 -2.72 -3.27 - -2.73 -1.58 -0.5 86609 Alpha-Cubebene -7.77

-2.31 -3.44 -3.72 -3.46 -2.2 -2.06 12304273 Gamma-Curcumene -7.76 -2.3 -3.18 -4.16 -4.05 -1.51 -3.41 10421034 1-Bisabolone -7.74 -2.41 -3.32 -3.42 -5.45 -2.35 -3.25 519762 cis-β-elemenone -7.73 -2.74 -3.54 -2.86 -2.97 -1.64 -2.95 6432312 Gamma-Elemene -7.72

-1.82 -3.61 -3.5 -3.09 -1.02 -2.31 1742210 Caryophyllene oxide -7.69 -2.06 -3.44 -3.15 -3.69 -2.4 -1.09 10104370 Beta-Bisabolene -7.67 -2.04 -3.7 -4.03 -4.52 -1.76 -2.86 196216 Curlone

-7.65 -3.68 -3,7 -3.4 -5.09 -2.1 -2.94 56927990 7-epi-sesquithujene -7.64 -2.06 -3.11 -3.93 -2.7 -1.54 -2.66 10857025 Bisabolone -7.61 -3.78 -3.99 -3.6 -5.11 -1.55 -2.85 101967134 Dihydrocostunolide

-7.6

-3.97 -3.47 -3.45 -2.37 -2.24 -1.61 17750987 Alpha-cedrene -7.59 -1.49 -2.99 -3.16 -3,86 -1.64 - - 2,8-epoxy-5-hydroxybisabola-3,10-diene-9-one -7.56

-4.37 -3.64 -3.25 -5.3 -2.07 -2.98 1548883 (Z)-ferulic acid

-7.56

-6 -3.97 -3.61 -3.74 -2.59 -4.11 189061 Procurcumenol -7.55

-3.2 -4.82 - -4.44 -3.4 -2.63 10586 Bisabolol -7.55

-1.87 -4.31 -3.15 -5.72 -2.45 -3.27 5368797 α-santalol -7.55

-3.49 -4.41 -3.31 -4.02 -2.72 -3.6 10703 o-cymene

-7.9 -1.93 -4.95 -4.84 -6.24 -3.74 -3.54 91698329 (Z)-gamma-Atlantone -7.48 -3.77 -3.72 -3.35 -5.17 -2.32 -3.47 14014430 Beta-Curcumene -7.47 -2.27 -3.21 -4.1 -4.09 -1.66 -3.18 12305301 1. Curzerene

-7.46 -2.64 -4.01 -3.01 -2.87 -1.84 -2.63 5280435 Phytol -7.44

-1.46 -2.49 -2.92 -4.96 -1.88 -1.88 90475698 Beta-Germacrene C -7.44 -2.12 -3.53 -3.26 -3.3 -2.08 -1.69 - (E)-4-(4-hydroxy-3-methoxyphenyl)but-3-en-2-one

-7.43

-5.82 -4.07 -4.09 -4.22 -2.03 -3.42 5373727 Germacrene D -7.43

-2.2 -3.58 -3.6 -3.62 -1.88 -2.26 31211 Zingerone -7.43 -3.33 -5.45 -4.21 -4.72 -3.31 -5.12 - Curcumenol

-7.4

- -3.39 -4.37 -3.57 -1.32 -2.01 92776 Alpha-Zingiberene -7.38

-2.11 -3.24 -3.81 -4.7 -1.51 -2.83 - γ-curcumene

-7.37

- -3.45 -3.88 -4.8 -2.05 -3.71 - 4-hydroxybisabola-2,10-diene-9-one

-7.33

-4.3 -4.04 -0.92 -3.41 -3.63 -2.93 91751355 Geranyl-p-cymene -7.32 -2.27 -4.11 -4.13 -5.27 -1.73 -3.65 10263440 Epiprocurcumenol -7.32 -3.96 -3.49 -3 - -3.46 -2.7 - β-pinene

-7.3

-3.45 -2.73 -3.13 -2.3 -1.48 14287397 Bisacurone -7.28 -4.1 -5.43 -4.08 -6.98 -3.52 -5.91 24834047 1,10-dehydro-10-deoxy-9-oxozedoarondiol -7.24

-2.62 -4.65 - - -4 -2.67 12315492 Beta-Sesquiphellandrene -7.24 -1.98 -3.55 -3.89 -4.54 -1.67 -2.64 14633011 Procurcumadiol -7.23

-3.3 -4.27 - -4.59 -3.1 -3.02 102165198 curculonone B -7.23

-3.15 -4.17 -3.68 -5.89 -2.88 -3.05 90351 Acoradiene -7.23

-2.28 -3.35 -3.73 -2.97 -2.32 -1.77 14287395 Bisacurone-A -7.2 -3.24 -5.05 -4.13 -6.15 -3.23 -4.23 21675005 Agarospirol -7.18

-2.79 -3.96 -3.14 -4.86 -2.77 -2.56 - γ-terpineol

-7.18 - -3.09 -3.6 -3.16 -1.68 -2.77 - 3-carene

-7.17

-4 -4.3 -3.12 -3.84 -2.28 -3.93 - vanillic acid

-7.17

-3.76 -4 -3.37 -3.99 -3.15 -3.87 196216 Curlone -7.16 -3.68 -3.7 -3.4 -5.09 -2.1 -2.94 6641 dicumyl peroxide -7.15 -3.18 -3.45 -4.13 -4.53 -1.79 -1.97 985 Palmitic acid

-7.12

-2.35 -2.12 -0.61 -3.95 -0.76 -1.77 - α-oxobisabolene

-7.08

- -4.29 -3.28 -4.98 -3.54 -3.76 6918391 Beta-elemene -7.08 -2.22 -2.83 -3.33 -2.78 -1.38 -2.49 5281522 Caryophyllene -7.08 -1.98 -3.26 -- -3.31 -1.55 -1.5 3033866 Gamma-Bisabolene -7.07 -2.92 -3.52 -3.75 -5.03 -1.97 -2.75 94164 Alpha-Santalene -7.04 -1.6 -2.91 -3.84 -3.82 -0.98 -1.89 12315160 Cis-Sabinol -7.03

-3.96 -3.76 -3.52 -3.5 -2.98 -2.98 14543198 Isoprocurcumenol -7.02

-2.75 -5.19 -3.2 -4.48 -3.85 -2.59 - 2-methyl-6-(4-formylphenyl)-2-hepten-4-one

-7.01

-3.77 -3.16 -2.94 -3.16 -1.58 -2.31 5318673 Isoshyobunone -7.01

-2.54 -3.12 -3.07 -3.67 - -2.72 5362828 Curdione -6.99 -2.54 -3.15 -3.56 -2.68 -2.53 -1.63 10856614 Alpha-selinene -6.95 -2.32 -3.7 -3.51 -3.31 -1.64 -2.35 153845 Curcumenone -6.95 -3.24 -4.6 -3.89 -4.24 -2.63 -2.57 6365122 (E)-Atlantone -6.92 -3.03 -3.83 -4.45 -4.96 -2.2 -2.88 10545 Ascaridole

-6.89

- -3.07 -3.01 -4.31 -1.45 -2.56 - 2-norpinanone

-6.89

- -2.36 -3.49 -3.36 -2.37 -3.11 - iso-artemisia ketone

-6.86 - -3.47 -3.37 -3.25 -1.78 -1.12 608753 β-vatirenene -6.81

-2.72 -3.17 -3.67 -3.83 -2.11 -2.08 102165200 Curculonone-D -6.81 -3.87 -4.43 -3.74 -5.33 -5.6 -5.74 6989 Thymol

-6.77

-2.47 -3.53 -3.15 -3.64 -1.87 -2 5318101 Alpha-Humulene -6.76 -1.88 -2.95 -3.34 -3.42 -1.97 -1.85 12311096 Isolongifolol -6.73

-3.2 -4.02 -2.44 -3.53 -2.15 -0.88 44409528 dehydrosaussurea lactone -6.72 -3.14 -4.53 -3.49 -3.69 -1.96 -2.29 14191394 Curcumanolide B -6.72

-4.3 -3.75 -2.62 -3.38 -2.02 -2.32 - 4-terpinol

-6.69

- -3.25 -2.42 -2.39 -2.25 -1.75 636458 Furanodiene -6.68 -2.16 -3.56 -3.96 -3.09 -2.56 -2.79 7462 α-terpinene

-6.66

-1.91 -3.69 -3.5 -1.89 -1.81 -2.72 10812 10812

-6.65

- -2.98 -2.6 -2.82 -1.9 -1.39 102165197 Curculonone-A -6.61 -2.91 -5.06 -3.78 -5.4 -2.81 -3.49 16217350 (–)-isolongifolol -6.6 -3.03 -3.27 -2.87 -3.18 -2.42 -2.31 90475698 β-germacene

-6.6

-2.12 -3.53 -3.26 -3.3 -2.08 -1.69 1183 Vanillin

-6.58

-3.68 -4.15 -4.26 -4.67 -2.64 -3.75 596375 m-eugenol -6.58 -4.62 -4.1 -3.46 -4.14 -2.8 -3.32 91698330 (E)-gamma-Atlantone -6.57 -2.51 -4.35 -3.41 -4.7 -2.65 -3.61 98037 1,2-Hexadecanediol -6.54 -2.14 -2.78 -3.73 -4.86 -2.68 -1.56 6429303 (Z)-α-bergamotene -6.54 -2.47 -3.44 -3.83 -2.63 -1.8 -1.93 261491 Alpha-Thujone -6.53 -3.44 -2.98 -3.31 -3 -1.9 -2.58 64685 Borneol

-6.47

-3.44 -2.07 -2.13 -3.56 -1.47 -1.86 - 1,10-dehydro-10-deoxy-9-oxozedoarondiol

-6.42

- -4.23 -4 -4.58 -3.07 -3.82 637429 calebin-A -6.39

-6.65 -2.5 -3.6 -3.73 -1.37 -1.86 5354238 dehydrozingerone -6.37 -3.71 -5.65 -3.89 -4.76 -3.21 -4.45 10686 Hemellitol -6.36 -1.79 -3.07 -2.89 -3.51 -1.68 -2.12 530421 Aristolene -6.34 -1.8 -2.99 -3.08 -2.07 -1.22 -0.52 5281516 1. (E,E)-alpha-farnesene -6.33 -0.56 -2.31 -2.66 -3.35 -0.16 -1.84 6448 bornyl acetate

-6.29

-4.02 -1.81 -4.47 -2.67 -1.71 -1.67 14191393 Curcumanolide A -6.28 -2.48 -3.24 - -2.71 -2.99 -2.52 7463 p-cymene -6.27 -1.79 -3.34 -3.12 -4.72 -2.98 -3.43 8500 p-methylacetophenone -6.27 -2.93 -3.53 - -2.74 -3.1 -3.06 10889018 β-santalene -6.23

-2.41 -3.24 -3.73 -3.31 -1.56 -2.21 98403 Farenal -6.19 -1.31 -2.17 -1.91 -2.56 -0.4 -1.98 5317319 (Z)-beta-Farnesene -6.02 -0.44 -1.91 -2.47 -3.18 -0.1 -1.68 - 5-hydroxyl-7-(4-hydroxy-3-methoxyphenyl)-1-(4-hydroxyphenyl)-4,6-heptadiene-3-one -6.02

-7.09 -4.07 -3.01 -2.89 -3.88 -1.48 - p-mentha-1,4(8)-diene -5.94

- -3.11 - - - -1.17 7127 Methyleugenol -5.92

-3.23 -3.76 -3.25 -4.25 -1.65 -3.5 11463 Terpinolene -5.75

- -3.26 -1.38 -2.94 -3.4 -2.81 129762283 Tetrahydroxycurcumin -

-8.48 - - - - - - 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one -

-8.14 - - - - - - 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one -

-7.93 - - - - - - 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one -

-7.39 - - - - - - 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione -

-6.8 - - - - - 637429 Calebin-A -

-6.65 - - - - - - 2-methyl-6-(4-formylphenyl)-2-hepten-4-one -

-6.51 - - - - - 1548883 Cis-ferulic acid -

-6 - - - - -

4.2 Protein-Ligand interaction and Lig-Plot The protein-ligand complexes in .pdb format are displayed, edited and run via the software LigPlot+ (version v.1.4.5) for generation of LigPlot schematic diagrams. The protein-ligand interaction along with the hydrogen bonding and hydrophobic interactions with the complex binding residues are given for the lead phytochemicals. The table-2 shows the hydrogen bonding and hydrophobic interactions of the ligands with the protein histidine decarboxylase. The LigPlot structures are also given below (Figure 4.4).

1 2

3 4

5 6

7 8

9 10

11 12

13 14

15 16 Fig 4.4- Lig Plots of Histidine decarboxylase protein interactions with the ligands or lead phytochemicals. The interaction of histidine decarboxlylase enzyme and ligands including- (1) tetrahydroxycurcumin (2) 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (3) 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (4) 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one (5) 5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one (6) 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione (7) 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1E,4E)-1,4-dien-3-one (8) 2-methyl-6-(4-formylphenyl)-2-hepten-4-one (9) calebin-A (10) curcumin I (11) curcumin II (12) 1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione (13) 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (14) 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one (15) 4"-(4"'-hydroxyphenyl)-2"-oxo-3"-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate (16) 4"-(4"'-hydroxyphenyl-3-methoxy)-2"-oxo-3"-butenyl-3-(4'-hydroxyphenyl)-propenoate

The LigPlot shows the amino acid of the target protein which is involved in the interaction with the ligand. The interaction profile of lead phytochemicals and the interface between heterodimers of the protein histidine decarboxylase with lowest binding energy to its known standard inhibitor is shown. The amino acids involved in hydrogen bonding and hydrophobic interaction with the lead phytochemicals are given in table 4.2 Table 4.2- The amino acid residues involved in the Hydrogen bonding and hydrophobic interaction with the ligands Sl. No. Protein-Ligand interaction Hydrogen Bonding Hydrophobic Interaction 1 Histidine decarboxylase and tetrahydroxycurcumin Gln347, Gln350, Tyr334

Pro332, Asn331, Val330, Leu335, Leu353, Pro352, Ile351, Phe328 2 Histidine decarboxylase and 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one Ser354, Gln350, Met347, Ile351, Tyr334

Pro352, Leu353, Pro328, Val330, Leu335, Pro332 3 Histidine decarboxylase and 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one Ile351, Asn331

Leu353, Pro352, Leu335, Tyr334, Pro332, Val330 4 Histidine decarboxylase and 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one Tyr334 Ile351, Val330, Pro332,

Pro352, Leu353, Leu335, Phe104 5 Histidine decarboxylase and 5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one Met347,Tyr334, Gln350

Pro332, Val330, Ile351, Leu353, Pro352, Leu335, Phe304 6 Histidine decarboxylase and 3-hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione Tyr334 Leu335, Leu353, Pro352, Met347, Ile351, Phe328, Val330, Pro332 7 Histidine decarboxylase and 1,5-bis(4-hydroxy-3-methoxyphenyl)-penta-(1E,4E)-1,4-dien-3-one Gln350, Pro352, Ile351, Met347, Asn331, Tyr334 Leu353, Phe328, Val330, Pro332 8 Histidine decarboxylase and 2-methyl-6-(4-formylphenyl)-2-hepten-4-one Gln350, Met347

Val330, Leu353, Pro352, Ile351, Phe328, Leu159, Thr327 9 Histidine decarboxylase and calebin-A Ile351, Met347, Tyr334

Phe328, Pro352, Leu335, Leu353, Val330, Pro332 10 Histidine decarboxylase and curcumin I Tyr334, Met347, Ile351, Gln350 Pro332, Leu353, Val330, Pro352, Phe328 11 Histidine decarboxylase and curcumin II Tyr334, Met347, Pro352, Ser354 Ile351, Leu335, Leu353, Val330 12 Histidine decarboxylase and 1-(4-hydroxyphenyl)-7-(3, 4-dihydroxyphenyl)-1, 6-heptadiene-3, 5-dione Gln350, Ile351, Met347, Tyr334

Val330, Leu353, Leu335, Phe104, His348, Pro332, Pro352 13 Histidine decarboxylase and 5-hydroxyl-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one Gln152, Ser148, Asp312, Arg358

Gln350, Pro352, , Ser354, Arg356, Arg355 14 Histidine decarboxylase and 1,5-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one Pro352, Met347, Tyr334, Ser354

Leu102, Phe104, Val330, Leu353, Ile351, Leu335 15 Histidine decarboxylase and 4"-(4"'-hydroxyphenyl)-2"-oxo-3"-butenyl-3-(4'-hydroxyphenyl-3'-methoxy)-propenoate Tyr334, Met347, Gln350, Ile351

Leu335, Pr332, Phe328, Val330, Leu355, Pro352 16 Histidine decarboxylase and 4"-(4"'-hydroxyphenyl-3-methoxy)-2"-oxo-3"-butenyl-3-(4'-hydroxyphenyl)-propenoate Tyr334

Leu353, Ile351, Met347, Phe328, His348, Pro352, Val330, Pro332

4.3 Protein-Ligand Binding Surface-Structure Determination The binding of the modified ligands with active sites of the targeted protein was determined and visualized by surface structure determination of protein and ligand binding. The surface structure of the protein and ligand is determined through PyMOL Molecular Graphics System software (version 1.1). The best docking score of proteins are chosen with the ligands having the best binding affinity and the surface structure was prepared. Proteins like Histamine H1 receptor (3rze) and histidine decarboxylase (4e1o) showed better interaction with the ligands. These phytochemicals have a higher affinity of binding to the targeted protein which resulted into a change in the conformation of the targeted protein. The high binding affinity of the interaction between protein-ligand complexes increased the greater intermolecular force between the ligands and its protein. Also, the high-affinity binding resulted in a higher degree

of occupancy for the ligand at its protein binding sites. The following are the surface structure of the target proteins and best lead compounds in figure 4.5.

1 2

3 4

5

Fig 4.5- The surface structures with molecular interaction of the protein and ligands. The surface structures of the lead phytochemicals with highest dock score are shown- (1) Histidine decarboxylase and tetrahydroxycurcumin (2) Histidine decarboxylase and 1,5-dihydroxy-1,7-bis(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (3) Histidine decarboxylase and 1,5-dihydroxy-1-(4-hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl)-4,6-heptadiene-3-one (4) Histidine decarboxylase and 1,5-dihydroxy-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene-3-one (5) Histidine decarboxylase and 5-hydroxyl-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl)-4,6-heptadiene-3-one

4.4 In vitro validation 4.4.1 Histamine detection in fermented curd by biochemical tests for colour formation In accordance with United States Pharmacopeia 23, the red colour formation in the final solution detects the presence of histamine. The results of the experiment are shown below in the following images. Figure 4.6 shows the red colouration which confirms the presence of histamine.

Figure 4.6- Detection of histamine by red color formation

4.4.2 Qualitative analysis and detection of histamine After performing the qualitative analysis in different samples along with the presence of turmeric we performed the tests for histamine detection. The control samples showed the presence of histamine by the formation of red colour although in very small amount. The test samples turned red after addition of NaOH due to degradation of curcumin in basic pH medium thus giving us false negative results. Figure 4.7 and 4.8 shows the various test samples before and after incubation period and subsequent biochemical analysis respectively. The negative and vehicle control samples are shown in Figure 4.9.

Figure 4.8- The samples after biochemical analysis

Figure 4.9- Biochemical analysis in negative control (c) and vehicle control (V)

The expected result was the red colour formation in the controls and not in the test samples. But as we know, turmeric is a natural pH indicator it gave false negative results in our experiment. Practically, turmeric is insoluble in water under neutral or acidic conditions but it becomes soluble in the alkaline condition being an oil-soluble pigment. Thus it gives a discolouration from yellow to red in the alkaline pH as it gains instability in its structure due to the conversion of stable predominant keto-form to enol form of its several phytoconstituents. As a result, when NaOH was added to the samples, red colour formation took place which concurred with the actual result of our experiment. The expected result was the inhibition or

reduction of histamine formation in the samples by lesser or no colour formation as observed in the negative and vehicle control.

4.5 In vitro anticancer activity (MTT assay) To access the antiproliferative activity of compound 002, the colon adenocarcinoma cell lines (HT-29) were used using MTT assay and the results are shown in figure 4.10. The result showed that 002 exhibited moderate anticancer efficacy at test concentrations (0.5-0.04 mg/ml). About 50% growth inhibition activity was observed at higher test concentration (0.5 mg/ml) against colon cancer cell line. The log(x) values were plotted in terms of molar concentrations. The IC50 values can be determined from the graph which distinctively shows the % inhibition with respect to the concentrations.

Figure 4.10- Graphical representation of withaferin treatment on MTT assay in Log(x) v/s % viability of cells

4.6 DPPH radical scavenging activity The antioxidant activity of 005 was measured at different concentrations (1, 2, 5, 10, 20 and 30 l/mL) by using DPPH assay and compared with standard antioxidant compound BHA (30 l/mL). Result showed that the antioxidant activity increased with increasing concentration of the sample (Figure 4.11). The radical scavenging activity of 005 was found to be 75-80% at different test concentration (1–20 l/mL). It was found at higher concentrations (100 l/mL).

Figure 4.11- Percentage scavenging activity of 005

4.7 Antibacterial activity by Disk Diffusion Method Antimicrobial activity of certain phytochemicals was determined by using Kirby-Bauer disc diffusion method against Gram-negative Escherichia coli. The LB agar plates containing Escherichia coli bacterial strains were retained and the paper disks saturated with the phytochemicals were placed in the media. After 24hour incubation, the zone of inhibition (ZOI) was observed in the plates with standard antibiotic disks of penicillin (0 µg/disc) and norfloxacin (10 µg/disc). However, the efficient zone of inhibition could not be seen in the agar plates with the phytochemicals (Bauer et al.,1966). The figures 4.12 and 4.13 show zone of inhibition in the bacterial colonies with disks of penicillin and norfloxacin respectively. Norfloxacin showed a greater zone of inhibition than penicillin. There was no ZOI observed in the control i.e DMSO as shown in figure 4.14.

Figure 4.13- Culture containing Norfloxacin Figure 4.12- Culture containing Penicillin

Figure 4.14- Culture containing DMSO

4.8 Discussion

From the time beginning it has been recorded that C. longa plant plays an important role in various diseases/ailments. Anti-inflammatory properties of C. longa phytochemicals are well documented in Ayurvedic literature and in another medicinal system of the world. The present study was designed to assess the potential of C. longa phytochemicals against allergic rhinitis. The literature showed the involvement of different proteins in the pathophysiology of disease (Figure 1.1, 1.2 and 1.3). Based on these we have targeted those proteins in the present study. The present study determines the in silico potential screening of the literature based

phytocomponents of C. longa and their molecular docking with significant proteins involved in the biosynthetic pathway of histamine formation and subsequent generation of allergic symptoms. The proteins involved in this pathway including histamine H1 receptor (3rze), histidine decarboxylase (4e1o), leukotriene C4 synthase (3hkk), 5-lipoxygenase (3o8y), adenylate kinase (2c9y), phospholipase C (4qj4) were targeted and molecular docking was performed. However, only the protein histidine decarboxylase showed efficient binding with the ligands in comparison to its standard inhibitor HME. The commonly prescribed treatment options for allergic rhinitis mainly include drug therapies like H1-antihistamines, LTRAs and intranasal glucocorticoids. Apart from these, there are alternatives like oral decongestants, newly inhaled steroids, intranasal mast cell stabilizers, intranasal anticholinergics, monoclonal anti- Ig E antibody treatment and immunotherapy are also opted for but less frequently. Although these are ideal for treatment of allergic rhinitis, they sometimes can lead to side effects along with longer duration for a cure. For instance, the prescribed drugs are carried out for a longer period of time yet there is no sign of recovery and the symptoms still prevail, there may be other conditions related to it like histamine intolerance. Histamine intolerance or pseudo-allergy, unlike allergies with IgE mediated histamine response to allergens, is a toxic histamine response by the body because of accumulation of excessive endogenous or exogenous histamine in the body. It is mainly caused due to defected or mutated enzymes involved in its biosynthesis or degradation. Histamine is a biogenic amine and its accumulation may also result from the intake of various foods exogenously. Various foods resulting in increased histamine levels include fish, meat or dairy products, fermented foods, beer, wine etc. The symptoms of histamine intolerance mainly coincide with those of allergic rhinitis which is why it is often confused or misinterpreted as allergic rhinitis whereas on the other hand it is barely allergy and is involved in the increased level of histamine or its accumulation. Because of this, the treatments opted for show less or no improvement failing to target the enzymes involved in histamine intolerance. The enzyme histidine decarboxylase plays a crucial role as it leads to histamine biosynthesis from histidine. Thus targeting this enzyme along with other enzymes like diamine oxidases and HMTs is significant in treating histamine intolerance. Histidine decarboxylase is the enzyme responsible for histamine formation from histidine in the granules, the release of which results in the generation of allergic symptoms commonly in allergic rhinitis. The lead phytochemicals including tetrahydroxycurcumin, cyclocurcumin, 1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene etc showed the G-score with % inhibition of 162%, 155% and 151% respectively as compared to its standard inhibitor HME. Although the remaining proteins showed efficient binding their G-scores are comparatively lesser in comparison to the scores of their respective standard inhibitors. The targeted enzymes involving allergic rhinitis could not be potentially inhibited by the ligands based on their dock score. But, the enzyme HDC showed efficient binding and percentage of inhibition by the ligands. Thus, this enzyme was targeted for further in vitro validation. Since the synthesis of histamine is dependent on the HDC enzyme, so it was targeted for in vitro studies and its potential inhibition by phytochemicals present in turmeric. The enzyme histidine decarboxylase (HDC) is responsible for the conversion of the amino acid histidine present in curd by the action of lactic acid bacteria. Most of the species of lactic acid bacteria present in curd possess the hdc gene which is responsible for the formation of the enzyme histidine decarboxylase. The activity of the enzyme is mainly to

convert histidine to histamine with the help of certain cofactors like vitamin B6, Mg and Zn. In silico validation of the phytoconstituents of turmeric show that the enzyme histidine decarboxylase is potentially inhibited by ligands like tetrahydroxycurcumin, cyclocurcumin, 1,5-epoxy-3-carbonyl-1,7-bis(4-hydroxyphenyl)-4,6-heptadiene etc based on which the expected result was the inhibition or reduction of histamine formation in the samples showing lesser or no color formation as observed in the negative and vehicle control. Turmeric is a natural pH indicator which directed false negative results in the in vitro experiment performed. Turmeric consists of several active compounds including curcumin which exhibits an extended conjugation and keto-enol tautomerism besides having a two-ringed aromatic phenolic structure. These properties in combination give turmeric its yellow colouration. Being chemically acidic in nature, curcumin can lose its proton easily in an alkaline environment. A sudden change in pH alters the stable predominated keto-form of their structure alters to unstable enol-form. Due to the loss of proton in the alkaline environment especially from its phenolic site, structural changes occur from benzenoid to quinonoid due to which alterations in extended conjugation, as well as tautomerism, occurs. Due to this structural alteration, a bathochromic shift occurs i.e., the quinonoid form emerges with a longer wavelength resulting in red colour rather than yellow. Practically, turmeric is an oil-soluble pigment and insoluble in water under neutral or acidic conditions. It becomes soluble in alkaline condition and changes its colour from yellow to red. This disco.louration occurs mainly due to the alterations in pH mainly from the range of 7.4 and above. As a result, the colour of turmeric in neutral or acidic pH i.e yellow or deep yellow is changed to dark pink or red colour. Thus when we added NaOH, which is a strong base to the samples; there was an instant irreversible change in colour to red which directed false negative results. The degradation of curcumin is slower in acidic medium and rapid in physiological pH or basic medium which makes it an intricate drawback in case of therapeutic utility.

4.9 Experimental Setup (Future Perspective)

Histidine decarboxylase is the enzyme responsible for histamine formation from histamine in the granules, the release of which results in the generation of allergic symptoms commonly in allergic rhinitis. The targeted enzyme histidine decarboxylase is also mainly involved mainly in the conversion of histamine from histidine resulting in accumulation of histamine or its increased levels leading to histamine intolerance. It is a toxic histamine response by the body because of accumulation of excessive endogenous or exogenous histamine in the body. Histamine is a biogenic amine and its accumulation may also result from the intake of various foods exogenously. Various foods resulting in increased histamine levels include fish, meat or dairy products, fermented foods, beer, wine etc. The enzyme HDC is responsible for the conversion of the amino acid histidine present in many fermented foods including curd by the action of lactic acid bacteria. Most of the species of lactic acid bacteria present in curd possess the hdc gene which is responsible for the formation of the enzyme histidine decarboxylase. The activity of the enzyme is mainly to convert histidine to histamine with the help of certain cofactors like vitamin B6, Mg and Zn. Based on in silico validation along with in vitro detection tests we confirmed the presence of histamine. The tests performed are rudimentary detection and confirmation tests. Based on these tests we have prepared a qualitative experimental setup. The gradation can be visually detected but due to the reaction of NaOH with curcumin,

we have obtained a false negative result. This can be quantitatively analyzed by means of the experimental setup mentioned below. 4.9.1 Histamine production and detection Isolation of Lactobacillus from curd is carried out and must be inoculated in MRS media for bacterial growth and it is incubated for 48hours. Pure culture colonies will be obtained from which a pure Lactobacilli strain must be inoculated in MRS broth along with additional components like 1%histidine, 0.6%vitamin B6 and 0.003% bromocresol (for detecting the presence of histamine). The bacterial culture is then incubated for around 1-2 weeks (Pham and Nguyen, 2016).

4.9.2 Extraction of histamine and its quantitative analysis by HPLC The bacterial culture is retained and centrifuged separating the supernatant. Addition of chloroform and ethanol further separates and subsequent heating leads to extraction of histamine. Quantitative analysis of histamine is done by HPLC against standard histamine for comparison (Pham and Nguyen, 2016).

5. Conclusion

Natural products are the popular remedies against a number of diseases and allergic rhinitis is no exception. Curcuma longa is a common medicinal herb with numerous medicinal properties. The anti-inflammatory potential is another commonly known property of turmeric that has been reported in various traditional medicinal systems including Ayurveda. The current study explores the anti-inflammatory potential of turmeric for its therapeutic usage against allergic disorders or histamine intolerance by in silico and in vitro validation. Various in vitro and in vivo studies are going on and a lot must be carried out in order to explore its absolute efficacy or medicinal potential. Moreover, the phytochemicals present in the herb is yet to be considered in modern medicine and pharmacology which can unwrap numerous utilities and potential in modern pharmacological drug therapies with lesser side effects and more efficacies.

Becker, G. S. and Murphy, K. M. (1988). A theory of rational addiction. Journal of

Political Economy 96: 675-700.

Barnes, P.J. (2011). Pathophysiology of allergic inflammation. Immunological Reviews. 242:31–50.

Bauer, W., Kirby, W.M., Sherris, J.C., Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 45: 493-496.

Dallakyan, S., and Olson, A. J. (2015). Small-molecule library screening by docking with PyRx. Chemical Biology: Methods and Protocols. 1263: 243-250.

Dempsey, O.J. (2000). Leukotriene receptor antagonist therapy. Postgraduate Medical Journal. 76: 767–773.

He, Y., Yue, Y., Zheng, X., Zhang, K., Chen, S., Du, Z. (2015). Curcumin, inflammation, and chronic diseases: how are they linked? Molecules. 20: 9183-9213.

Jurenka, J.S. (2009). Anti-inflammatory properties of curcumin, a major constituent of Curcuma longa : a review of preclinical and clinical research. Alternative Medicine Review. 14:141-153.

Kumar, S., Kumar, R., Dwivedi, A., & Pandey, A. K. (2014). In vitro antioxidant, antibacterial, and cytotoxic activity and in vivo effect of Syngonium podophyllum and Eichhornia crassipes leaf extracts on isoniazid induced oxidative stress and hepatic markers. BioMed Research International. 2014: 1- 11.

Kumar, S., Chashoo, G., Saxena, A.K., Pandey, A.K. (2013). Parthenium hysterophorus: a probable source of anticancer, antioxidant and anti-hiv agents. BioMed Research International. 2013. Kumar, S.; Kumar, R.; Dwivedi, A.; Pandey, A.K. (2014). In vitro antioxidant, antibacterial, and cytotoxic activity and in vivo effect of Syngonium podophyllum and Eichhornia crassipes leaf extracts on isoniazid induced oxidative stress and hepatic markers. BioMed Research International. 2014: 459452.

Lahlou, M. (2013). The success of natural products in drug discovery. Pharmacology & Pharmacy 4: 17.

Min, Y.G. (2010).

0: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 100%

The pathophysiology, diagnosis and treatment of allergic rhinitis. Allergy Asthma

Immunology

Research. 2: 65-76.

O'Boyle, N. M., Banck, M., James, C. A., Morley, C., Vandermeersch, T., Hutchison, G. R. (2011). Open Babel: An open chemical toolbox. Journal of Cheminformatics. 3:1.

Rachelefsky, G.S. (1998). Pharmacologic management of allergic rhinitis. Journal of Allergy and Clinical Immunology. 101:367-369.

Thornhill, S.M. and Kelly, A.M. (2000). Natural Treatment of Perennial Allergic Rhinitis. Alternative Medicine Reviews. 5: 448-454.

Walekar, A., Chodankar, D., Naqvi, M., Trivedi, C. (2016). Assessment of bioequivalence of fexofenadine and montelukast fixed dose combination tablet versus separate formulations of the individual components at the same dose levels. Indian Journal of Pharmaceutical Sciences.78:651-656

Wheatley, L.M. and Togias, A. (2015). Allergic rhinitis. New England Journal of Medicine. 372:456-463.

Min, Y.G. (2010).

0: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 100%

The pathophysiology, diagnosis and treatment of allergic rhinitis. Allergy Asthma &

Immunology

Research. 2:65-76.

Brozek, J.

0: https://biblio.ugent.be/publication/8538875 88%

L., Bousquet,

J.

et al. (2017).

Allergic rhinitis and its impact on asthma (ARIA)

guidelines 2016 revision.

Journal of Allergy and Clinical Immunology. 140: 950-958.

Lee, B.J., Kim, Y.J., Kim, J.H., Shin, H.S., Chung, Y.S. (2001). A comparative study of intranasal budesonide and oral terfenadine in perennial allergic rhinitis: effect on the symptom score and nasal secretion eosinophils. Journal of Asthma Allergy and Clinical Immunology. 21:216-22.

Javed S, Michael AK (2003) Decongestants. Medscape. https://emedicine.medscape.com/article/134825-medication#5

Druce, H.M. and Kaliner, M.A. (1988). Allergic rhinitis. Journal of the American Medical Association. 259:260-3

Lieberman, P. (1988). Rhinitis: allergic and nonallergic. Hospital Practice. 23:117-45. Owen, J.A., Punt, J. (2013). Overview of the immune system. In: Freeman WH (ed) Kuby Immunology, 7th edn. Macmillan, New York. Busse, W.W. and Lemanske, K.F. (2001). Asthma. New England Journal of Medicine. 344: 350-362

0: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3434398/ 100%

Pawankar,

R., Mori, S., Ozu, C., Kimura, S. (2011). Overview on the pathomechanisms of allergic rhinitis. Asia Pacific Allergy. 1: 157-167

Aaronson, D.W. (1998). Side effects of rhinitis medications. Journal of Allergy and Clinical Immunology. 101: 379-382. White, M.V. (1990). Role of histamine in allergic diseases.

0: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 41%

Journal of Allergy and Clinical Immunology. 86: 599-605 Sin, B. and Togias, A. (2011) Pathophysiology of allergic and non allergic rhinitis. Proceedings of the American Thoracic Society. 8: 106-114.

Bousquet, J., Vignola, A.M., Campbell, A.M., Michel, F.B. (1996). Pathophysiology of allergic rhinitis. International Archives of Allergy and Immunology. 110: 207-218. Rachelefsky, G.S. (1998). Pharmacologic management of allergic rhinitis. Journal of Allergy and Clinical Immunology. 101:367-369. Mygind, N., Dahl, R., Bisgaard, H. (1999). Leukotrienes, leukotriene receptor antagonists and rhinitis. Allergy. 55: 421-424.

Islam, F., Gopalan, V., Lam, A.K. (2017). RETREG1 (FAM134B): A new player in human diseases: 15 years after the discovery in cancer. Journal of Cellular Physiology. 233: 4479-89.

0: http://recipp.ipp.pt/bitstream/10400.22/7861/1/DM_H%C3%A9lioGuedes_2015.pdf 100%

Spector, S. (1999). Ideal pharmacotherapy for allergic rhinitis. Journal of Allergy and Clinical Immunology. 103: 386-7.

Braido, F., Lagasio, C., Piroddi, I.M.G., Baiardini, I., Canonica, G.W. (2008). New treatment options in allergic rhinitis: patient considerations and the role of ciclesonide. Therapeutics and Clinical Risk Management. 4: 353-61. Parnes, S.M. (2002). The role of leukotriene inhibitors in allergic rhinitis and paranasal sinusitis. Expert Opinion on Pharmacotherapy. 3: 33-8.

Chazot, P.L. (2009). Advances in histamine pharmacology reveal new drug targets. British Journal of Pharmacology. 157:1-3 Kalpaklioglu, F., Baccioglu, A. (2012). Efficacy and safety of H1-antihistamines: an update. Antiinflammatory & Anti-allergy Agents in Medicinal Chemistry. 11: 230-7. Slater, J.W., Zechnich, A.D., Haxby, D.G. (1999). Second-generation antihistamines: a comparative review. Drugs. 57: 31-47 Dempsey, O.J. (2000). Leukotriene receptor antagonists therapy. Postgraduate Medical Journal. 76: 767-773. Stella, R. and Donnell, O. (1999). Leukotriene-biosynthesis and mechanisms of action. Australian Prescriber. 22: 55-7. Matsuse, H., Kohno, S. (2014). Leukotriene receptor antagonists pranlukast and montelukast for treating asthma. Expert Opinion on Pharmacotherapy. 15: 353-63. Church, M.K. (2011). Safety and efficacy of bilastine: a new H(1)- antihistamine and utricaria. Expert Opinion on Drug Safety. 10: 779-93.

2

Hit and source - focused comparison, Side by Side:

Left side: As student entered the text in the submitted document.
Right side: As the text appears in the source.

Instances from: https://emedicine.staging.medscape.com/article/134825-overview

0: https://emedicine.staging.medscape.com/article/134825-overview 87%	0: https://emedicine.staging.medscape.com/article/134825-overview 87%
workshop by World Health Organization, allergic rhinitis and its impact on asthma (	Workshop Group., World Health Organization. Allergic rhinitis and its impact on asthma.

Instances from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3434398/

4: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3434398/
100%

Pawankar,

R., Mori, S., Ozu, C., Kimura, S. (2011). Overview on the pathomechanisms of allergic rhinitis. Asia Pacific Allergy. 1: 157-167

4: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3434398/
100%

Pawankar R, Mori S, Ozu C, Kimura S. Overview on the pathomechanisms of allergic rhinitis. Asia Pacific Allergy. 2011;1 (3):157–167. [

Instances from: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf

1: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 100%

The pathophysiology, diagnosis and treatment of allergic rhinitis. Allergy Asthma

Immunology

Research. 2: 65-76.

1: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 100%

The Pathophysiology, Diagnosis and Treatment of Allergic Rhinitis. Allergy, Asthma & Immunology Research. 2(2): 65-76. [85]

2: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 100%

The pathophysiology, diagnosis and treatment of allergic rhinitis. Allergy Asthma &

Immunology

Research. 2:65-76.

2: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 100%

The Pathophysiology, Diagnosis and Treatment of Allergic Rhinitis. Allergy, Asthma & Immunology Research. 2(2): 65-76. [85]

5: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 41%

Journal of Allergy and Clinical Immunology. 86: 599-605 Sin, B. and Togias, A. (2011) Pathophysiology of allergic and non allergic

5: https://repositorio-aberto.up.pt/bitstream/10216/107621/2/216409.pdf 41%

Journal of Allergy and Clinical Immunology. 128(6): 1139-1150. [88] Greiner A and Meltzer E (2011). Overview of the Treatment

rhinitis. Proceedings of the American Thoracic Society. 8: 106-114.

of Allergic Rhinitis and Nonallergic Rhinopathy. Proceedings of the American Thoracic Society. 8(1): 121-131.

Instances from: https://biblio.ugent.be/publication/8538875

3: https://biblio.ugent.be/publication/8538875 88%

L., Bousquet,

J.

et al. (2017).

Allergic rhinitis and its impact on asthma (ARIA)

guidelines 2016 revision.

Journal of Allergy and Clinical Immunology. 140: 950-958.

3: https://biblio.ugent.be/publication/8538875 88%

L, Jean Bousquet, Ioana Agache, et al. “Allergic Rhinitis and Its Impact on Asthma (ARIA) Guidelines : 2016 Revision.” JOURNAL OF ALLERGY AND CLINICAL IMMUNOLOGY 140.4 (2017): 950–958.

Instances from: http://recipp.ipp.pt/bitstream/10400.22/7861/1/DM_H%C3%A9lioGuedes_2015.pdf

6: http://recipp.ipp.pt/bitstream/10400.22/7861/1/DM_H%C3%A9lioGuedes_2015.pdf 100%	6: http://recipp.ipp.pt/bitstream/10400.22/7861/1/DM_H%C3%A9lioGuedes_2015.pdf 100%
Spector, S. (1999). Ideal pharmacotherapy for allergic rhinitis. Journal of Allergy and Clinical Immunology. 103: 386-7.	Spector, S. (1999). Ideal pharmacotherapy for allergic rhinitis. Journal of Allergy and Clinical Immunology, 103(3,