Determination of Anti-Histaminic and Anti-Allergic Activities of Traditionally used Ayurvedic Preparation Consisting Medicinal Plants

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Dedicated

To

My Beloved

Father, mother, wife

and

daughter Ashabari

JADAVPUR UNIVERSITY

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- Participated in "National Workshop on Good Clinical Practice" (NWGCP-2024), held on 27th July, 2024, organized by ABS Clinical Solution in collaboration with Bioequivalence Study Centre, Jadavpur University, Kolkata.
- Participated in "National Seminar on Pharmacovigilance and its importance in Unani Medicine" held on 3rd February 2024 at Dr. K.P. Basu Hall, Jadavpur University, Kolkata.
- Participated in 3rd International Conference and Buyers Sellers Meet For Medicinal Plants used in Lifestyle Products on 6th December, 2023, held at Dr. Triguna Sen Auditorium, Jadavpur University, Kolkata, Theme: "Streaming of Supply Chain and Decentralized Value Addition of Medicinal Plants," organized by Jadavpur University and sponsored by National Medicinal Plants Board (NMPB), Ministry of AYUSH, Govt. of India.
- Participated in "National Workshop on GCP and Clinical Trial Conduct" held on 24th June, 2023, at Dr. K.P. Basu Memorial Hall, Jadavpur University, Kolkata.
- Participated in Workshop on "PreCon-2K23, Pre-Conference Workshop on BA/BE Studies" of the 30th Annual Conference of Indian Pharmacological Society and Eastern Zonal Colloquium, held on 3rd March, 2023, at Dr. K.P. Basu Memorial Hall, Jadavpur University, Kolkata.
- Participated in Unani Day 2023-Pre-activities conducted by Regional Research Institute of Unani Medicine (Govt. of India), Kolkata in collaboration with RCFC (NMPB, Ministry of AYUSH), Jadavpur University, Kolkata & Peripheral Pharmacovigilance Centre (Unani), Kolkata on "Seminar/Awareness Programme on Pharmacovigilance in AYUSH" held on 7th February 2023, Jadavpur University, Kolkata.
- Poster Presentation titled "Determination of antihistaminic activity of traditionally used Ayurvedic formulation" at the 9th International Congress of Society for Ethnopharmacology, at JSS Academy of Higher Education and Research, Mysuru, Karnataka, India, 22nd April–24th April, 2022.
- Poster Presentation titled "Determination of antihistaminic working principle of a polyherbal Ayurvedic Preparation: Haridra Khanda" at the 7th International Congress of Society for Ethnopharmacology, at Jamia Hamdard, New Delhi, India, 15th Feb–17th Feb, 2020.
- Participated in the National Seminar on "Clinical Research: Present Scenario in Pharmacovigilance and Clinical Trials" held in the Department of Pharmaceutical Technology, Jadavpur University, 2018.

STATEMENT OF ORIGINALITY

I, Rudranil Bhowmik, registered on 17/06/2019 do hereby declare that this thesis entitled "**Determination of Anti-Histaminic and Anti-Allergic Activities of Traditionally used Ayurvedic Preparation Consisting Medicinal Plants**." contains literature survey and original research work done by the undersigned candidate as part of Doctoral studies.

All information in this thesis have been obtained and presented in accordance with existing academic rules and ethical conduct. I declare that, as required by these rules and conduct, I have fully cited and referred all materials and results that are not original to this work.

I also declare that I have checked this thesis as per the "Policy on Anti Plagiarism, Jadavpur University, 2019", and the level of similarity as checked by iThenticate software is 9 %.

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CERTIFICATE FROM THE SUPERVISOR

This is to certify that the thesis entitled " Determination of Anti-Histaminic and Anti-Allergic Activities of Traditionally used Ayurvedic Preparation Consisting Medicinal Plants." submitted by Shri. Rudranil Bhowmik, who got his name registered on 17/06/2019, Registration No:1021913006 for the award of Ph.D (Engg./Pharmacy) degree Jadavpur University is absolutely based upon his own work under the supervision of Prof. Sanmoy Karmakar and that neither his thesis nor any part of the thesis has been submitted for any degree/diploma or any other academic award anywhere before.

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Rudranil Bhowmik

PREFACE

This research work is carried out in partial fulfillment of Doctor of Philosophy (Pharmacy). The current research work entitled **" Determination of Anti-Histaminic and Anti-Allergic Activities of Traditionally used Ayurvedic Preparation Consisting Medicinal Plants."** demonstrated its antihistaminic and anti-allergic effect.

Allergic disorders, including allergic rhinitis, asthma, atopic dermatitis, and urticaria, have become a pressing global health concern in recent years. With increasing urbanization, industrialization, and environmental pollution, the prevalence of allergic diseases has significantly risen. Conventional therapies, primarily antihistamines and corticosteroids, remain the mainstay of treatment; however, these pharmacological interventions are often associated with limitations such as sedation, tolerance, and systemic side effects. In response to these challenges, there has been a growing interest in alternative and complementary medicine, particularly Ayurveda, which offers holistic, natural, and sustainable solutions to allergy management. This thesis explores the antihistaminic and immunomodulatory potential of two classical Ayurvedic formulations, Haridra Khanda (HK) and Maha Manjisthadi Kwatham (MMK), aiming to bridge the gap between traditional medicine and modern scientific validation.

The foundation of this study lies in understanding histamine biology and its critical role in allergic responses. Histamine is a biogenic amine primarily stored in mast cells and is released upon exposure to allergens, triggering inflammatory cascades. The interaction of histamine with its receptors specially H1, leads to a variety of physiological effects, including vasodilation, increased vascular permeability, and smooth muscle contraction, all of which contribute to the symptoms of allergic diseases. While synthetic H1 receptor antagonists have been widely used for symptom relief, their drawbacks have necessitated the search for alternative therapeutic approaches. Ayurvedic formulations, rich in bioactive phytochemicals, offer a promising avenue for allergy treatment with fewer side effects and potential long-term benefits.

This study initially examined both Haridra Khanda and Maha Manjisthadi Kwatham, assessing their antihistaminic properties through in vitro and in vivo experiments, metabolite profiling, and molecular docking studies. However, as the research progressed, Haridra Khanda emerged as the more suitable formulation for further investigation, and from that point onward, the study focused on its efficacy and mechanisms of action.

The experimental section of this research involved an array of biochemical assays, animal models, and computational studies to validate the therapeutic properties of Haridra Khanda. The pharmacological investigations reveal that Haridra Khanda has significant antihistaminic properties, inhibiting histamine release and stabilizing mast cells. In cell culture studies, Haridra Khanda extracts demonstrated inhibition of mast cell degranulation, reducing histamine release in response to allergen exposure. These effects were further confirmed through ELISA, showing downregulation of pro-inflammatory cytokines.

To deepen the understanding of its molecular mechanisms, metabolite profiling of Haridra Khanda was performed using LC-qTOF-MS, identifying active phytoconstituents responsible for its antihistaminic action. Additionally, molecular docking studies demonstrated the strong binding affinity of these bioactive metabolites to histamine H1 receptors and Fcε receptors, crucial mediators in allergic inflammation.

The in vivo experiments involved murine models of allergic asthma, where Haridra Khanda was administered to assess its effects on airway inflammation and immune response. The results demonstrated that Haridra Khanda significantly reduced airway hyperresponsiveness, suppressed eosinophil infiltration in lung tissues, and decreased plasma IgE levels, a hallmark of allergic conditions. These findings suggest that Haridra Khanda not only acts as an antihistaminic agent but also plays a broader role in immune modulation and inflammation control.

The results from this research emphasize the importance of integrating Ayurvedic medicine with modern pharmacological research, providing a compelling case for the inclusion of Haridra Khanda in evidence-based allergy treatment. Unlike conventional antihistamines, which often target only H1 receptors, Haridra Khanda exhibits multi-targeted effects, influencing immune modulation, mast cell stabilization, and inflammatory pathways. This multifaceted approach offers a holistic alternative for managing chronic allergic conditions.

This research would not have been possible without the guidance and support of my mentors, colleagues, and research collaborators. I extend my deepest gratitude to my guide, institutional supiriors and funding agencies, whose encouragement and invaluable insights have shaped the direction of this study. Special thanks to my family and friends for their unwavering support throughout this academic journey.

By merging the wisdom of Ayurveda with contemporary scientific validation, this study aims to provide a sustainable, effective, and natural approach to allergy management, paving the way for a broader acceptance of herbal medicine in modern healthcare. The findings presented here hope to inspire further research and encourage the adoption of integrative medicine for treating allergic disorders, ultimately improving patient outcomes worldwide.

Kolkata, February,2025

Rudranil Bhowmik

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
AAS	Angioedema Activity Score
ADGRE2	Adhesion G-protein-coupled receptor E2
AECT	Angioedema Control Test
AE-QoL	Angioedema-Quality of Life Questionnaire
ASST	Autologous Serum Skin Test
AU	Acute Urticaria
BCR	B Cell Receptor
BTK	Bruton's Tyrosine Kinase
C5aR	Complement Component 5 Receptor
CholU-QoL	Cholinergic Urticaria-Quality of Life Questionnaire
CholU	Cholinergic Urticaria
CholUAS	Cholinergic Urticaria Activity Score
CHRM3	Cholinergic Receptor M3
CIndU	Chronic Inducible Urticaria
ColdU	Cold Urticaria
ColdUAS	Cold Urticaria Activity Score
COX1	Cyclooxygenase 1
CRP	C-Reactive Protein
CRTh2	Chemoattractant Receptor-Homologous Molecule Expressed on T Helper 2 Cell
CSU	Chronic Spontaneous Urticaria
CU	Chronic Urticaria
CU-Q2oL	Chronic Urticaria-Quality of Life Questionnaire
dsDNA	Double-Stranded Deoxyribonucleic Acid
ECP	Eosinophil Cationic Protein
EPO	Erythropoietin
ESR	Erythrocyte Sedimentation Rate
FP	Fusion Protein
HIV	Human Immunodeficiency Virus
ICAM	Intercellular Adhesion Molecule
IL-6	Interleukin-6
ILC-2	Skin-Resident Group 2 Innate Lymphoid Cells
IV	Intravenous
LPS	Lipopolysaccharides
LYN	Lck/Yes Novel Tyrosine Kinase
mAb	Monoclonal Antibody

Abbreviation	Full Form
MBP	Myelin Basic Protein
MCP3	Monocyte Chemotactic Protein-3
MRGPRX2	Mas-Related G Protein Coupled Receptor × 2
NSAID	Non-Steroidal Anti-Inflammatory Drugs
OSMRβ	Oncostatin M Receptor-β
PAF	Platelet Activating Factor
PAR1	Protease-Activated Receptor-1
PAR2	Protease-Activated Receptor-2
PAR3	Protease-Activated Receptor-3
PECAM	Platelet Endothelial Cell Adhesion Molecule
RANTES	Regulated upon Activation Normal T Cell Expressed and Secreted
SC	Subcutaneous Injection
SCF	Stem Cell Factor
Siglec 8	Sialic Acid-Binding Immunoglobulin-Like Lectin 8
SM	Small-Molecule Drug
SYK	Spleen Tyrosine Kinase
TGF	Transforming Growth Factor
TNF	Tumour Necrosis Factor
TPO	Thrombopoietin
TSLP	Thymic Stromal Lymphopoietin
UAS	Urticaria Activity Score
UCT	Urticaria Control Test
UTI	Urinary Tract Infections
VCAM	Vascular Cell Adhesion Protein
VEGF	Vascular Endothelial Growth Factor
FcRI (IgE)	High Affinity IgE Receptor
ITAM	Immunoreceptor Tyrosine-based Activation Motif
PLCγ	Phospholipase C gamma
PIP2	Phosphatidylinositol 4,5-bisphosphate
IP3	Inositol trisphosphate
DAG	Diacylglycerol
PI3K	Phosphoinositide 3-kinase
LAT	Linker for Activation of T cells
PLC	Phospholipase C
MAPK	Mitogen-Activated Protein Kinase
IKK2	IκB kinase 2
NFkappa B	Nuclear Factor kappa-light-chain-enhancer of activated B cells
LTB4	Leukotriene B4

Abbreviation	Full Form
LTD4	Leukotriene D4
PGD2	Prostaglandin D2
COX2	Cyclooxygenase 2
IL-10	Interleukin-10
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
TGF-β	Transforming Growth Factor-beta
TNF-α	Tumor Necrosis Factor-alpha
PGE2	Prostaglandin E2
LTC4	Leukotriene C4
LTE4	Leukotriene E4
cPLA2	Cytosolic Phospholipase A2
5-LO	5-Lipoxygenase
ERK	Extracellular Signal-Regulated Kinase
IL-8	Interleukin-8
IL-5	Interleukin-5
IL-3	Interleukin-3
IL-1	Interleukin-1
D3	3,5-Diiodothyronine
IL-4	Interleukin-4
NK	Natural Killer
b.w.	Body Weight
C 48/80	Compound 48/80
EB	Evans Blue
HBSS	Hank’s Balanced Salt Solution
HK	Haridra Khanda
i.v.	Intravenous
LC	Liquid Chromatography
MMK	Manjishthadi Kwatham (brihat)
MRM	Multiple Reaction Monitoring
p.o.	Per os
QTRAP	Quadrupole Ion Trap
H1R	Histamine Receptor-1
FcεR	Fc Epsilon Receptor
Cas	Chemical Abstracts Service
Et	Ethanol
MeOH	Methanol
PBS	Phosphate Buffered Saline
TPC	Total Phenolic Content

Abbreviation	Full Form
LC-qTOF-MS	Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry
DMEM	Dulbecco's Modified Eagle Medium
FBS	Fetal Bovine Serum
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide
DMSO	Dimethyl Sulfoxide
OD	Optical Density
CYP	Cytochrome P450
HLM	Human Liver Microsomes
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
MeCN	Acetonitrile
IC50	Half Maximal Inhibitory Concentration
OVA	Ovalbumin
BALF	Bronchoalveolar Lavage Fluid
ELISA	Enzyme-Linked Immunosorbent Assay
HRP	Horseradish Peroxidase
H&E	Hematoxylin and Eosin
PAS	Periodic Acid Schiff
IFN-γ	Interferon Gamma
mg	Milligram
µg	Microgram
ng	Nanogram
mmol	Millimole
nmol	Nanomole
µmol	Micromole
mN	Millinewton
g	Gram
KgF	Kilogram-force
psi	Pounds per Square Inch
°C	Degree Celsius

CHAPTER I

Introduction

Introduction

Allergic disorders are increasingly recognized as a significant health challenge, affecting millions globally. These conditions are characterized by hypersensitivity reactions triggered by allergens such as pollen, dust mites, or specific foods. The hallmark symptoms—pruritus, rhinitis, bronchoconstriction, and dermatitis are mediated by histamine and other inflammatory mediators. Antihistaminic and antiallergic therapies are central to symptom management, yet conventional treatments often pose limitations, including sedation, tolerance, and adverse systemic effects (1). This has driven interest in complementary approaches, particularly Ayurvedic formulations, which offer holistic and sustainable alternatives.

Histamine is a biogenic amine produced through the enzymatic decarboxylation of the amino acid histidine (2,3). It is primarily synthesized and stored in mast cells, which are present in nearly all tissues and organs. These cells are particularly concentrated in areas prone to injury, such as the nose, mouth, feet, internal surfaces of the body, and blood vessels. Additionally, histamine exists in non-mast cell locations, including the brain, where it acts as a neurotransmitter. Another key site of histamine storage and release is the enterochromaffin-like (ECL) cells in the stomach (4).

Mast cell activation and degranulation occur when an antigen crosslinks IgE antibodies bound to FcεRI receptors on mast cells. This process leads to the release of histamine and various inflammatory cytokines. Among these substances, histamine is the most potent vasoactive mediator and plays a crucial role in the acute phase of hypersensitivity reactions (5,6). Histamine exerts its effects by binding to specific histamine receptors on target cells, influencing processes such as gastric acid secretion, smooth muscle contraction, vasodilation, and increased vascular permeability(7).

Ayurveda, the ancient Indian system of medicine, emphasizes the management of allergic disorders by restoring balance among the body's doshas—Vata, Pitta, and Kapha. Several classical formulations, including *Haridra Khanda* and *Manjishtadi Kwath (Brihat)*, have been traditionally recommended for their antihistaminic and antiallergic properties. *Haridra Khanda*, a polyherbal formulation, is rich in curcumin from *Haridra* (*Curcuma longa*) and other botanicals that possess anti-inflammatory, immunomodulatory, and mast cell-stabilizing effects (8). Similarly, *Manjishtadi Kwath (Brihat)*, containing *Manjistha* (*Rubia cordifolia)* and other herbs, is recognized for its blood-purifying, anti-inflammatory, and detoxifying properties, which contribute to reducing allergic manifestations and chronic inflammation (9).

Recent Scientific studies are involved in validating the therapeutic potential of these formulations. *Haridra Khanda* has been reported to inhibit histamine release and reduce IgE-mediated hypersensitivity, making it effective in conditions like allergic rhinitis and urticaria (10). Likewise, *Manjishtadi Kwath (Brihat)* has shown promise in modulating immune responses and controlling inflammatory cytokines, thus alleviating symptoms of allergic dermatitis and other atopic conditions (11). The active phytochemicals in these formulations, such as curcumin and flavonoids, have demonstrated potent antioxidant, anti-inflammatory, and immunoregulatory activities, which work synergistically to counter allergic pathophysiology (12).

Despite these scientific claims, comprehensive studies on the efficacy, safety, and molecular mechanisms of Ayurvedic formulations like *Haridra Khanda* and *Manjishtadi Kwath (Brihat)* are still limited. This thesis aims to evaluate the antihistaminic and antiallergic activities of these formulations through preclinical studies. By integrating traditional Ayurvedic wisdom with modern pharmacological insights, this research seeks to develop robust, evidence-based strategies for managing allergic disorders.

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CHAPTER II

Histamine, other Mediators of Allergy and its Remedy from the Medicinal Plants

Introduction

Allergies are on the rise. Despite advances in our understanding of the mechanisms of allergic disease, the prevalence of seasonal allergic rhinitis, asthma, and atopic dermatitis has increased [1]. In this context this chapter will try to present an overview of our understanding about the mechanisms and management of allergy using natural compound.

Diverse pathways of molecular pathogenesis of Allergy:

Mast cells

Mast cells play an important role in allergic reactions. Mast cells, rich with cellular granules, originated from bone marrow progenitors and responsible for innate and adaptive immunity. These cells have tendency to accumulate at site of inflammations with atopy, malignancy etc. since their function, phenotypical expression and maturation depends on the surrounding microenvironment. Mast cells play an important role in the initiation and modulation of allergic, anaphylactic, and several inflammatory reactions. These reactions include vasodilation, increasing vascular permeability, influencing inflammatory cells proliferation, facilitating adaptive immune responses, modulating angiogenesis and fibrosis. They exhibit a diverse set of receptors, including FcRI (IgE), histamine receptors (H1,H4), FcR (IgG), stem cell factor (SCF) or KIT receptor(CD117), complement (including C5aR) and cytokines, which when activated, activate a variety of signalling pathways. Release of a broad variety of allergic mediators classified into three classes as a result of ligand receptor bound mast cell activation. Firstly, mediators remain stored in granules of mast cells and liberated upon mast cell activation, secondly, enzymes like chymase, tryptase. Third group of mediators are synthesised after activation by specific stimulus, including, leukotrienes (LTB4, LTD4), prostaglandins (PGD2), platelet activating factor and cytokines like several interleukins (IL-10, IL-8, IL-5, IL-3, IL-1), GM-CSF, VEGF, TGF-β and TNF-α. According to stimulation mast cells assess their configuration of mediator liberation, regulates allergic inflammatory amplitude and assist in immune response agreement.

FcRI (IgE)

Fc receptor plays the crucial role for initiating any IgE mediated allergic manifestation. These receptors are found on mast cells, basophils and eosinophils. Only alpha subunit present on cell surface winds with IgE, while beta, gamma subunits are responsible for intra cellular signalling.

Initially antigen bound IgE cross links with two Fc receptor on cells surface leading to phosphorylation of tyrosine residue in immuno-receptor tyrosine-based motif (ITAM) by LYN.

Subsequently activation of ITAM leads to auto phosphorylation of SYK.

SYK phosphorylates linker for activation of T cells (LAT), a membrane scaffold protein that act as a site of activation of several other signalling molecule like PLCγ. PLCγ being phosphorylated by SYK, hydrolyses PIP2 in IP3 and DAG. IP3 triggers calcium release in endoplasmic reticulum. Increased cytosolic calcium level trigger must cell and liberates several mediators of inflammation[2].

SYK also activates other inflammatory pathways which includes phosphorylation of AKT via PI3k. Activated Akt in turn leads to transcription of proinflammatory cytokines and chemokines. Another pathway involves phosphorylation via RAS and RAF. This leads to the production of arachidonic acid by phospholipase A2, subsequently COX and LOX pathway. Therefore inflammatory mediators like PGD2, PGE2, LTC4, LTD4 are produced.

Besides this there are other cytokine receptors on mast cell surface that contribute to late phase inflammatory process. For example, activation of TNF alpha receptor leads to MAPK activation followed by IKK2 and NFkappa B. Activated NFkappaB translocates to nucleus inducing transcription of cytokines and chemokines. MAPK can also activate P-38 and c-JUN by phosphorylation which also regulates transcription.

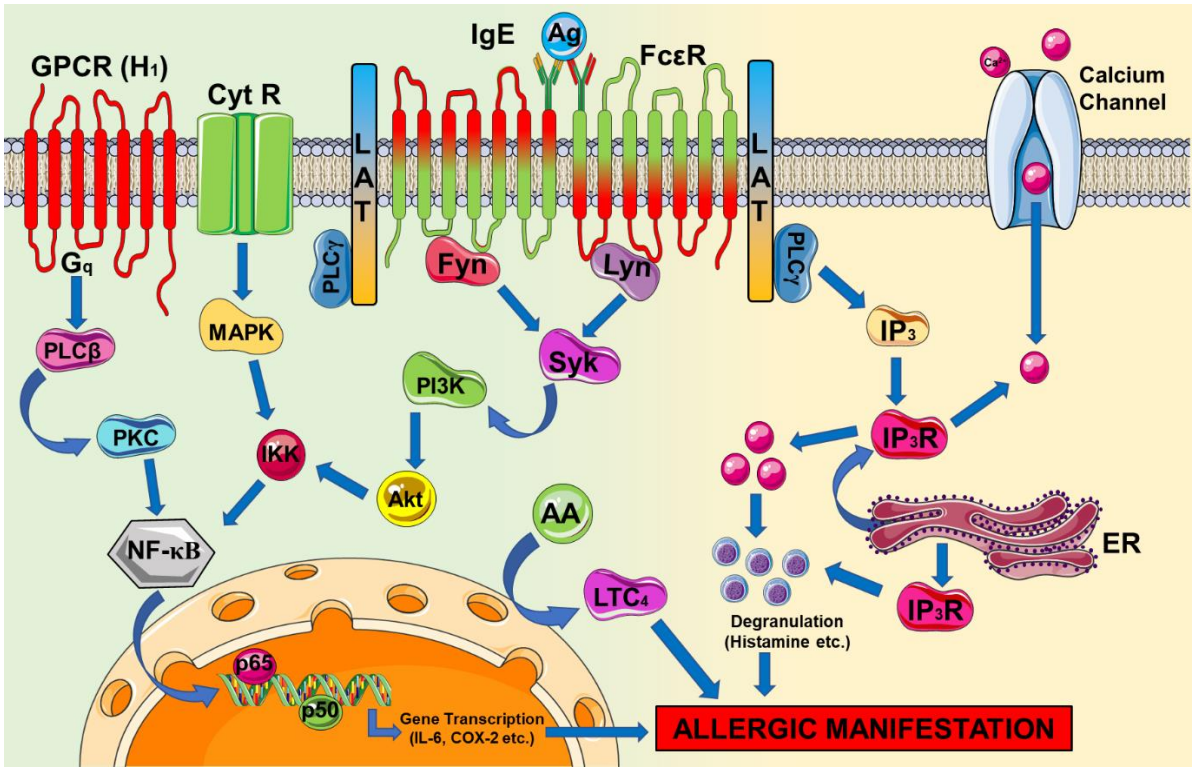

Fig. 1. A schematic overview of the molecular mechanisms involved in allergic reactions. The binding of IgE to high-affinity Fc receptors found on mast cells, basophils, and eosinophils

triggers the process of degranulation. Additionally, mast cells can be directly activated by histamine through H1 receptors, as well as by peptides, chemokines, complement-derived anaphylatoxins, FcϵRI molecules lacking bound IgE, or through the chemical crosslinking of IgE. The "late-phase response" occurs several hours later and is primarily driven by cytokines and chemokines. The release of these mediators enhances endothelial cell permeability and induces smooth muscle contraction. Key mediators involved in this response include histamine, heparin, serotonin, leukotrienes, prostaglandins, and bradykinin.

Histamine and Histamine Receptor

Histamine is a key contributor to allergic reactions and inflammatory processes. Its release triggers inflammatory responses, which were traditionally believed to be primarily mediated through the H1 receptor. Antihistamines, which act as H1 receptor antagonists, have been widely used for many years to manage allergic conditions. The importance of histamine in the pathophysiology of illnesses such as broncho-constriction and chronic pruritus, on the other hand, may have been underestimated. In this work, we examine the growing body of evidence showing histamine plays a role in inflammation and immune function modulation in these disorders. The discovery of a fourth histamine receptor (H4) and its expression on a variety of immune and inflammatory cells, in particular, has prompted a re-evaluation of histamine's actions, implying a new potential for H4 -receptor antagonists as well as a possible synergy between H1 and H4 -receptor antagonists in targeting various inflammatory conditions.

Histamine was discovered as a modulator of biological activities in the early 1900s, and medicines targeting its receptors have been used in clinical trials for over 60 years. Histamine has a wide spectrum of physiological and pathological consequences, and new roles are continually being discovered. Histamine's most well-known functions includes inflammation, gastric acid secretion, and as a neurotransmitter. Histamine is released from predefined reserves in mast cells and basophils during inflammation. Histamine causes vasodilation and an increase in vascular permeability via acting on vascular smooth muscle cells and endothelial cells. The 'triple response' in the skin is an immediate local reddening due to vasodilation, a wheal due to enhanced vascular permeability, and a flare response due to indirect vasodilation via axonal reflex activation[3]. Histamine is required for stomach acid secretion in the gastrointestinal system. Entero-chromaffin like cells in the stomach release histamine in response to gastrin and vagal stimulation. This histamine can then drive H^+, K^+ ATPases in parietal cells, resulting in H^+ release and subsequent acidification, which aids digestion processes[4]. Histamine is a neurotransmitter that has a role in sleep–wake cycles, appetite, learning, and memory in the

central nervous system. It is produced by a subpopulation of neurons in the hypothalamic tuberomammillary nucleus, and its effects are broadly distributed throughout the brain[5]. Unlike many other drug side effects, adverse effects of antihistaminic has been clinically utilised to reduce events of motion sickness and insomnia. Histamine, on the other hand, has been linked to a variety of different ailments. For example, histamine levels in bronchoalveolar lavage fluid from allergic asthma patients are higher, and this higher level is negatively related to airway function[6–10].Histamine levels have been found to be higher in the skin and plasma of atopic dermatitis [11,12] and chronic urticaria patients [13,14]. Multiple sclerosis and psoriatic skin both have high histamine levels. Rheumatoid arthritis patients' plasma and synovial fluid, as well as psoriatic arthritis patients' plasma, have higher histamine levels [15,16]. Traditional antihistamines are now considered to be less efficacious to such an extent that histamine in many occasions is no longer considered to be involved in the above mentioned pathophysiologies.

The varied biological effects of histamine are now known to be mediated by four distinct histamine receptors, including the most recently discovered histamine H4 receptor. The significance of histamine in disorders including asthma and chronic pruritus (itch) should be reconsidered, based on this developing role for the H4 receptor and new studies on the H1 receptor in animal models.

Histamine receptors are all G protein coupled receptors (GPCRs), and they all have shared patterns with other monoamine GPCRs. Despite sharing several critical residues essential in activation and histamine binding, the receptors' sequence homology is surprisingly modest, ranging from 16–35 percent at the protein level.While most histamine receptors share a high degree of homology across species, this is not the case for the H4 receptor, which has a homology of 65–72 percent, resulting in significant variances in histamine affinity [15,17,18]. However, all of these receptors communicate by interacting with and/or activating certain G proteins. Phospholipase C is activated, inositol phosphate is produced, and calcium is mobilised when H1 receptors bind to Gq proteins. Gs are activated by H2 receptors, which leads to an increase in cyclic AMP production [19]. H3 receptors stimulate mitogen-activated protein kinases (MAPKs) and ion channels by inhibiting cAMP production, increasing calcium mobilisation, and activating MAPKs [20]. The activation of the H4 receptor in primary cells appears to be primarily linked to pertussis toxin-sensitive Gi/o proteins, which signal through intracellular calcium increases. However, suppression of cAMP can also be seen in H4 receptor transfected cells [21]. MAPKs and the transcription factor AP1 have both been reported to be activated. The activation of the H4 receptor in primary cells appears to be primarily linked to

pertussis toxin-sensitive Gαi/o proteins, which signal through intracellular calcium increases. However, suppression of cAMP can also be seen in H4 receptor transfected cells[21]. MAPKs and the transcription factor AP1 have both been reported to be activated[22,23]. In transfected cells, all of the receptors have been demonstrated to have constitutive activity, raising the prospect that inverse agonists may have distinct features than neutral antagonist[19,21]. H1 receptors are found on a variety of cell types, including endothelial cells and smooth muscle cells, and play a role in vasodilation and bronchoconstriction. For many years, H1 receptor antagonists such as diphenhydramine and loratadine have been utilised to treat allergic inflammatory responses. Furthermore, H1 receptors mediate many of histamine's CNS effects, including as sleep–wake cycles, and the initial generation of H1 receptor antihistamines had sedative effects due to blood–brain barrier penetration. H1 receptor-deficient mice had lower locomotor activity, exploratory behaviour, cognitive functions, pain sensibility, and eating behaviour[24].The finding of histamine actions that were not blocked by antihistamines acting on the H1 receptor led to the theory of two histamine receptors [25,26]. The discovery of histamine functions that were not blocked by antihistamines acting on the H1 receptor led to the theory of two histamine receptors, which was supported by the development of H2 receptor-specific ligands. H2 receptors, like H1 receptors, are expressed on a variety of cell types, and one of the H2 receptor's key tasks is to mediate gastric acid release. As a result, H2 receptor antagonists such as cimetidine and ranitidine have been developed for the treatment of stomach acid problems. Histamine effects that were not stopped by H1 or H2 receptor antagonists provided evidence for the presence of a third histamine receptor (H3), but the sequence was not identified until many years later. Histamine effects that were not stopped by H1 or H2 receptor antagonists provided evidence for the presence of a third histamine receptor (H3), but the sequence was not identified until many years later [22,27]. Histamine effects that were not stopped by H1 or H2 receptor antagonists provided evidence for the presence of a third histamine receptor (H3), but the sequence was not identified until many years later. Histamine, in example, was discovered to limit its own release as well as the release of other neurotransmitters. The H3 receptor, which works as a presynaptic autoreceptor and is expressed largely in the nervous system, is the receptor that mediates this reaction. It is involved in both central and peripheral neurotransmission. Several H3 receptor ligands, such as thioperamide and clobenpropit, have been actively employed as research tools, despite the fact that they are not currently in clinical usage. Clinical trials with H3 receptor antagonists have been reported, however they all appear to be in the early stages [28]. These antagonists, on the other hand, are thought to be useful in the treatment of cognitive problems, obesity, sleeplessness, and myocardial ischemic arrhythmias [20]. H3 receptors have recently been

discovered to be involved in blood–brain barrier function and thus have a role in neuro-inflammation [29]. The H4 receptor was found using a genetic technique based on searches using the H3 receptor sequence, unlike the other histamine receptors [30,31]. Previous findings on eosinophils, however, suggested the presence of a nonH1, H2, or H3 receptor, though this was not recognised as a fourth receptor at the time [32–34]. The H4 receptor has a more selective expression pattern than the H1 and H2 receptors, being found mostly in cells of haematopoietic origin, such as dendritic cells, mast cells, eosinophils, monocytes, basophils, and T cells. The H4 receptor has low affinity for the majority of ligands that target the H1 and H2 receptors. H1 receptor antagonists such as diphenhydramine, cetirizine, desloratadine, and fexofenadine, which are routinely used to treat allergies, have showed no inhibition of the H4 receptor at concentrations up to 10 M [33,35,36]. Several H1 receptor ligands were found to exhibit affinity for the H4 receptor in one investigation; however, these findings have not been verified in other studies and should be treated with caution [37,38]. Some H3 receptor ligands and H2 receptor agonists, on the other hand, are also effective H4 receptor ligands [36]. In addition, current research has identified a small number of selective H4 receptor ligands, such as JNJ 7777120, which have proved effective in identifying aspects of the receptor's physiological roles [36]. CCL16 binding to the H4 receptor has been reported, however it has not been validated in other laboratories [39]. Although more research is needed to fully understand the function of the H4 receptor, it has been linked to mast cell, eosinophil, and dendritic cell chemotaxis, as well as T cell and dendritic cell cytokine production. Furthermore, in vivo studies have linked the receptor to inflammatory and pruritic responses [21,39,40,40,41]. These recent findings have sparked a rethinking of histamine's role in immunological and inflammatory responses, as well as the relative responsibilities of the H1 and H4 receptors in mediating histamine's function. Many cells involved in inflammatory reactions have been shown to express H1, H2, and H4 receptors. As a result, depending on the quantity of histamine receptors triggered, histamine can have variable and occasionally opposing effects on a given cell. Furthermore, receptor levels can fluctuate between species and change at different stages of cell development or pathophysiological circumstances. In vitro development of monocytes into macrophages, for example, enhances H1 receptor expression, and inflammatory stimuli can upregulate H4 receptor expression in monocytes [42,43]. Understanding receptor modulation is clearly crucial in the context of chronic human diseases.

Anaphylaxis is a severe, IgE-mediated hypersensitivity reaction triggered by the sudden release of chemical mediators from mast cells and basophils. These mediators include histamine,

serotonin, and lipid-derived compounds such as prostaglandin D2 (PGD2), leukotriene B4 (LTB4), cysteinyl leukotrienes (LTC4, LTD4, and LTE4), platelet-activating factor (PAF), and various cytokines. The binding of IgE to FcRI receptors on mast cells initiates this process, leading to the release of these mediators[44,45]. During this reaction, the Lyn kinase phosphorylates tyrosine residues within the immune receptor tyrosine-based activation motif (ITAM) of the FcRI subunits, which in turn activates the tyrosine kinase Syk. Syk is crucial for phosphorylating the linker for T cell activation (LAT), which then interacts with phospholipase C (PLC) and adaptor proteins Gads and Grb2. This interaction facilitates the production of inositol-1,4,5-triphosphate (IP3), which mobilizes intracellular calcium from endoplasmic reticulum stores. The rise in intracellular calcium levels promotes degranulation and facilitates the translocation of cytosolic phospholipase A2 (cPLA2) and 5-lipoxygenase (5-LO) to the nuclear membrane, further amplifying the inflammatory response[46]. The release of free arachidonic acid (AA) from membrane phospholipid by cPLA2 initiates the synthesis of cyclooxygenase-2 (COX-2)-dependent PGD2 and 5-LO-dependent LTC4 in mast cells [47,48]. Furthermore, COX-2 dependent PGD2 is well known to play an important role in the development of inflammation and allergic diseases such as asthma [49].LTC4 plays critical roles in inflammatory and allergic diseases, and there is growing evidence that it may also play a role in cancer and cardiovascular disease [50–52]. As a result, inhibiting eicosanoid production is an important therapeutic strategy for a variety of allergic inflammatory diseases.

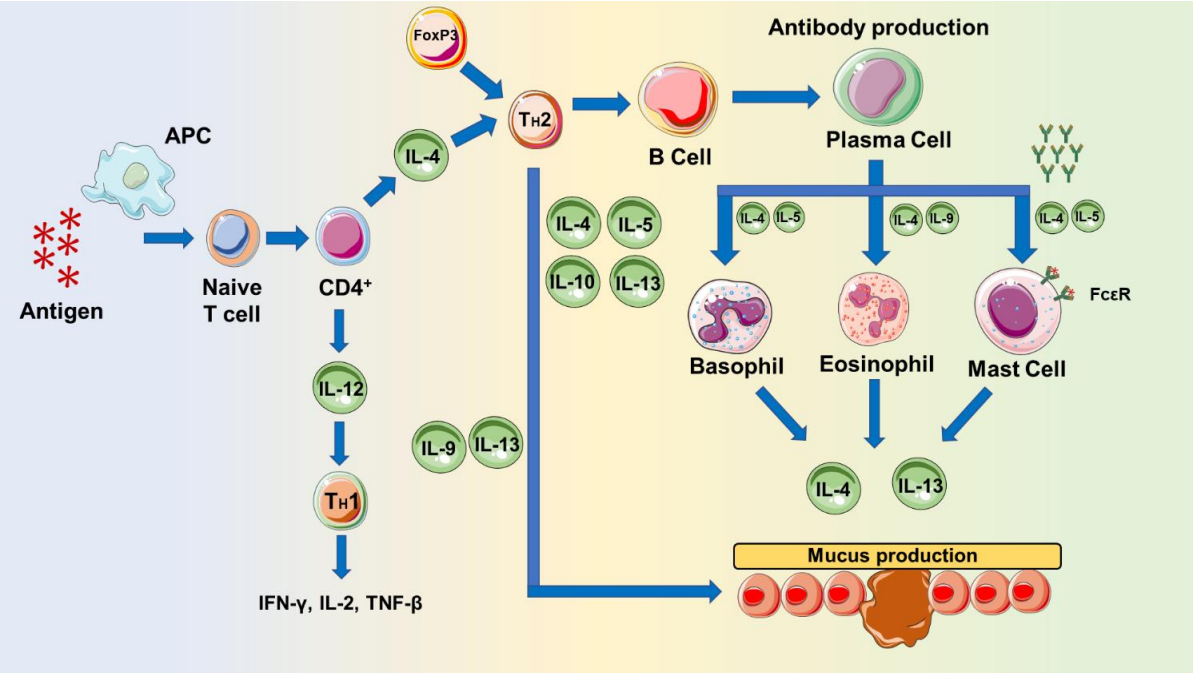

Fig. 2. Activation of immune response through antigen exposure to the dendritic cells which become antigen presenting cells(APC). APCs attracts naïve T cells and transform them to the

CD4+ cells, which eventually leads to differentiation and proliferation of B cells. Proliferated B cells produce antibody for humoral immunity**.**

SCF

Stem cell factor or SCF is a growth factor that is responsible for mast cell survival, proliferation as well as differentiation. SCF also induces as well as differentiation. SCF also induces degranulation in mast cells and its expression is significantly increased in allergic patients like asthmatics. It has been observed that over-expression of SCF in allergic diseases is associated with local increase in the number and activation of mast cells. Thus, it is considered as one of the potential therapeutic targets in type 1 hypersensitivity[53]. SCF acts through a membrane bound protein called c-kit [54] via the tyrosine kinase pathway. This leads to activation a variety of intracellular signalling proteins like PLC Gamma [55] , PI3K [56], Akt, MAP kinase, p-38, ERK etc. The MAP kinase ERK 1/2 pathway activates PI3 kinase and Akt to induce cell proliferation through augmented levels of D3, E and A2 cyclins [57]. SCF prevents apoptosis of bone marrow derived mast cells as c-kit activation leads to activation of PKB/Akt and inhibition of Bad phosphorylation, a pro-apoptotic protein[58]. SCF promotes Fc epsilon receptor dependant mast cell activation by activating PI3 kinase thus potentiating calcium release and subsequent degranulation [59]. Besides, it also leads to NF-kappaB activation and expression of cytokines and chemokines [60]. SCF contributes to haematopoietic cell chemotaxis by activating Lyn protein that interacts with Cdk-2 [61]. Hence SCF plays a critical role in the infiltration of mast cell precursors in tissue.

Ayurveda and its approach towards allergic conditions

Natural remedies with anti-allergic and antihistamine qualities are used in Ayurveda, the traditional Indian medical system, to treat allergies holistically. Haridra Khanda and Mahamanjistha Kwath are two well-known Ayurvedic preparations that are widely used because of their effectiveness in treating allergic conditions and boosting immunity. A traditional Ayurvedic remedy, Haridra Khanda is prepared with turmeric (*Curcuma longa*), sugar, ghee, and other healing herbs. The main ingredient, turmeric, is well known for its strong anti-inflammatory, antioxidant, and antihistamine qualities, which make it a great remedy for skin rashes, itching, and respiratory allergies. People with long-term illnesses like asthma, urticaria, and allergic rhinitis benefit most from Haridra Khanda. A potent blood purifier and anti-inflammatory concoction, Mahamanjistha Kwath is made from *Rubia cordifolia* (Manjistha) and other complementary herbs. Its antihistamine-like qualities support

detoxification, reduce swelling, and skin allergies. Mahamanjistha Kwath is particularly suggested for eczema, psoriasis, and acne because it balances the Pitta and Kapha doshas and supports a healthy immune system and skin. The significance of natural, holistic treatments for allergies is highlighted by these Ayurvedic remedies, which target the underlying cause of the condition rather than just its symptoms. When taken regularly as directed by an Ayurvedic practitioner, these formulations can promote long-term immune resilience and general health.

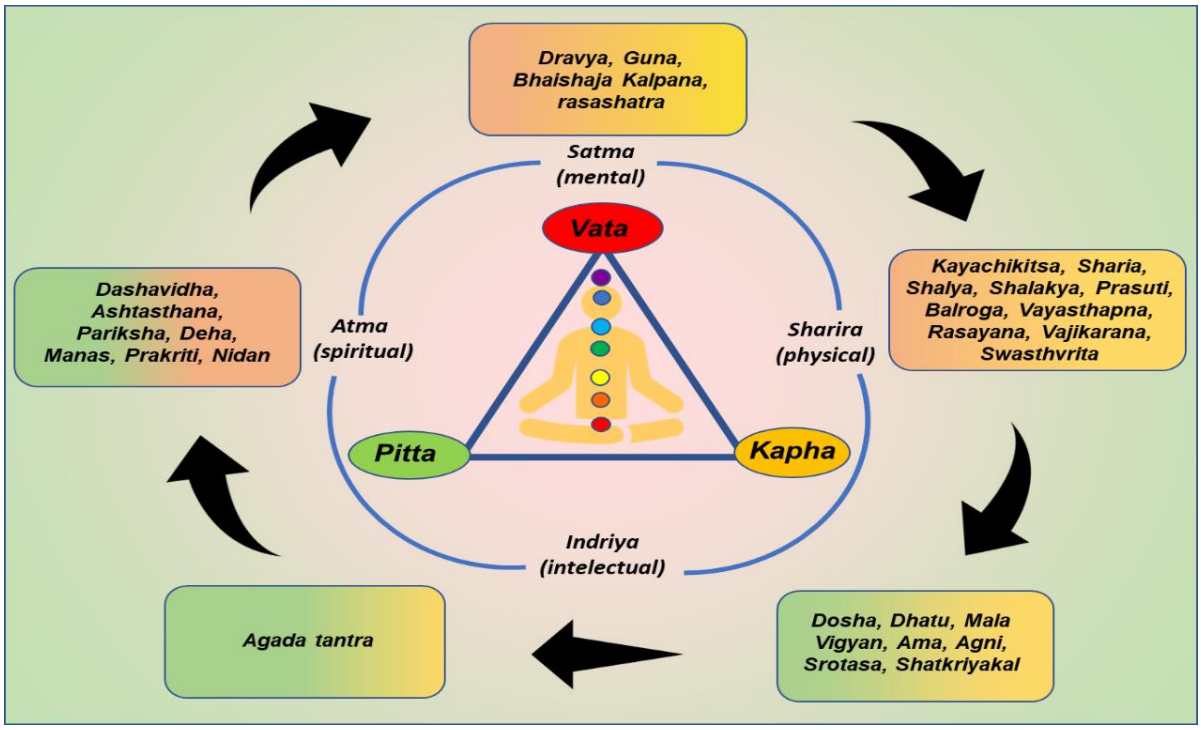

Fig. 3. A basic diagram Ayurvedic principal of Kapha, Pitta and Vata.

Table 1: Constituents (plant components) of Haridra Khanda

Plant name (Scientific name)	**Part used**	**Amount of that part used(g)**
Haridra (*Curcuma longa*)	Rhizome	384 gm
Shunti (*Zingiber officinale*)	Rhizome	48 gm
Maricha(*Piper nigrum*)	Fruit	48 gm
Pippali (*Piper longum*)	Fruit	48 gm
Twak (*Cinnamum zeylanicum*)	Stem Bark	48 gm
Ela (*Elettaria cardamomum*)	Seed	48 gm

Patra (*Cinnamomum tamala*)	Leaf	48 gm
Haritaki (*Terminalia chebula*)	Pericarp	48 gm
Vibhitaki (*Terminalia bellerica*)	Pericarp	48 gm
Amalaki (*Emblica offinalis)*	Pericarp	48 gm
Nagakeshar (*Mesua ferrea*)	Stamens	48 gm
Musta (*Cyperus rotundus*)	Root Tumber	48 gm
Lauha (incinerated iron)	-	48 gm
Havisha (Cow ghee), Ksheera (Cows milk) and Khanda (sugar candy) were used during preparation of this formulation.		
The Ayurvedic Pharmacopoeia of India, part 1, 2nd ed., Section 3:31, pg no. 48.		

Table 2: Constituents (plant components) of Manjishthadi Kwatham (Brihat)

Plant name (Scientific name)	**Part used**	**Amount of that part used(g)**
Manjistha *(Rubia cordifolia)*	Root Tuber	48gm
Kutaja *(Wrightia antidysenterica)*	Stem Bark	48gm
Amrita *(Tinospora cordifolia)*	Stem	48gm
Ghana *(Cyperus rotundus)*	Root Tuber	48gm
Vacha *(Acorus calamas)*	Root	48gm
Sunthi *(Zingiber officinale)*	Rhizome	48gm
Haridra *(Curcuma longa)*	Rhizome	48gm
Daruharidra *(Berberis aristate)*	Root	48gm
Kshudra *(Solanum anguivi)*	Root	48gm
Aristha *(Azadirachta indica)*	Stem Bark	48gm
Patola *(Trichosanthes lobata)*	Plant (Whole)	48gm

Tiktakatuka*(Neopicrorhiza scrophulariiflora)*	Root	48gm
Bharngi *(Rotheca serrata)*	Root	48gm
Vidanga (*Embelia ribes)*	Seed	48gm
Amlikam (*Tamarindus indica)*	Root	48gm
Kalinga (*Wrightia antidysenterica*)	Seed	48 gm
Murya (*Chonemorpha fragrans)*	Root	48gm
Daru (*Cedrus deodara)*	Root	48gm
Bhringa (*Eclipta prostrata)*	Plant (whole)	48gm
Magadha (*Piper longum)*	Fruit	48gm
Trayanti (*Gentiana kurroo)*	Root	48gm
Patha (*Cyclea peltate)*	Olant (whole)	48gm
Vari (*Asparagus racemosus)*	Root Tuber	48gm
Gayatri (*Acacia catechu)*	Root	48gm
Pathya (*Terminalia chebula)*	Heart Wood	48gm
Dhatri (*Phyllanthus emblica)*	Fruit Rind	48gm
Vibhitakai (*Terminalia bellirica)*	Fruit Rind	48gm
Kirataka (*Swertia chirayita)*	Fruit Rind	48gm
Mahanimba *(Melia azedarach)*	Root	48gm
Asana *(Pterocarpus marsupium)*	Stem Bark	48gm
Aragwadha *(Cassia fistula)*	Heart Wood	48gm
Syama *(Operculina turpethum)*	Stem Bark	48gm
Avalguja *(Cullen corylifolium)*	Root	48gm
Chandana *(Santalum album)*	Seed	48gm

Varanaka *(Crataeva magna)*	Heart Wood	48gm
Danti *(Balospermum mondanum)*	Root	48gm
Sakhotaka *(Streblus asper)*	Stem Bark	48gm
Vasa *(Justicia beddomei)*	Root	48gm
Parpata *(Hedyotis corymbose)*	Root	48gm
Sariba *(Hemidesmus indicus)*	Plant (whole)	48gm
Prativisha *Aconitum heterophyllum)*	Root	48gm
Ananta *(Tragia involucrate)*	Root	48gm
Vishala *(Citrullus colocynthis)*	Root	48gm
Jala *(Plectranthus vettiveroides)*	Plant (whole)	48gm
The Ayurvedic Pharmacopoeia of India, part 1, 2nd ed., Section 4:24, pg no. 59.		

Preclinical studies of the components of HK and MMK

Turmeric

Curcumin (diferuloyl methane) is the primary active compound found in the rhizome of *Curcuma longa*. It has been traditionally utilized for its therapeutic properties in managing inflammation, digestive issues, liver disorders, diabetic wounds, skin injuries, rheumatism, sinusitis, and various other health conditions. [62]. Curcumin also inhibits histamine release and the secretion of tumour necrosis factor- (TNF-) and interleukin-4 (IL-4) from mast cells activated by IgE, calcium ionophore A23187, or compound 48/80 [63–65]. In addition, phorbol ester inhibited COX-2 gene expression in human gastrointestinal epithelial cells and mouse skin. Curcumin has also been shown to inhibit IgE-induced type I hypersensitivity and ovalbumin-induced airway hyperreactivity, as well as to inhibit COX-2 gene expression in phorbol ester-treated human gastrointestinal epithelial cells [64,66,67] and mouse skin [49,68], as well as in vitro lipoxygenase and cyclooxygenase activities in mouse epidermis [69]. Curcumin's effect on IgE/Ag-induced COX-2 dependent PGD2 and 5-LO dependent LTC4 production in mast cells, as well as IgE-mediated systemic anaphylactic response, has not been well explored.

Cinnamon

Cinnamaldehyde, the principal chemical constituent of Cinnamomum plants, inhibits the expression of NF-kB through its antioxidant activity, which is the key mechanism of its anti-inflammatory effects [70]. Cinnamaldehyde also inhibits pro-inflammatory mediators associated in Alzheimer's disease, such as chemokines, interferons, interleukins, lymphokines, eicosanoids (prostaglandins and leukotrienes), and reactive oxygen species (ROS) [71,72]. Major components from cinnamon is considered as p-cymene and trans-cinnamaldehyde. These compounds reduce maturation of dendritic cells but also stimulates proliferation of anti-CD3/CD28 T cells. Cinnamaldehyde reduce sulfidoleukotriene release and CD63 expression in human basophils also reduce hyperreactivity of nasal airway in mice [73].

Mesua ferrea

M. ferrea contains xanthines like mesuaxanthone-A, mesuaxanthone-B, calophyllin-B, dehydrocycloguanandin, euxanthone, jacareubin and 6-desoxy jacareubin [74]. These components exhibited anti-inflammatory property in different animal models. In paw oedema model Mesua ferrea inhibit carrageenan induced paw oedema while given along with other herbs containing Ayurvedic formulation. While ethanol extarct alone produce anti-inflammatory activity in different type of in vitro bio assays [75]. A study suggests that M. ferrea (as an Ayurvedic formulation Bresol®) decelerates anti-inflammatory activity in airway constriction and ameliorates COPD condition [76].

Trikatu

Piper nigrum (black pepper), *Piper longum* (long pepper) and *Zingiber officinale* (ginger) are the three herbs found in Trikatu. Piperine is the main chemical as well as a biological marker in the component herbs *Piper longum* (P. longum) and *Piper nigrum* (P. nigrum), with minor amounts of other constituents. *Zingiber officinale* (Z. officinale) contains chemical constituents like Gingerols, Gingiberene, Shagols, and others. Trikatu, an herbal compound is used to treat inflammatory disorders. Trikatu suppressed the cell mediated and humoral immune response and decrease macrophage phagocytic index. Trikatu also maintains the cytokine homeostasis [77].

Piperine inhibited interleukin, prostaglandin E2, and matrix metalloproteinase at doses of 20 and 100 mg/kg/day [77]. Piperine, along with other components, has been shown to inhibit the expression of enzymes such as 5-lipoxygenase and cyclooxygenase 1 that are responsible for the biosynthesis of leukotrienes and prostaglandins, thereby preventing degenerative disorders

such as rheumatoid arthritis [78]. Piperine inhibited the expression of toll-like receptor (TLR)-2 and TLR-4, as well as the activation of the NF-B and mitogen-activated protein kinase pathways, preventing excessive secretion of TNF-, IL-1 and IL6 in mice models of *Staphylococcus aureus*-induced endometritis [79]. Piper Nigrum fruit extract improves allergic response regulation by suppressing inflammatory cell accumulation, improving nasal histopathology, and inhibiting NFkBp65 and STAT3 signalling activation as well as inflammatory related cytokines [80]. P-glycoprotein (P-gp) plays an important role in drug pharmacokinetics and pharmacodynamics. Piperine has been shown to increase the bioavailability of curcumin as a P-gp substrate by at least 2000%. Aside from these, at least 50 other P-gp substrates and inhibitors have been identified [81].

Ginger rhizomes (Zingiber officinale) have been shown to have potent antiemetic properties. Two of its active ingredients, gingerol and shogaol, have been shown in various in vivo studies to be at least partially responsible for the drug's antiemetic properties. Gingerol and shogaol were discovered to have antiemetic properties by acting on the 5-HT receptor ion-channel complex, most likely by binding to a modulatory site distinct from the serotonin binding site [82,83].

Gingerols are anti-inflammatory compounds found in ginger. These substances are thought to explain why so many people with osteoarthritis or rheumatoid arthritis experience pain relief and improved mobility when they consume ginger on a regular basis. One mechanism by which ginger may exert its beneficial effects is the inhibition of prostaglandin and leukotriene biosynthesis [84]. A study refers that essential oil of ginger inhibited chronic adjuvant arthritis in rats. Ginger and its pungent constituents inhibit both cyclooxygenase (prostaglandin synthetase) and lipoxygenase enzymes of the prostaglandin and leukotriene biosynthetic pathways, making them dual inhibitors of arachidonic acid metabolism [85]. Ginger administration at 50 mg/kg caused significant changes in serum prostaglandin-E2 (PGE2) in rats, whereas high doses of ginger were significantly effective in lowering serum PGE2. TBX2 levels were also significantly lower in rats given while ginger given orally. These findings suggest that ginger has anti-thrombotic and anti-inflammatory properties [86].

Trifala

Triphala is a polyherbal blend composed of dried fruits from three plants: *Terminalia chebula* Retz, *Terminalia bellirica* (Gaertn.) Roxb, and *Emblica officinalis* L. The fruits of these plants contain various bioactive phytochemicals, including phenolic compounds (such as gallic acid, ellagic acid, chebulinic acid, chebulagic acid, and emblicanin A and B), flavonoids (like

quercetin and kaempferol), alkaloids (such as phyllantidine and phyllantin), ascorbic acid, carbohydrates, and proteins [87].

Gallic acid and quercetin are well-recognized antioxidants, particularly in studies related to allergic rhinitis (AR). Research indicates that gallic acid can help reduce nasal inflammation by shifting the immune response toward a Th1-dominant profile in an ovalbumin-induced allergic rhinitis mouse model. This shift results in decreased levels of Th2 cytokines (IL-4, IL-5, IL-13, and IL-17) and an increase in Th1 cytokines (IFN-γ and IL-12) in nasal lavage fluid (NALF) following treatment. Additionally, studies suggest that gallic acid inhibits proinflammatory cytokines and histamine release through mechanisms involving cyclic adenosine monophosphate (cAMP), intracellular calcium regulation, NF-κB, and p38 mitogen-activated protein kinase (p38 MAPK) pathways [88,89].

Quercetin, a flavonoid aglycone, is another significant bioactive compound with anti-allergic properties. It has been shown to suppress the production of AR-related mediators at both transcriptional and translational levels in vitro and in vivo. In IL-4-induced human nasal epithelial cells, quercetin reduces nitric oxide (NO) production, inducible nitric oxide synthase (iNOS) mRNA expression, and the activation of signal transducer and activator of transcription 6 (STAT6) [90,91]. Additionally, in animal models of antigen-induced AR, quercetin inhibits the expression of pro-inflammatory cytokines and inflammatory mediators, including cyclooxygenase-2 (COX-2), vasoactive intestinal peptide (VIP), substance P, calcitonin gene-related peptide (CGRP), nerve growth factor (NGF), and the HIR gene [92].

Triphala also contains ascorbic acid (vitamin C) and kaempferol, both of which have therapeutic potential. Studies indicate that plasma levels of ascorbic acid tend to be lower in individuals with allergic conditions. Supplementing with vitamin C has been shown to alleviate respiratory and skin-related allergy symptoms. One study found that combining vitamin C with exercise reduced inflammatory cytokines in nasal secretions, decreased malondialdehyde (MDA)—a key marker of oxidative stress—and improved physiological function in rhinitis patients. Similarly, kaempferol has been shown to mitigate AR-related inflammation by modulating various cytokines and inflammatory markers in both eosinophil cell lines and mouse models of ovalbumin-induced allergic rhinitis [93-96].

Cedrus deodara

Cedrus deodara (Roxb.) Loud. (C. deodara) volatile oil inhibited compound 48/80-induced degranulation of isolated rat peritoneal mast cells significantly. C. deodara wood oil also

inhibited the enzyme lipoxygenase, which inhibited leukotriene synthesis [72,97]. Himachalol is one of the best anti-allergic constituents derived from C. deodara [98,99].

Cyclea peltata

Cyclea peltata (C. peltata) alkaloids inhibited mRNA expression of iNOS, COX-2, and TNF-a in LPS-treated RAW cells, demonstrating a significant anti-inflammatory effect. Bisbenzyl isoquinoline alkaloids, tetrandrine, fangchinoline, and coclaurine have all been found in C. peltate [100,101]. The main compound found in C. peltata is tetrandrine, which contributes phytochemical and in vitro pharmacological activity [102,103]. The unsaturated fatty acids in the C. peltata methanolic extract would aid in cell integrity, preventing the spread of venom components from the bite site [104,105].

Asparagus racemosus

The effect of saponin-rich fractions of Asparagus racemosus on LPS-treated macrophages was significant in lowering cytokine titres. The inhibition of two cytokines, IL-6 and TNF, was observed to be dose dependent [106,107].

Acacia catechu

Researchers tested the anti-inflammatory activity of the proprietary *A.catechu* along with *S.baicalensis* in animal and human immortalised cell lines and primary human cells using lipopolysaccharide as the pro-inflammatory agent. The combination all cell models, had a normalizing effect on the pro-inflammatory genes cyclooxygenase, tumour necrosis factor, IL-1, and IL-6. Nuclear factor kappa B (NF-kB), the central controlling factor for these genes, was also downregulated [108,109]. There are numerous references in Ayurveda that portray it as a valuable tree with numerous medicinal properties. It has antipyretic, anti-diarrheal, hepatoprotective, and hypoglycemic properties [110]. Many polyphenols have been isolated, including catechin, rutin, isorhamnetin, and epicatechin, which could explain its diverse medicinal value [111,112]. Catechin, derived from catechu, was found to be a specific inhibitor of histidine decarboxylase in vitro and to have low toxicity in clinical trials [113]. Catechin and its stereo-isomers have been shown to be anti-inflammatory, anti-diabetic, anti-cancer, anti-neuroprotective, bactericidal, memory enhancer, anti-arthritic, and hepatoprotective, primarily by altering the pathway by NF-KB, Nrf-2, TLR4/NF-B, COMT, and MAPKs [114].

Swertia chirayita

Swertia chirayita (S. chirayita) contains xanthones, which suppress inflammatory mediators (TNF- and IL-6) and PGE2/COX-2 in LPS-stimulated RAW 264.7 murine macrophages by inhibiting the NF-B signalling pathway. S. chirayita yields prominent xanthones such as bellidifolin and swerchirin. Bellidifolin inhibits the NF-kB intracellular signal transduction pathway by preventing IKK, Akt, and MAPK phosphorylation. According to this study, bellidifolin from S. chirayita has a high potential for development as a therapeutic drug for acute and chronic inflammatory disorders [115]. The effect of an aqueous extract of Swertia chirata stem on the balance of pro and anti-inflammatory cytokines in the primary joint synovium of arthritic mice has been studied. Oral administration of this extract resulted in dose-dependent reductions of tumour necrosis factor (TNF- α), interleukin-1 (IL-1), interferon γ(IFN-γ), and elevation of interleukin-10 (IL-10) in arthritic mouse joint homogenates. This aqueous extract contained two polar compounds, amarogentin and mangiferin, but no swerchirin, chiratol, methyl swetianin, or swertanone. Mangiferin has been shown to have potent anti-inflammatory activity, and its presence in extract is thought to be responsible for the above changes in arthritic mouse joint homogenates [116,117].

Cullen corylifolium

In India, Cullen corylifolium (L.) Medik (also known as Psoralea corylifolia) is frequently discovered growing as a herb by the roadside and in trash bins. The plant has been used for centuries as an aphrodisiac and anti-inflammatory, as well as for leukoderma, psoriasis, vitiligo, asthma, ulcers, and kidney disorders. It is said to contain terpenoids, flavonoids, alkaloids, coumarins, and essential oil. [118]. Two substances isolated from Cullen corylifolium (L.) Medik, corylifol H and epi-bavacoumestan C, have been shown to have anti-inflammatory properties and to inhibit NO production in LPS-activated RAW 264.7 macrophages in a dose-dependent manner [119,120]. The seeds of Cullen corylifolium were the source of the first bakuchiol isolation [121]. In DNA microarray testing, bakuchiol increased the synthesis of collagen types I and IV and stimulated the production of type III collagen in a model of mature fibroblasts. The effects of a bakuchiol containing skin care product were also investigated. After using the product for 12 weeks, the skin became more elastic and firm, the number of wrinkles decreased, and the facial contours improved. Additionally, photoaging and skin discoloration were reduced [122].

Balospermum montanum

β-Hexosaminidase is a marker of mast cell degranulation mediated by IgE that is associated with the symptoms of itchy eczema. Results of screening for the anti-allergic activity of the crude extract of Balospermum montanum and the isolated pure compounds against the release of β-hexosaminidase from RBL-2H3 cells [123]. Nitric oxide(NO) is a pro-inflammatory mediator that macrophages release when there is inflammation. Inflammatory responses are associated with an excessive concentration of NO produced by inducible NO synthase (iNOS) in macrophages. When it comes to inflammatory diseases like rheumatoid arthritis and osteoarthritis, NO plays a significant role alongside a number of other mediators [124,125]. The in vitro activity of NO production from the LPS-induced RAW 264.7 cell line is decreased by the crude extract of Balospermum montanum [125].

Streblus asper Lour.

In animal testing, Streblus asper demonstrated positive anti-allergic activity. S. asper was tested in mice and rats for its anti-PCA (passive cutaneous anaphylaxis) and mast cell stabilising properties. Streblus asper exhibited anti-PCA activity in mice. It displayed dose-dependent anti-PCA activity in rats. Rats with mast cell stabilising activity displayed resistance to degranulation brought on by compound 48/80. S. asper provided protection from egg albumin-induced degranulation in sensitised rats [126].

Rubia cordifolia

Inflammation is a biological defense response involving many cells and cytokines. Immune cells such as monocytes and macrophages release pro-inflammatory cytokines, enzymes, and secondary inflammatory mediators during inflammation. Furthermore, monocytes and macrophages perform critical roles in the elimination of exogenous microbial pathogens and release a variety of growth factors and inflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β.and IL-6. 6-hydroxyrubiadin, an anthraquinone derived from *Rubia cordifolia*, inhibited LPS-induced NFκB activation as well as c-Jun N-terminal kinase phosphorylation in RAW 264.7 macrophages. Furthermore, 6-hydroxyrubiadin reduced the expression of TNF-α, IL-1β, and IL-6 in PMA-primed U937 and RAW 264.7 cells. Furthermore, 6-hydroxyrubiadin therapy lowered cytokine production *in vivo* [127]. Another literature suggested that mollugin, isolated from *Rubia cordifolia*, could considerably lower weight loss and the disease activity index in the DSS-induced UC mouse model. Histological studies also revealed that mollugin therapy significantly reduced tissue damage. Mollugin

therapy at 20 and 40 mg/kg dosages significantly decreased IL-1β and TNF-α overproduction. TLR4 levels in colon tissues were also considerably lower in the mollugin-treated groups relative to the DSS group. Mollugin alleviates DSS-induced UC by reducing the generation of pro-inflammatory chemocytokines[128]. The said phytoconstituent also found to inhibit pro-inflammatory mediators iNOS and cyclooxygenase-2 [128,129]. Another phytoconstituent obtained from *R. cordifolia* is 2-carbomethoxy-2,3-epoxy-3-prenyl-1,4-naphthoquinone, a naphthoquinone (CEPN) was found to have a prominent anti-inflammatory effect. CEPN inhibited LPS-induced NO and PGE2 production by reducing iNOS and COX-2 gene expression and also lowered IL-1β, IL-6, and TNF-α production by lowering mRNA levels. TLR4-mediated Myeloid Differentiation Factor 88 (MyD88)-dependent processes were blocked by CEPN in these circumstances, including MyD88 association with IRAK1, phosphorylation of IRAK1, TAK1, and MAPKs (ERK, JNK, and p38 MAPK), and activation of NF-κB and AP-1. In addition, CEPN reduced TRIF-dependent TLR4 signaling events such as phosphorylation of IRF3 and activation of iNOS protein production [130].

Tinospora cordifolia

Tinospora cordifolia is mentioned in *Charak, Sushruta,* and *Ashtang Sangraha,* as being useful in treating leprosy, fever, asthma, anorexia, jaundice, gout, skin infections, diabetes, chronic diarrhea, and in dysentery [131]. An abundant polyphenolic compound (-)-epicatechin has been identified abundantly in Tinospora cordifolia using LC-MS [132]. In another study it was observed that (-)-epicatechin may enter cells and act on both intracellular and plasma membrane targets. (-)-Epicatechin may reduce NADPH-oxidase and consequent superoxide generation by binding directly to the enzyme, modulating calcium influx, or perhaps preventing the binding of ligands that activate NADPH-oxidase, such as TNFα, to their receptors. (-)-epicatechin may also directly scavenge free radicals and associated oxidants at larger concentrations. Reduced cell oxidants may block the redox-sensitive release of the LC8 inhibitory peptide, permitting IκB phosphorylation and degradation, as well as the related release of the active NF-κB complex. Inside the nucleus, (-)-epicatechin can attach to the DNA-binding site in NF-κB proteins, preventing NF-κB from interacting with κB sites in gene promoters and therefore reducing gene transcription [133]. Berberine, an isoquinoline alkaloid, significantly reduced arachidonic acid or lipopolysaccharide-induced TNF-α, MCP-1, IL-6, IL-8, and COX-2 biomarkers in THP-1 cells via reducing NF-κB translocation into the nucleus [134]. Guo & Sun et al. recently examined the anti-inflammatory impact of magnoflorine, aporphine alkaloid found in *T. cordifolia*, *in vivo* and *in vitro* on

lipopolysaccharide-induced acute lung damage. The results demonstrated that magnoflorine reduced the production of numerous pro-inflammatory cytokines including TNF-α, IL-1, and IL-6 in a dose-dependent manner, and the likely mechanisms are connected with the suppression of TLR4-mediated NF-κB and MAPK signaling pathways[135].

Cyperus rotundus

Terpenoids derived from rhizomes of *Cyperus rotundus* were very efficient as anti-inflammatory, antipyretic, and analgesic tonics. Generally, sesquiterpenes phytochemicals including nootkatone, α-cyperone, β-selinene, and valencene, contribute to its anti-inflammatory activity through their action on hemeoxygenase-1 pathway [136]. These sesquiterpenes, were tested for anti-inflammatory efficacy against lipopolysaccharide (LPS)-stimulated RAW 264.7 cells. Among them, nootkatone and α-cyperone, inhibited these cells strongly by decreasing the quantity of iNOS and COX-2 [137]. α-Cyperone also found to reduce PGE2 synthesis in RAW 264.7 cells. It inhibited LPS-induced inducible COX-2 production at both mRNA and protein levels. Furthermore, α-cyperone inhibited the mRNA expression and production of the inflammatory cytokine IL-6 in LPS-stimulated RAW 264.7 cells through decreasing the NF-κB pathway [138]. Isocyperol, a sesquiterpene isolated from the rhizomes of *Cyperus rotundus*, greatly reduced the LPS-induced production of nitrite oxide (NO) and PGE2. It also inhibited LPS-induced production of iNOS and COX-2 at the mRNA and protein levels in RAW 264.7 macrophages. Not only, isocyperol inhibited the production of many pro-inflammatory cytokines induced by LPS, including IL-1β, IL-6, and monocyte chemotactic protein-1 (MCP-1) , but also in macrophages, it inhibited LPS-induced nuclear translocation and transcriptional activation of NF-κB and also inhibited activated STAT3[139]. In another study, *C. rotundus* was found to suppress leukotriene synthesis and β-hexosaminidase release. It was also shown that the most active sesquiterpene, valencene prevented β-hexosaminidase degranulation in IgE-stimulated RBL-2H3 cells by blocking the initial activation process, Lyn phosphorylation, making it a potent antiallergic ageny both *in vitro and in vivo*[140].

Azadirachta

Azadirachta indica is generally known as neem and can be found in Bangladesh, India, Pakistan, and Nepal. This plant is widely employed in traditional therapeutic methods such as Ayurvedic, Unani, and Homeopathic medicinal practices [141]. Many studies have demonstrated medicinal benefits, most notably the anti-inflammatory effect of neem extracts and their metabolites. Cotton pellet-induced granuloma and carrageenan-induced paw edema

were both reduced in rats by Indian neem leaf extract and neem seed oil, respectively[142]. Nimbidin, a tetranortriterpene, is the main active ingredient in *Azadirachta indica* seed oil. Oral injection of 5-25 mg/kg nimbidin to rats for three days decreased macrophage migration to peritoneal cavities in response to inflammatory stimuli, as well as phagocytosis and phorbol-12-myristate13-acetate (PMA) driven respiratory burst in these cells. Nimbidin also decreased NO and PGE2 production in LPS-activated macrophages after *in vitro* exposure but only marginally inhibited IL-1. The mechanism of NO inhibition suggested that nimbidin improved the induction of iNOS without inhibiting its catalytic activity. Thus, nimbidin may be useful in the treatment of inflammation[143]. Epoxyazadiradione, a limonoid isolated from neem fruits, suppressed Macrophage migration inhibitory factor (MIF)-mediated pro-inflammatory activities in RAW 264.7 cells considerably. It inhibited MIF-induced macrophage chemotactic movement, NF-κB nuclear translocation, iNOS upregulation, and NO generation in RAW 264.7 cells. The anti-inflammatory action of epoxyazadiradione *in vivo* following co-administration of LPS and MIF in mice resembled the clinical state of sepsis or bacterial infection. When LPS and PyMIF were co-administered to BALB/c mice, epoxyazadiradione inhibited the release of pro-inflammatory cytokines such as IL-1, IL-1, IL-6, and TNF-α[144]. Another limonoid obtained from neem, nimbolide, was found to possess anti-inflammatory potential in both *in vitro* and *in vivo* models. In intestinal epithelial cells and macrophages, nimbolide significantly reduced the production of inflammatory cytokines viz. IL-6, IL-8, IL-12, and TNF-α. It also decreased the phosphorylation of IκBα and the DNA-binding affinity of NF-κB, making it a helpful anti-inflammatory agent for the near future [145].

Eclipta prostrata

Eclipta prostrata Roxb. also known as *Eclipta alba* (L.) Hassk. belongs to the Asteraceae family and is generally known as false daisy in English and bhringoraj or bhringraj in Bangladesh and India. It is considered to be an ethnomedicinal plant. It is recognized as bhringoraaja, bhangraa, and karissalaankanni in the three primary kinds of traditional medical systems in the Indian subcontinent, namely Ayurveda, Unani, and Siddha [146].

Using a mouse model of allergen-induced asthma, a Brazilian research team investigated the effects of different dosages of a standardized extract of *E. prostrata*. Chemical markers were present in the standardized methanol extract at amounts of 0.02 % oroboside, 1.69 % demethylwedelolactone, and 1.71 % wedelolactone. Treatment with 250 mg/kg of extract decreased respiratory resistance and elastance considerably by providing 0.745, 4.22, and 4.30 mg/kg per day of oroboside, demethylwedelolactone, and wedelolactone, respectively. These

effects were equivalent to those of dexamethasone. The methanol extract of *E. prostrata* considerably decreased the total number of inflammatory cells and eosinophils in bronchoalveolar lavage and the amounts of interleukin (IL)-4, IL-5, and IL-13 in lung homogenate [147].

The main constituent of *E. prostrata*, wedelolactone, a coumestan, is known to be an inhibitor of IKK, a master regulator of the NF-kB inflammatory pathway. Leukemogenesis is aided by ongoing inflammation and active inflammasomes. It was observed that *E. prostrata* and its active ingredient WDL had anti-leukemogenic effects on the marrow cells of experimentally leukemic mice. Interestingly, when compared to WDL alone, the plant mixture showed the most remarkable results. In the same study, IL-1β was found to be three times more abundant in the leukemic state than in the control group. Furthermore, the wedelolactone-treated groups showed remarkable improvement in the expressional level of cleaved IL-1β by more than 1.5 folds[148]. Another research work suggested that with an IC50 value of 4.6 μM, orobol showed the most potent inhibition of NO, surpassing the NF-κB inhibitor caffeic acid phenethylester (IC50 = 5.0 μM), the non-steroidal anti-inflammatory agent indomethacin (IC50 = 20.1 μM), and the NO synthase inhibitor L-nitroarginine (IC50 = 59.0 μM). However, orobol was inactive against TNF-α but reduced the formation of PGE2 (IC5049.6μM)[149].

Santalum album

East Indian sandalwood, also known as *Santalum album* L., is a slow-growing hemiparasitic tree across South Asia. This species has made significant contributions to the market for fragrances. While the essential oil emulsion or paste of sandalwood is routinely used in India as Ayurvedic medicine to treat inflammatory skin diseases, such as acne, much of the information available about the anti-inflammatory properties of sandalwood oil (SOs) is anecdotal, as a consequence of inadequate standardization and poor characterization of most preparation [150,151]. Lipopolysaccharides induced the release of 26 cytokines and chemokines, 20 significantly inhibited by concurrent exposure to ibuprofen and one of the two essential oils of sandalwood. Indication suggested that cytokines/chemokines were reduced to the same extent by purifying α-santalol and β-santalol at concentrations corresponding to the santalol contents of the oils. Additionally, lipopolysaccharide-induced synthesis of the arachidonic acid metabolites prostaglandin E2 and thromboxane B2 by skin cell co-cultures was inhibited by purified α-santalol and β-santalol. It was proposed as a potential mechanism for the observed anti-inflammatory activities of topically applied SOs. It justifies using goods

needing anti-inflammatory benefits because SOs can imitate non-steroidal anti-inflammatory medicines like ibuprofen that work by inhibiting cyclooxygenases[151].

According to a study, S. album extract can reduce the neuroinflammatory response that the TLR3 agonist polyinosnic-polycytidylic acid (PolyI:C) causes in human neuroblastoma cells. In SH-SY5Y cells, S. album extracts differently altered the TLR3-mediated immune response. Additionally, the mRNA levels of IFN-β, IFN-α, MxA, and OAS-1 were dramatically elevated, while IL-6, CXCL8, CCL2, and IP-10 were significantly lowered in cells treated with S. album extract. The expression of IFNs and inflammatory cytokines in SH-SY5Y cells have been indirectly influenced by S. album extract[152]. The histologic characterization and expression of the keratinocyte proliferation markers Ki67 and psoriasin given by Sharma et al. revealed that SO therapy of the psoriasis tissue model also reversed psoriatic pathogenesis. The phenotypic effects were associated with decreased levels of GM-CSF, IL-1β, IL-6, IL-8, MCP-1, and ENA-78. The capacity of EISO to alleviate psoriasis symptoms *in vitro* tissue models of the disease that have been well established lends credence to the idea that the clinically observed symptom relief is caused by the inhibition of intrinsic tissue inflammatory responses in affected lesions [153].

***Berberis aristata* DC**

Allergic conjunctivitis is a frequently encountered ocular condition in routine ophthalmic practice. In addition to phlyctenular conjunctivitis, which arises from endogenous allergies, and spring catarrh, an exogenous allergic reaction, the conjunctiva can also respond to various sensitizing agents, including external, physical, and chemical factors. However, the role of allergies in causing conjunctival congestion is often overstated.

The decoction of Daruharidra has shown effectiveness in managing both acute and chronic conjunctivitis of different origins. Its beneficial effects can be attributed to its anti-allergic, anti-inflammatory, and antibacterial properties. Additionally, it possesses netrarujahara (analgesic effects for ocular discomfort), kaphajabhisyandahara (efficacy in allergic eye conditions), and netrya (eye-nourishing) properties, making it a valuable therapeutic option for allergic conjunctivitis [154].

Carrageenan induced edema in a biphasic response. The first phase was mediated through the release of histamine, serotonin and kinins, whereas the second phase is related to the release of prostaglandins and slow reacting substances which peak at 3 hr. The alcoholic and aqueous extract of Berberis aristata showed their maximum effect in 4th hour [155].

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CHAPTER III

Immunopathogenesis of Urticaria: A Clinical Perspective on Histamine and Cytokine Involvement

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REVIEW

Immunopathogenesis of urticaria: a clinical perspective on histamine and cytokine involvement

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Introduction

Urticaria encompasses a collection of disorders characterized by a distinctive pattern of cutaneous reactions. It is estimated that approximately 20% of individuals worldwide will experience urticaria at some point during their lifespan [1, 2]. Urticaria occurs when mast cells in the skin become hyperactive and degranulate, releasing several mediators along with histamine that stimulates sensory nerves, cause vasodilation and eventually, plasma leaks out of the blood vessels and recruits more cells [3, 4]. The emergence of the classic symptoms of this disorder include itchy wheals (hives) and angioedema. Compared to its more transient counterpart, acute urticaria (AU), chronic urticaria (CU) typically persists for a duration exceeding six weeks [4]. There are two other categories of CU: inducible and spontaneous. In inducible urticaria, an external stimulus, such as cold in cold urticaria (ColdU), triggers an urticarial reaction. It is unclear what causes spontaneous urticaria; however, stress, infections, and other aggravating factors may precipitate symptoms in certain people. A single patient can simultaneously experience spontaneous and induced urticaria [4–6]. Though most cases of AU clear up after a week, a significant percentage (40%) persists for a longer duration. In many cases, CU continues for years before going into spontaneous remission. However, some patients may have a connection to diseases or certain drugs or foods. Most cases of AU arise spontaneously and without apparent reason. Chronic spontaneous urticarial (CSU), also known as chronic idiopathic urticaria, seems to have two known causes: involvement of autoantibodies; IgE (type I or autoallergy) and IgG (type IIb or autoimmunity). There is currently no very well-known explanation for what causes chronic inducible urticaria (CIndU) [7, 8]. Autoantibodies that activate mast cells, promote cell infiltration, agglomeration, and activate complementary systems that play essential roles in CSU pathogenesis [9, 10]. Patients and society at large bear a disproportionate share of the cost of CU. There is substantial evidence that CU symptoms negatively impact health-associated living standards [11, 12]. These symptoms include trouble sleeping, decreased physical and mental well-being, and subpar academic and occupational performance. About a third of those with CU are not helped by the current therapeutic choices. Several potential medications are already in the developmental stage, but new targeted treatments are urgently needed. This study aims to examine the present state of knowledge on the immunopathogenesis of urticaria and its diagnosis and treatment. Additionally, this article also aims to serve as a comprehensive resource for clinicians and researchers, particularly dermatologists, in the effective management of urticarial conditions, with the ultimate goal of enhancing the quality of life for

affected patients. Rate of occurrences In 2017, it was projected that there were 160 million new cases of urticaria worldwide, making the prevalence estimate for the year 86 million. However, the frequency of each form of urticaria varies throughout populations. CU, particularly CSU, has a higher occurrence and incidence among women aged 30 years and older, while the highest AU prevalence is observed in children under the age of 5 [6, 13–21]. Generally, patients suffering from CSU were found to be older in age than CIndU patients with an average age range of 30–70 years for the former and 20–40 years for the latter. CSU typically manifests between the ages of 30 and 50 years, while CIndU occurs in patients of 20–35 years of age. Both men and children are more likely to get cholinergic urticaria (CholU), but all other types of urticaria have a female predominance in adults [21]. While both AU and CU can affect individuals from diverse racial backgrounds, several studies [15, 19, 22, 23] have reported a higher prevalence of these conditions among patients of colour. In general, AU has a lifetime prevalence ranging from 6 to 19%, while other forms of urticaria have a prevalence ranging from 3 to 22%. The overall lifetime incidence is approximately 4.4%. Point prevalence, which typically considers prevalence after one year, is around 1.5% in the United States and Europe, and between 3 and 4% in Korea, China, and Mexico [22]. It is possible that distinctive demographic, environmental, and behavioural trends in places like Italy, Taiwan and South Korea [21, 24–27], among others, contribute to the rising CU rates. Compared to CSU [6, 28] CIndU is much rarer. Across all CU cases, the combined incidence of all CIndU subtypes was 13% [29] whereas the incidence of CSU was between 60 and 90%. From the symptomatic dermographism, it was found that CholU and ColdU are the most prevalent types of CIndU in both adults as well as children. Heat urticaria, sun urticaria, aquagenic urticaria, contact urticaria, and vibratory angioedema collectively represent only 2% to 3% of all CU cases [21, 30]. Up to 36% of CU patients also experience delayed pressure urticaria, but this condition typically occurs concurrently with CSU. The median duration of an AU episode is around one week. Progression from AU to CU varies among studies, with estimates ranging from approximately 5% to 39%. CSU has a shorter mean or median disease duration than CIndU, and the cumulative weighted average estimates remission on their own 17% after 1 year, 45% after 5 years, and 73% after 20 years [31]. Recurrence of symptoms is reported in only 3% to 31% of individuals with CSU. The average or median duration of CIndU was 2–12 years, while its three most common subtypes symptomatic dermographism lasted 2–5 years, CholU lasted 3–8 years, and ColdU lasted 2–9 years. Within 5 years, one-third of CIndU patients experience remission. The remission percentage is highest in the case of symptomatic dermographism. Both CholU and ColdU have the lowest remission percentage. Phenotypic, endotype, clinical,

laboratory aspects and therapy response in urticaria have all been linked to different causes and indicators of the disorder [31].

Factors associated with urticaria

High population density [15], an individual's or family history of allergy disease [32], or both, [33] have all been cited as possible contributors to the development of AU. Some scientific investigators have found a correlation between high income and low risk for CU, whereas others have found a correlation between high risk for AU with poverty or low socioeconomic status [34, 35]. In a twin study, genetic variables may contribute to urticaria susceptibility. Several polymorphic genes, including TNFRS11A, TBXA2R, and PLA2G4A, have been linked to susceptibility to NSAIDinduced AU and/or angioedema [36, 37]. There is evidence that genes showing polymorphism and encoding interferon-gamma, interleukin-6, interleukin-17 receptor antagonist, interleukin-10, transforming growth factor beta, tumour necrosis factor (TNF), interleukin-2, interleukin-1, HLA class I and II alleles and PTPN22 contribute susceptibility to chronic uveitis. Susceptibility to autoimmune disorders can also be attributed to specific genes like HLA alleles and PTPN22 [38]. Examples of additional autoimmune disorders are type 1 diabetes mellitus, rheumatoid arthritis and HLA- DR4 that have been linked to autoimmune CSU, as characterized by a positive release of histamine from basophils [39]. The risk of having an autoimmune disorder within 10 years of a CSU diagnosis is significantly greater, especially in women of middle age with autoimmune CSU [40]. Compared to the control group, CSU patients' odds of hypothyroidism or rheumatoid arthritis were 23 and 20 times higher, respectively. Systemic lupus erythematosus, celiac diseases, rheumatoid arthritis and type I diabetes mellitus [41] were diagnosed before CSU in 80% of patients and after CSU in 20%. Autoimmune thyroid disease patients, especially women, had a much greater risk of developing CSUs [42]. The prevalence of a family history of CSU was highest among individuals with positive indicators of autoimmune urticarial [43–46], affecting up to 25% of patients with CSU. Additionally, women who suffer from peptic ulcer disease or irregular uterine bleeding are more likely to develop CU [47, 48]. According to reports, CholU and ColdU occur at different rates in different parts of the world, indicating that environmental factors like altitude and temperature may increase susceptibility to CIndU [49].

Treatment and annual healthcare

expenditures

Significant healthcare consumption and economic hardship are linked with urticaria, especially CU. This includes both planned and unplanned doctor's visits, laboratory spending fees, and lost productivity and income as a result of missed work [11, 50]. Compared to the general population, those with CU and related conditions such as angioedema were more likely to seek medical attention, including more visits to primary care physicians, specialists in allergy and dermatology, and hospitals [12, 49, 51]. In children (15.5% vs. 9.9%) and adults (7.8% vs. 4.6%), individuals with CIndU were more likely to require hospitalization than those with CSU.

Immunopathogenesis of urticaria

The skin's numerous mast cells are critical in urticaria's aetiology. Besides the superficial and deep dermis, the subcutis also contains these cell types, and are concentrated around the sensory nerves and cutaneous blood vessels. Itchy wheals and/or angioedema result from their activation and subsequent degranulation [3], which causes acute urticaria. ASU, wheals, and/or angioedema have been associated with type I hypersensitivity reactions produced in response to food, medications, and other types of allergens in patients with anaphylaxis [52]. By attaching to a preformed complex of an IgE antibody attached to its high-affinity receptor, FcεRI, exoallergen activates and degranulates mast cells and basophils [53]; this is known as type I hypersensitivity or an instantaneous IgE-mediated reaction. Pharmacological inhibition of cyclooxygenase 1 (COX1) and elevated levels of cysteinyl leukotrienes are common causes of NSAID-induced urticaria and/or angioedema [54]. Direct contact with allergens after sensitization [55] or urticariogenic compounds such as stinging nettles [4] can cause acute contact urticaria in unsensitized individuals. Current and future therapies for CSU aim to specifically target the activation of mast cells, including their signals, receptors, signalling pathways, inhibitory receptors, and mediators. These components are crucial in developing wheals and angioedema associated with CSU [3, 10, 13]. Different signals activate the many activation receptors found in mast cells [13]. Examples include MRGPRX2, FcεRI, PAR1, C5aR, PAR2, PAR3, cytokine receptors and chemoattractant receptor-homologous molecules produced on helper T cells (CRTh2). The interaction between stem cell factor (SCF) and its receptor KIT (CD117) on mast cells significantly impacts various aspects of mast cell biology, including differentiation, survival, migration, proliferation, and apoptosis. SCF is produced by

multiple cell types, including fibroblasts, endothelial cells, and mast cells themselves [56]. Several cytoplasmic signalling proteins, for instance, spleen tyrosine kinase (SYK), bruton's tyrosine kinase (BTK), and LYN phosphorylate downstream signalling targets, causing mast cells to activate and degranulate must work together to activate FcεRI. [57]. Signaling through the FcεRI begins with LYN phosphorylating the β and γ chains of the receptor, which in turn activates SYK and BTK (Fig. 1). FcεRI-mediated mast cell activation and production of cytokines are positively regulated by the cytosolic tyrosine kinase BTK. It has been demonstrated the critical role of Bruton's tyrosine kinase (BTK) in the activation of the B cell receptor (BCR), which is also involved in the activation of mast cells [57, 58]. Mast cells not only produce a small number of inhibitory receptors that, once bound to their ligands, can shut down mast cells and stop them from becoming active e.g. CD200R, siglec 8, FcγRIIb and CD300a [59]. Histamine is the primary player in the onset of CSU symptoms; however, other modulators such as tryptase, prostaglandin D2 (PGD2), tumour necrosis factor (TNF), IL-5, IL-13, IL-4, IL-17, and IL-31 are also involved. These mediators influence both the skin cells that are already there and the Basophils, T cells and eosinophils which get triggered during immune reaction [3, 13] (Fig. 1).

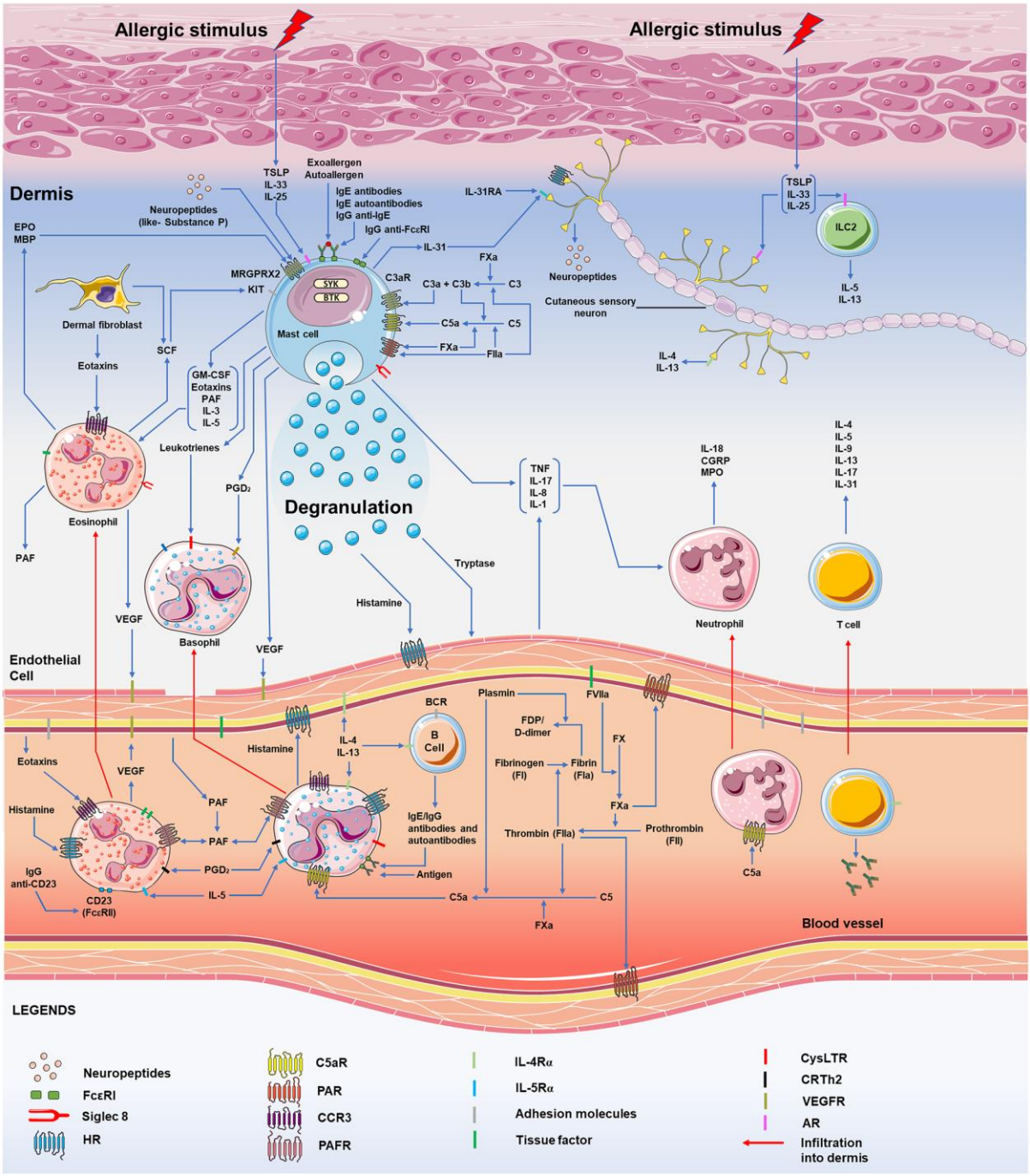

Fig. 1 The immuno-pathogenesis of urticaria and its development is based on the sequential activation and degranulation of histamine and other involved mediators, leading to various activities such as dilation of dermal blood vessels, sensory nerve activation (itch) and induction of plasma extravasation (oedema and cellular infiltration). In aller- gic urticaria, liberation of alarmins which includes IL-25, IL-33, and TSLP, lead to the activation of epithelial cytokines and result in the activation of ILC-2 and the differentiation of T cells into T helper 2 (TH2) cells that subsequently secrete TH2 cytokines. Activated aller- gen specific TH2 cells are primarily responsible for producing IL-13, IL-4 and IL-5. After that, B cells cause allergens to form a cross-link- ing with IgE–FcεRI complexes on the mast cells' surface, resulting in its

activation. Complex interlinked multistep molecular events char- acterize CSU. These events encompass cellular infiltration (eosino- phils, basophils and T cells are primarily involved), IgE/IgG mediated release of histamine, Itch signalling molecular mechanisms regulated by cutaneous pruriceptive sensory nerves, which include both hista- mine-dependent and independent pathways collectively contributing to inflammation of sensory neurons, complementary cascade activa- tion such as through anaphylatoxin C5a production and tissue factor- initiated extrinsic pathway belonging to the coagulation cascade. It is pertinent to mention the different receptors such as FcεRI, signalling pathways (such as SYK and BTK) and mediators (such as histamine) responsible for the activation of the mast cells, eosinophils, immune cells, and /or different immune cells that are involved in the patho- genesis of urticarial. These are potential therapeutic targets presently being targeted for currently available therapies or developing a novel therapeutic agent. Similarly, autoallergy and/or autoimmunity have been implicated in the development of CIndU

Cellular infiltration

CSU exhibits perivascular and interstitial inflammatory cellular infiltrates comprising eosinophils, neutrophils, lymphocytes, and basophils, resembling an allergic late-phase reaction [9, 60–62]. MCP3, Eotaxins, IL-5, RANTES, IL-17, C5a, C3a, platelet-activating factor (PAF), activated endothelial cells, and TNF from mast cells, TH2 cells, dermal fibroblasts, and other sources potentially facilitate the migration of infiltrating cells from blood to skin. Histamine, TNF, thrombin, and other factors increase cell adhesion molecule expressions (E-selectin, P-selectin, VCAM, PECAM, ICAM) on endothelial cell surfaces and CSU-affected skin areas, contributing to this phenomenon (Fig. 1) [9]. Eosinopenia and basopenia in blood, found in around 10–15% of CSU patients, may indicate cellular migration into the skin. These symptoms are associated with autoimmunity, CSU activity, and suboptimal response to H1 antihistamines (H1-AH) and omalizumab [63, 64]. Within 30 min of injecting autologous serum intradermally, wheals exhibited perivascular neutrophils and eosinophils, succeeded by a rise in T lymphocytes [65]. Activated basophils that have migrated to blood and skin can release leukotrienes, histamine, and cytokines via FcεRI and C5aR activation, contributing to CSU's pathophysiology [66, 67]. CSU patients show increased eosinophil granules in wheals [68, 69] particularly involving MBP, which activates and degranulates mast cells. This suggests a potential interaction mechanism between eosinophils and mast cells [68]. Eosinophil activation can result from IgG autoantibodies targeting the low-affinity IgE receptor or mast cell mediators like TNF, IL-5, eotaxin, and PAF (Fig. 1) [70, 71]. Activated eosinophils can also release SCF, a mast cell growth factor. In summary, eosinophils play a vital role in

activating the coagulation cascade, mast cells, and Mas-related G-protein-coupled receptor X2 (MRGPRX2) through tissue factor production and MRGPRX2 agonist release [68, 71]. CSU skin biopsy samples reveal not only TH2 cells but also TH1 and TH17 cells [9]. TH2 cells are common in allergic conditions due to their cytokine production, which stimulates IgE synthesis and activates basophils, mast cells, and eosinophils. CSU patients' blood and lesional skin have elevated cytokine levels and expressions. These include IFN, TNF, TGF, IL-1, IL-3, IL-4, IL-5, IL-6, IL-13, IL-17, IL-23, IL-24, IL-31, and IL-33.

Coagulation cascade and complement system

Inducers like histamine, LPS, TNF, VEGF, IL-6, IL-1, and IL-33 can exhibit substantial tissue factor expression in eosinophils and cutaneous microvascular endothelial cells [72]. Activation of coagulation factors like factor Xa (FXa) and FIIa occurs through the extrinsic coagulation pathway, triggered by tissue factor inducers [73, 74]. Tissue factor inducers and mediators activate mast cells, causing wheal and flare through vascular plasma leakage. Extravascular plasma holds autoantibodies to FcεRI and IgE-bound mast cells under the skin [9, 75]. Mast cell degranulation can result from thrombin and FXa action, impacting pseudoautosomal regions [10, 76]. Extrinsic coagulation, fibrinolysis, or IgG anti-FcεRI binding to basophils and mast cells are necessary for generating complement component C5a [10]. Active coagulation and plasmin factors create C5 derivatives (C5a & C5b) and C3 derivative (C3b). Leached plasma holds C5a and C3a, which activate basophils and mast cells through C5aR and C3aR, respectively [10, 77]. In summary, tissue factor-stimulated peripheral basophils release leukotriene C4 in the presence of functional-specific IgE antibodies to tissue factor [78]. Coagulation and fibrinolysis activation is believed to stem from CSU. Blood biomarkers like D-dimer, indicative of thrombin production and fibrinolysis, rise in severe CSU cases and decrease during remission [79]. Furthermore, a study revealed that D-dimer could be a possible marker in a subset of patients suffering from CSU [80]. Elevated D-dimer levels in severe autoimmune urticaria may not always indicate the need for anticoagulant medication. Reducing D-dimer levels can lower the proinflammatory state by successfully controlling the symptoms of the patients. Researching the substantial association between elevated D-dimer levels and type IIb autoimmune CSU, together with resistance to omalizumab, might be promising for early diagnosis. It is been further noted that in individuals suffering from severe autoimmune CSU, cyclosporine medication reduces D-dimer levels more than anticoagulant therapy does during immunosuppressive therapy [81]. In CSU patients, elevated CRP levels were positively correlated with increased C3, C4, IL-6, and D-dimer levels, Autologous Serum Skin Test (ASST) positivity, and CSU activity [79, 82]. This underscores the close connection between

autoimmune, inflammation, complement, and coagulation pathways in chronic urticarial syndrome's pathogenesis, potentially contributing to the perpetuation and exacerbation of urticarial inflammation (Fig. 1).

Interplay of autoantibodies in CSU

IgE, IgA, IgM, and IgG antibodies are pivotal in CSU's pathophysiology [83–85]. Adequate IgE concentrations bind to FcεRI's α subunit, activating basophils and mast cells, regardless of the antigen [86]. In allergic, autoallergic, and autoimmune urticaria, IgE cross-linking by allergens, autoallergens, and IgG anti-IgE antibodies can trigger mast cell and basophil activation, resulting in mediator release [8, 85, 87, 88]. Many CSU patients exhibit IgE antibodies targeting autoantigens like TPO, EPO, tissue factor, dsDNA, ECP, FcεRI, IL-24, and thyroglobulin. In vitro research suggests that specific antibodies, such as anti-IL-24, IgE, and IgE anti-TPO, can activate mast cells and/or basophils [8, 89, 90]. Effective passive transmission of IgE anti-thyroid peroxidase (TPO) has been observed. CSU patients with elevated IgE anti-TPO levels show high positive TPO skin prick test rates, providing further evidence of IgE antibodies' role in CSU's origin [91]. Autoallergens like IL-24 are produced in the skin. Cross-reactivity between proteins such as TPO (absent in the skin) and EPO (present in the skin) could clarify why IgE and autoallergens activate mast cells only in the skin and not other organs [92, 93]. Recent studies indicate that around 20% to 50% of CSU patients possess these autoantibodies [94]. Diagnosing autoimmune CSU linked with these autoantibodies necessitates ELISA-detected antibodies (IgG), confirmed skin auto-reactivity via ASST, and basophil activation testing. However, only 8% of CSU patients meet autoimmune CSU criteria [7, 95]. The clear distinction and separation of autoIgE and IgG endotypes remain debatable. It's increasingly evident that a single patient might exhibit IgG autoantibodies alongside other types like IgA, IgE, and IgM. Yet, actual overlap rates remain uncertain [84, 85]. Blood autoantibodies, especially IgE autoantibodies, may arise initially, while over time, different classes of autoantibodies can develop during the disease progression.

Inflammatory nerves in CSU

Histamine, interleukin-31 (IL-31), neuropeptides, and mediators like that have been postulated to mediate the non-monodirectional interaction between immune cells, sensory nerves and mast cells in CSU [68, 96, 97]. Mast cell activation through MRGPRX2 could perpetuate the symptom cascade in urticaria, including pruritus, vasodilation, plasma leakage, and neurogenic inflammation [98]. MRGPRX2, reacting to various chemicals, triggers mast cell activation without IgE [99]. Application of MRGPRX2 agonists worsens skin reactivity in CSU-

diagnosed individuals. CSU patients have an excess of mast cells expressing the MRGPRX2 gene. Elevated levels of neuropeptide-like substance P and MRGPRX2 agonist are observed in CSU patients [100, 101].

Pathogenesis of chronic induced urticaria

Passive transfer and/or omalizumab effectiveness show that autoallergic IgE-mediated mast cell activation is evident in conditions like ColdU, symptomatic dermographism, CholU, and solar urticarial [102]. Autoantibodies of IgE type might form in reaction to skin-secreted proteins, as seen with cold-induced protein secretion [103]. Mast cell activation in solar urticaria is linked to chromophore attachment, molecularly altered by sunlight, to IgE on mast cells [104]. CholU symptoms may arise from sweat antigen generation due to blocked sweat gland ducts with serum antibodies against MGL 1304 sweat antigen detected [105]. CholU might induce acetylcholine escape, degranulating mast cells via reduced M3 receptor expression in the epithelial cells of the sudoriferous gland [105]. ColdU shows IgM and/or IgG antibodies against IgE. Heat urticaria individuals rarely react positively to intradermal testing using hot autologous serum, likely due to inactivated complement and denatured IgE in the serum [103, 106]. Gain-of-function mutations in the G-protein-coupled receptor E2 on mast cells can lead to autosomal dominant hereditary vibratory angioedema. This mutation might weaken the connection between the receptor's α and β subunits, increasing mast cells' susceptibility to vibration-triggered degranulation [107]. Histamine is one of multiple pro-inflammatory mediators in delayed pressure urticaria, along with TNF and interleukin family members. Delayed pressure urticaria, distinct from other CIndUs, shows significant dermal leukocyte infiltration due to a delayed onset stimulus [108]. Contact urticaria can result from either a non-immune reaction or an immunological response involving IgE or T cells [109]. Multiple theories have emerged to explain aquagenic urticaria's origin, including factors beyond a histamine-dependent mechanism. Enhanced passive diffusion of water and water-soluble antigens (epidermis) are also suggested contributors [110].

Prevention, screening, and diagnosis

Diagnosis

Urticaria presents consistently across age, race, and gender. Angioedema and wheals share the same distribution regardless of skin colour, although erythema from wheals can be harder to detect on darker skin [111]. Diagnosis involves personal history and physical examination, considering patient photos or symptom documentation. Despite subtypes, urticaria is typically diagnosed accurately and swiftly. Acute urticaria Acute urticaria (AU) is self-limiting and often

doesn't require extensive diagnostics. Immediate symptom onset after allergen contact might warrant allergy testing to prevent re-exposure in patients with hypersensitivity or food allergies caused by drugs. Chronic spontaneous urticaria Wheals are the predominant symptom in CSU patients (57%), followed by angioedema and wheals (37%), and simple angioedema. Circadian fluctuations in mast cell activation and underlying pathophysiology may explain the spontaneous occurrence of CSU symptoms, with a higher likelihood during evening and nighttime [112, 113]. Night-time symptoms, for instance, have been linked to the autoimmune endotype CSU [114]. Wheals are most frequently observed on the arms and legs but can appear anywhere. Angioedema commonly occurs on the lips and eyelids, though it can manifest on other body parts like the feet and hands [1]. Moderate to severe CSU typically manifests as daily, near-daily, or intermittent-recurrent wheals and/or angioedema [4]. CSU exacerbations are associated with triggers such as stress, specific foods, medications (especially NSAIDs), and infections. Over 30% of CSU patients experience NSAID hypersensitivity, verified by oral drug provocation testing that induces CSU exacerbation following COX1 inhibitor intake. Selective COX2 medications are usually better tolerated [54, 115]. Specialised treatment includes assessing total serum IgE and IgG anti-TPO levels in all CSU patients. Elevated IgG anti-TPO levels could indicate autoimmune CSU [7, 40, 116]. Recent studies suggested that thyroid autoimmunity is not useful for classifying CSU patients. Baseline total IgE levels have emerged as the most reliable prognostic indicator for predicting omalizumab response in individuals with autoimmune CSU [117, 118]. Chronic inducible urticaria CIndU patients usually have shorter-lasting wheals (1 h) than CSU patients (up to 24 h). Frequent exposure to triggers and low sensitivity contribute to significant pathological activity [4, 109]. Systemic reactions like anaphylaxis can occur; high-risk patients should have adrenaline autoinjectors, even if lesions are mainly confined to triggerexposed skin areas [119]. Approximately half of individuals experience improvement in their CU during pregnancy, while about a third experience worsening [120]. Pregnancy can worsen the severity of CU, particularly in cases of combined CIndU and CSU. Diagnosis of CIndU involves a thorough history and provocation testing to identify triggers and assess disease activity. Validated provocation testing tools (Fig. 2) are available for most CIndU subtypes, aiding in quantifying disease activity and tracking treatment effectiveness.

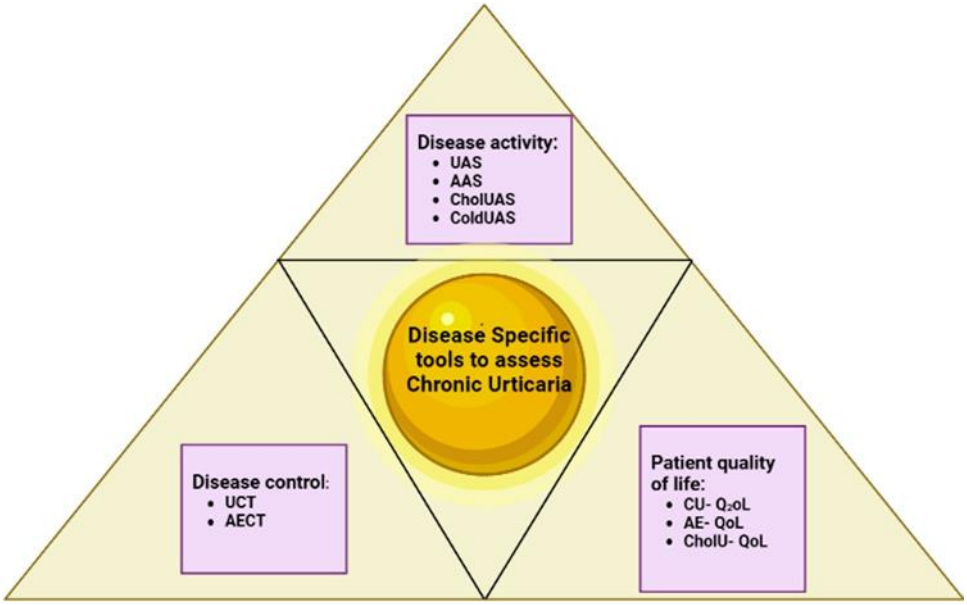

Fig. 2 Depicts tools to assess the control, activity and patient's quality of life in case of chronic urticarial.

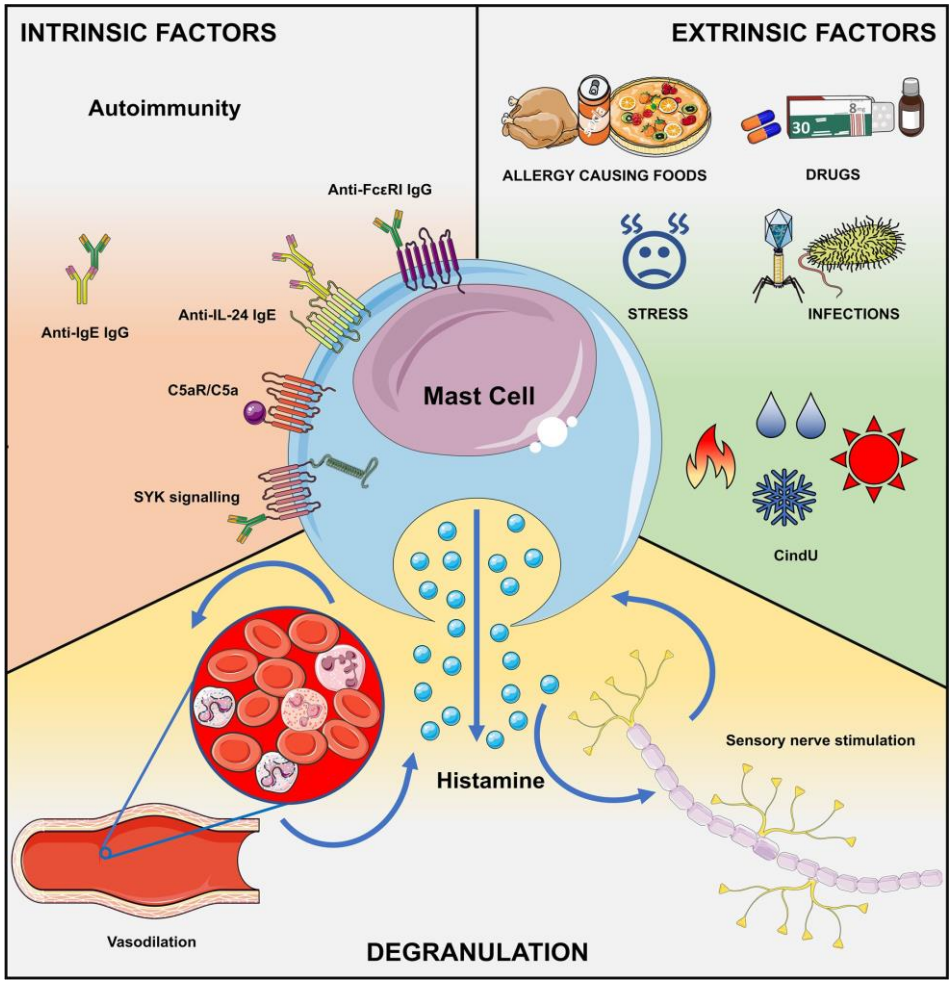

Fig. 3. Factors affecting the degranulation process leading to the release of histamine eventually causing sensory nerve stimulation and peripheral vasodilation.

Comorbidities

Acute urticaria

Reactions caused by foods, stress, Hymenoptera stings, and physical factors are less frequent compared to triggers like NSAIDs and antibiotics. Infections can complicate diagnosis, as anti-inflammatory drugs used for infections can obscure the cause of urticaria. While inducible factors play a minor role in childhood AU, they contribute significantly to around 15% of adult AU cases [121]. Patients aged 0–6 years were more likely to have their AU's cause identified than those aged 7–18 years [52]. In children younger than 13, AU was primarily triggered by food and infection, whereas in adolescents aged 13–18, these factors were less commonly associated [52]. Chronic spontaneous urticaria (AU) often disappears after treating the underlying infection or stopping the triggering medication (Fig. 3).

Chronic spontaneous urticaria

Most studies reveal rates of CIndU that are more than 10%; this indicates that it is a common comorbidity of CSU. Patients diagnosed with CU showed a prevalence of CSU ranging from 29–93%, CIndU ranging from 6–35%, and 1–43% having both CSU and CIndU. 36% of the 245 individuals diagnosed with CSU had CIndU, which was determined by a positive challenge test. Most patients exhibited symptomatic dermographism (25%), followed by ColdU (13%) [48]. A patient can have more than one form of CIndU besides CSU [122].Patients suffering from CSU, likely to have an autoimmune disease than the general population (28% vs. 1–2%, respectively). Rheumatoid arthritis, autoimmune gastritis, vitiligo and diabetes mellitus are the most common autoimmune diseases seen in patients at CSU. Autoimmune thyroid diseases account for 25% of all cases, with the most common form being Hashimoto's thyroiditis, which can occur with or without hypothyroidism [40, 123]. While up to 77% of individuals with CSU5 are reported to have Helicobacter pylori and dental infections, the connection between bacterial infections and CSUs remains limited and contradictory [5]. Viral infections (hepatitis, HIV) and fungal infections are unlikely contributors to CSU development, with unclear clinical significance [5]. CU didn’t affect the progression of COVID-19, but in a trial, about one-third of patients, particularly those with severe cases, experienced worsened CU symptoms during COVID-19 infection [124]. The link between CSU and parasitic infections remains unclear, but treating proven parasitic infections with antiparasitic medications improved CSU symptoms in about one-third of cases [5]. Increased rates of allergic conditions haven't consistently been shown in CSU patients [5]. Three extensive studies have indicated that

patients with CSU are more likely to have allergic diseases than the general population or healthy groups without urticaria [34, 125, 126]. CSU patients have a history of cancer, mainly non-hematologic types, and sometimes CSU resolves after the patient enters remission [5, 26, 127]. Cancer-induced immune system dysfunction can activate complement and coagulation cascades, potentially contributing to CSU development in cancer patients. The resolution of CSUs after cancer treatment might reflect the restoration of immune homeostasis [128]. Around 60% of CSU patients face mental health challenges, primarily depression and anxiety, which considerably impact their overall quality of life [5].

Chronic inducible urticaria

The Coexistence of CIndU and CSU is possible. Among individuals with symptomatic dermographism, 71% also reported CSU, while the figures were 25% for CholU and 10% for ColdU [119, 129, 130]. CholU, solar urticaria, ColdU, and symptomatic dermographism often coexist with allergic disorders, with a prevalence ranging from 26 to 48% [131–134].

Differential diagnosis

Wheals and/or angioedema can be related or prodromic signs in patients with conditions beyond urticaria [135]. Comprehensive evaluation involving history, physical examination, and tests is necessary to diagnose or rule out other potential causes of CSU. In cases with wheals but no angioedema, it's crucial to consider excluding autoinflammatory conditions like Schnitzler syndrome and cryopyrin-associated periodic syndromes [4]. Wheals in individuals with autoinflammatory syndromes often do not respond well to antihistamines, leading to a distinct condition called neutrophilic urticarial dermatosis. This condition is characterised by concentrated perivascular and interstitial infiltration of neutrophils infiltration around blood vessels, known as leukocytoclasia, without vessel wall necrosis. [136]. Urticarial vasculitis can present with prolonged wheals and angioedema lasting over 24 h. Diagnosis is confirmed through histological criteria, including fibrin deposits, leukocytoclasia, and extravasated erythrocytes, which are distinctive markers of this condition [137, 138]. Both long-lasting wheals (lasting more than 24 h) and angioedema can be caused by this condition. Histologically, CSU shows skin swelling and inflammation with eosinophils, neutrophils, lymphocytes, and nuclear debris, while vasculitis is rare in this context [61, 137].

Prevention and management of disease

Patient-reported outcome measures are essential tools for assessing CU activity and treatment effectiveness [139]. Prospective use of the Urticaria Activity Score (UAS) over seven days (UAS7) is considered the gold standard for evaluating CSU (number of wheals and itching sensations) [4, 140]. The Angioedema Activity Score (AAS) is a prospective tool resembling a diary, aiding clinicians in quantifying angioedema activity for patients with various recurrent angioedema forms [141]. Modified versions of the Urticaria Activity Score (UAS), such as the Cholinergic Urticaria Activity Score and the Cold Urticaria Activity Score, are utilised in collecting daily data for chronic inducible urticaria (CIndU). These scores assess the severity of wheals, itching, and trigger encounters within 24 h [139, 142, 143]. Retrospective tools with a 4-week recall time, such as the Urticaria Control Test (UCT) and the Angioedema Control Test (AECT), are used to evaluate the success of treatment in preventing disease flare-ups in all types of CU (Fig. 2) [144, 145]. Several therapeutics in the management of CU are listed in Table 1.

Management

Second-generation antihistamines (sgAHs) that effectively block the H1 receptor are affordable and considered the primary treatment option for urticarial [148, 149]. Although they do not exhibit antagonistic effects on histamine binding, they function as inverse agonists, so shifting the equilibrium towards an inactive state [150]. The use of first-generation H1-AH is generally discouraged due to its sedative and anticholinergic properties, and its potential for drug-drug interactions [4, 151]. Systemic glucocorticoid hormones are efficacious in treating urticaria when administered at standard doses, but this effect is not seen universally [4]. However, a study revealed that a short course of oral corticosteroids such as prednisone, for a maximum of 10 days, can be beneficial in treating AU and acute exacerbations of CSU by reducing the duration of the illness [152–154]. The safety and efficacy of off-label high-dosage sgAH therapy, administered at a dosage up to four times the approved daily dose, has been shown for the following antihistamines: cetirizine, bilastine, desloratadine, ebastine, fexofenadine, levocetirizine, and rupatadine [155–161]. In the context of urticaria treatment, it is suggested to consider high-dose sgAHs as the first strategy when the standard dosage fails to control symptoms effectively. The optimal timing for adjusting the dosage of antihistamine medicine is decided by professional discretion, which may include gradual reduction or abrupt discontinuation. Based on half-life estimates, it is suggested that two weeks is sufficient to see

changes in antihistamine levels in CU [161]. Omalizumab, the first monoclonal antibody targeting IgE, can decrease the levels of unbound IgE and modulate the expression of FcεRI on basophils and mast cells [162–165]. The downregulation of FcεRI expression can decrease cellular activation mediated by IgE and IgG anti-FcεRI antibodies, inhibiting the release of histamine and mitigating inflammation [165]. Substantial data have supported the use of Omalizumab as an adjunctive treatment to antihistamines and is highly recommended for patients aged 12 years and above with CSU [166]. Omalizumab has been shown to improve the quality of life in individuals with chronic spontaneous urticaria (CSU) in clinical trials and real-world studies [167–170].

Table 1 The list includes prospective molecular targets, therapeutic agents, class, route of administration, and clinical trial number (identification) in chronic urticaria

Therapeutic agents	Alternative names of the therapeutic agent	Class of therapeutic agents	Route of administration of the therapeutic agents	Molecular target of the therapeutic agent	Class of CU	Stage of clinical trial	NCT number registered in clinicaltrials.gov database
Quilizumab	Anti M1 prime monoclonal antibody; MEMP-1972A; RG-7449	mAB	S.C	IgE	CSU	Phase II	NCT01987947
Eculizumab	ABP 959; BEKEMV;	mAB	–	C5a inhibitor	CSU	Not applicable	[146]
LY 3454738	CD200R Antibody	mAB	IV	CD200R	CSU	Phase II	NCT04159701
MTPS9579A	RG-6173	mAB	IV	Tryptase	CSU	Phase II	NCT05129423
Canakinumab	ACZ-885, Anti-IL-1 beta monoclonal antibody; Antibody A; Ilaris	mAb	SC	IL-1β	CSU	Phase	NCT01635127
Dupilumab	Dupilumab-Sanofi/Regeneron; Dupixent; REGN-668; SAR-231893, BAT-2406	mAB	SC	IL-4Rα	CSU	Phase II–III	NCT03749135–NCT04180488
					CholU	Phase II	NCT03749148
					ColdU	Phase III	NCT04681729
Benralizumab	Benra, Benralizumab—AstraZeneca/Kyowa Hakko Kirin, Benralizumab—Kyowa-Hakko/AstraZeneca, BIW-8405, BIW-8405-IL-5R; Fasenra; KHK 4563; MEDI-563	mAb	SC	IL-5Rα	CSU	Phase II	NCT04612725
						Phase IV	NCT03183024
Mepolizumab	240,563; Bosatria; Mepolizamab; Nucala; SB-240563; BAT-2606	mAb	SC	IL-5	CSU	Phase I	NCT03494881
Vixarelimab	KPL-716; RG-6536; RO-7622888	mAb	SC	IL-31 (via OSMRβ)	CSU	Phase II	NCT03858634
Rituximab	IDEC-102; IDEC-C2B8; IDEC-C2B8-anti-CD20; MabThera; R 105; RG 105; Ristova; Rituxan; Rituximab-EU; RO-452294	mAb	IV	CD20	CSU	Phase I–II	NCT00216762
Ligelizumab	QGE-031	mAb	SC	FcεRI (via IgE)	CSU	Phase II	NCT02649218
							NCT02477332
						Phase III	NCT03580356
							NCT03580369
							NCT04210843
							NCT03907878
					CSU,CholU,ColdU	Phase I	NCT04513548
					ClndU	Phase III	NCT05024058

Table 1 (continued)

Therapeutic agents	Alternative names of the therapeutic agent	Class of therapeutic agents	Route of administration of the therapeutic agents	Molecular target of the therapeutic agent	Class of CU	Stage of clinical trial	NCT number registered in clinicaltrials.gov database
Barzolvolimab	CDX-0159	mAb	IV	KIT	CSU	Phase I	NCT04538794
						Phase II	NCT05368285
					ColdU, CholU, symptomatic dermographism	Phase I	NCT04548869
UB-221	–	mAb	IV	FcεRI (via IgE)	CSU	Phase I	NCT03632291
							NCT04175704
							NCT04404023
						Phase II	NCT05298215
Lirentelimab	AK 002; Antolimab	mAb	IV	Siglec 8	CSU, CholU, symptomatic dermographism	Phase II	NCT03436797
UCB8600	–	mAb	Oral	FcεRI (via IgE)	CSU	Phase I	NCT04444466
Reslizumab	–	mAbs	IV	anti-IL5	CSU, ColdU	-	[147]
Tezepelumab	AMG 157; MEDI-9929; Tezepelumab-ekko; Tezspire	mAb	SC	TSLP	CSU	Phase II	NCT04833855
GSK2646264	–	SM	Topical	SYK	CSU, ColdU	Phase I	NCT02424799
TAS5315	–	SM	Oral	BTK	CSU	Phase II	NCT05335499
Tirabrutinib	GS-4059; ONO-4059; Tirabutinib hydrochloride—Gilead Sciences/Ono Pharmaceutical; Velexbru	SM	Oral	BTK	CSU	Phase II	NCT04827589
Rilzabrutinib	PRN-1008; SAR-444671	SM	Oral	BTK	CSU	Phase II	NCT05107115
Remibrutinib	LOU-064; LOU064-NXA; NVP-LOU064-NXA	SM	Oral	BTK	CSU	Phase II	NCT03926611
							NCT04109313
						Phase III	NCT05048342
							NCT05032157
							NCT05030311
Fenebrutinib	GDC-0853; RG-7845; RO-7010939	SM	Oral	BTK	CSU	Phase II	NCT03137069
							NCT03693625
JW 1601	LEO-152020; LP-0190	SM	Oral	H4R	CholU	Phase II	NCT04853992

Table 1 (continued)

Therapeutic agents	Alternative names of the therapeutic agent	Class of therapeutic agents	Route of administration of the therapeutic agents	Molecular target of the therapeutic agent	Class of CU	Stage of clinical trial	NCT number registered in clinicaltrials.gov database
Etanercept	mAbx01; Enbrel; p75TNFR-Ig; rhu TNFR-Fc; Soluble tumour necrosis factor receptor p75 Fc IgG1 fusion protein; TNF receptor fusion protein; TNFR-Fc-p75; TNR-001	FP	SC	TNF	CSU	Phase II–III	NCT01030120
Rilonacept	ARCALYST; Arcalyst; IL-1-Cytokine-Trap; IL-1-Trap; Interleukin-1 Trap; KPL 914; RGN-303	FP	SC	IL-1β, IL-1α	ColdU	Phase II	NCT02171416
AZD1981	–	Antagonist	Oral	CRTh2	CSU	Phase II	NCT02031679

–=Not Reported"

Conclusion

In conclusion, chronic urticaria represents a diverse spectrum of CSU and CIndU conditions. This comprehensive overview of the article sheds light on the complex interplay of cellular infiltration, immune responses, coagulation cascades, and autoantibodies underlying the pathophysiology of chronic urticaria. The intricate relationships between various immune cells, cytokines, autoantibodies, and triggers like histamine contribute to the persistent wheals and angioedema that characterize these conditions. Examining comorbidities associated with this condition emphasizes its systemic impact, including autoimmune disorders, infections, and mental health challenges. The concurrent existence of CIndU and CSU further underscores the need for a holistic diagnostic and therapeutic approach to address the complexity of triggers and symptoms. In management, the article highlights the pivotal role of assessment tools like UAS and AAS in measuring disease severity and guiding treatment strategies. The therapeutic options continue to expand from second-generation antihistamines to monoclonal antibodies. Omalizumab's emergence as an adjunctive treatment offers renewed hope, particularly for those who do not respond to traditional therapies. A list of potential molecular therapeutic targets and their targeting agents have been enlisted in Table 1, along with their NCT for identification. Understanding and managing chronic urticaria involves unravelling intricate immunological mechanisms, identifying relevant triggers, and addressing interconnected comorbidities. As research continues to uncover novel insights, healthcare providers are better equipped to provide accurate diagnoses, effective treatments, and improved quality of life for individuals battling chronic urticaria. A holistic approach encompassing immunology, neurobiology, and patient-centred care is essential in managing the diverse facets of this enigmatic condition.

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CHAPTER 4

Ayurvedic herbal formulations Haridra Khanda and Manjisthadi Kwath (brihat) in the management of allergic rhinitis: A pharmacological study

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Research article

Ayurvedic herbal formulations Haridra Khanda and Manjisthadi Kwath (brihat) in the management of allergic rhinitis: A pharmacological study

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1.Introduction

Although allergy disorders including asthma and allergic rhinitis have become more common over the past ten years, the precise reasons are still unknown. The increased prevalence of atopic disease has been attributed to both air pollution and dietary habit changes. Seasonal allergic rhinitis, asthma, atopic dermatitis, urticaria and itching are various forms of allergy disorders [1]. About 20 % of people experience acute urticaria throughout their lifetime. In a year, a mere 0.08 % of the population from every major region of the United States experienced chronic urticaria. However, this rate drastically increased in european countries, ranging from 0.38 % to 0.8 %. Notably, adolescents in China have a much higher prevalence of 2.7 % as revealed by a thorough cross-sectional investigation [2]. Itching is a common symptom observed in dermatology outpatient clinics across Europe. More than half (54.4 %) of the adult patients in these clinics experience itching, compared to just 8 % in the control group. The severity of itching is particularly high in patients diagnosed with prurigo. This symptom is not limited to hospital settings only; it is also common in private dermatology practices. In another private practice based study in Germany, over a third (36.2 %) of the patients reported itching over a one-week period, where the majority (87.6 %) of these cases were chronic in nature [3]. Children typically have a higher prevalence of atopic dermatitis than adults do, according to data on the incidence and prevalence of the condition. Abuabara et al. conducted a study in the UK to determine the prevalence of Atopic Dermatitis among 8,604,333 UK residents between 1994 and 2013. 12.3 % of children aged 17 and under, 5.1 % of people aged 18 to 74, and 8.7 % of individuals over 75 were reported to have the said condition [4]. In both the United States and Europe, 20–30 % of adults and possibly a somewhat greater number of children suffer from allergic rhinitis [5]. Despite advances in our knowledge of the pathogenesis of allergic diseases and the discovery of synthetic molecules, the prevalence of allergic rhinitis, asthma, atopic dermatitis, urticaria and itching has increased as evident from the prevalence data. WHO has recognized Ayurveda globally, addressing the issue of standardization of traditional medical systems in both academics and healthcare systems. Furthermore, WHO reports that 65 % of the global population predominantly depends on traditional medicines for their healthcare. Some multiple biological pathways or factors are involved in a disease. So, it is quite rational to use multiple components/ herbs to target multiple biological targets [6,7]. The Sarangdhar Samhita, an Ayurvedic text, promoted the use of many herbs to improve the effectiveness of treatments. The active phytochemical components in individual plants are not enough to provide the intended therapeutic effects. Combining different herbs in a specific ratio improves

the therapeutic impact and lessens the toxicity. Ayurveda finds its root origin in India and involves the concept of 3 basic balanced doshas and thus health is attained, but imbalance results in disease. An individual's Prakriti is identified based on these Panchamahabhutas and Tridosha, and a specific treatment regimen can be suggested by their particular constitution [8, 9]. The traditional Acharyas (traditional Ayurvedic practitioners) of India formulated traditional ayurvedic polyherbal drug formulations to address the various manifestations of allergy. The practice of these traditional formulations has been passed from one generation as a traditional practice. Haridra Khanda (HK) and Manjishthadi Kwatham (brihat) (MMK) are two ayurvedic polyherbal drug formulations that are frequently prescribed by ayurvedic practitioners for ages in and around India. The constituents of HK and MMK are summarized in Tables 1 and 2, respectively. In the ayurvedic system of medicine, HK has been used in the management of urticaria, itching and blister while MMK in gout, disease of the skin, facial palsy, a disorder of adipose tissue and eye [10,11]. HK is a bright yellow colored powdered solid dosage form while MMK is a dark brown colored tablet. This present research work aims to pharmacologically validate Haridra Khanda (HK) and Manjishthadi Kwatham (brihat) (MMK) in the management of allergies involving *invivo* and *invitro* studies to form a rational basis for the prescription of these two ayurvedic polyherbal drug formulations, currently in clinical use in Indian government hospitals.

Table 1 : Constituents of Haridra Khanda as per its label claim.

Plant name (Scientific name)	Part used	Amount present per 10 (g)
Haridra (*Curcuma longa*)	Rhizome	2.1 gm
Shunti (*Zingiber officinale*)	Rhizome	150 mg
Maricha(*Piper nigrum*)	Fruit	150 mg
Pippali (*Piper longum*)	Fruit	150 mg
Twak (*Cinnamum zeylanicum*)	Stem Bark	150 mg
Ela (*Elettaria cardamomum*)	Seed	150 mg
Patra (*Cinnamomum tamala*)	Leaf	150 mg
Haritaki (*Terminalia chebula*)	Pericarp	150 mg
Vibhitaki (*Terminalia bellerica*)	Pericarp	150 mg
Amalaki (*Emblica offinalis)*	Pericarp	150 mg
Nagakeshar (*Mesua ferrea*)	Stamens	150 mg

Plant name (Scientific name)	Part used	Amount present per 10 (g)
Musta (*Cyperus rotundus*)	Root Tumber	150 mg
Vidanga (*Embelica ribes*)	Fruit	150 mg
Trivrat (Operculina turpethum)	root	150 mg
Lauha (incinerated iron)	-	150 mg
Khanda (sugar)	-	7.5 gm
Sodium benzoate (Preservative)	-	q.s.

Table 2 : Constituents of Manjishthadi Kwatham (Brihat) as per its label claim.

Plant name (Scientific name)	Part used	Amount present per tablet
Manjistha *(Rubia cordifolia)*	Root Tuber	0.394 g
Kutaja *(Holarrhena pubescens)*	Stem Bark	0.394 g
Amrita *(Tinospora cordifolia)*	Stem	0.394 g
Khana *(Cyperus rotundus)*	Root Tuber	0.394 g
Bala (*Sida cordifolia*)		0.394 g
Vacha/shatgrandha *(Acorus calamas)*	Root	0.394 g
Sunthi *(Zingiber officinale)*	Rhizome	0.394 g
Haridra *(Curcuma longa)*	Rhizome	0.394 g
Daruharidra *(Berberis aristate)*	Root	0.394 g
Aristha *(Azadirachta indica)*	Stem Bark	0.394 g
Patola mula *(Trichosanthes cucumerina)*	root	0.394 g
Katuka *(Neopicrorhiza scrophulariiflora)*	Root	0.394 g
Bharngi (*Clerodendrum serratum*)	root	0.394 g

Plant name (Scientific name)	Part used	Amount present per tablet
Agni (*Plumbago zeylanica*)	root	0.394 g
Vidanga (*Embelia ribes)*	Seed	0.394 g
Murva (*Chonemorpha fragrans)*	Root	0.394 g
Daru (*Cedrus deodara)*	Root	0.394 g
Bhringa (*Eclipta prostrata)*	Plant (whole)	0.394 g
Magadha (*Piper longum)*	Fruit	0.394 g
Trayanti (*Gentiana kurroo)*	Root	0.394 g
Patha (*Cyclea peltate)*	plant (whole)	0.394 g
Sathi (*Kaempferia galanga*)	Underground stem	0.394 g
Gayatri (*Acacia catechu)*	Root	0.394 g
Pathya (*Terminalia chebula)*	Heart Wood	0.394 g
Dhatri (*Phyllanthus emblica)*	Fruit Rind	0.394 g
Vibhitakai (*Terminalia bellirica)*	Fruit Rind	0.394 g
Kirataka (*Swertia chirayita)*	Fruit Rind	0.394 g
Mahanimba *(Melia azedarach)*	Root	0.394 g
Asana *(Pterocarpus marsupium)*	Stem Bark	0.394 g
Aragwadha *(Cassia fistula)*	Heart Wood	0.394 g
Syama *(Operculina turpethum)*	Stem Bark	0.394 g
Avalguja *(Cullen corylifolium)*	Root	0.394 g
Chandana *(Santalum album)*	Seed	0.394 g
Varanaka *(Crataeva magna)*	Heart Wood	0.394 g
Puthika (oldenlandia corymbosa)	Plant (whole)	0.394 g

Plant name (Scientific name)	Part used	Amount present per tablet
Danti *(Balospermum mondanum)*	Root	0.394 g
Sakhotaka *(Streblus asper)*	Stem Bark	0.394 g
Vasa *(Justicia beddomei)*	Root	0.394 g
Parpata *(Hedyotis corymbose)*	Root	0.394 g
Sariba *(Hemidesmus indicus)*	Plant (whole)	0.394 g
Krishnasariba(ichnocarpus frutescens)	root	0.394 g
visha *(Aconitum heterophyllum)*	Root	0.394 g
Ananta *(Tragia involucrate)*	Root	0.394 g
Vishala *(Citrullus colocynthis)*	Root	0.394 g
Jala *(Plectranthus vettiveroides)*	Plant (whole)	0.394 g
Jastimadhu(*Glycyrrhiza glabra*)	root	0.394 g
Magalyapushpi (*Clitoria ternatea*)	roots	0.394 g

2. Materials and methods

2.1. Chemicals HK (Batch No.RJA21094, mfg by Revinto Lifescience Pvt.Ltd, Lic No. AUS-715) and MMK (Batch No.526994, manufactured by Arya baidya Sala, Kottakkal, Lic No.45/25D87) were donated by Central Ayurveda Research Institute for Drug Development, Kolkata. Dialysis membrane-70 (catalogue no. LA393, Himedia), 10X Phosphate Buffered Saline (PBS) (cat no. 78529, SRL), goat blood, standard Pheniramine maleate (cas.no.132-20-7, Merck), Evans Blue (Cas.no.314-13-6, Sigma Aldrich), formamide (Cas.no.75-12-7, Merck), Histamine (Cas.no. 56-92-8, Sigma Aldrich), Compound 48/80 (C 48/80) (Cat.no. C2313, Merck), ENG Scientific Eosin Y, 1 % aqueous Solution (Cat.no. ES8901), Acetone (Fisher Chemical™, Cat.no. A18-4), CD CHO Medium (Cat.no. 10743029, Gibco, ThermoFisher Scientific), Fluo-4 NW Calcium Assay Kit (Cat.no. F36206, ThermoFisher Scientific, USA), Hanks' Balanced Salt Solution (HBSS) without Ca2+, Mg2+ for Pierce™ Primary Cell Isolation Kits (Cat.no. 88284, ThermoFisher Scientific), Trypsin-EDTA (0.05 %),

phenol red, (Cat.no. 25300062, Gibco, ThermoFisher Scientific), DAPI (Cat.no. D3571, ThermoFisher Scientific), Metoprolol (Cas no. - 56392-17-7, Merck).

Equipments: magnetic stirrer (Remi 2MLH), Histamine chamber (Scientific Instrument Traders, Ambala, India), Bright field microscope (Olympus CH20i, Japan), Neubauer chamber (Superior, Marienfeld, Germany), 0.22 μm filter (Millipore®; Thermo Fisher Scientific, Waltham, MA, USA), SpectraMax (M5 Series Multi-Mode Microplate Readers, Molecular Devices, LLC, USA), flat black clear bottom 96 well plates (Cat.no. 266120, ThermoFisher Scientific, USA), Zeiss LSM 700 Confocal microscope (Carl Zeiss 700, Germany), Ab Sciex QTRAP (API-4000).

2.2. Animals

Swiss albino mice (20–25 g) and guineapigs (350–400g) were utilised in these experiments where n = 6 was assigned for each group. The mice were maintained under a 12 h cycle of light and dark phase, at a temperature of 25 ± 2 ◦C with accessible food and drinking water. All the involved animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of Dept. of Pharmaceutical Technology, Jadavpur University, through project proposal no. JU/IAEC-22/21.

2.3. Histamine-challenged mice (n = 6)

Control*H* group: Saline water administered p.o. for 14 days. Disease Control*H* group: Saline water (p.o.) administered for 14 days. HK*H* group: 1.38 g/kg/day (p.o.) HK administered concurrently for 14 days. MMK*H* group: 0.25 g/kg/day MMK (p.o.) administered for 14 days. Standard*H* group: [5.85 mg/kg (i.p.) Pheniramine maleate injected on the 14th day before histamine administration]. 0.1 mL of sterile 1 % EB dye and 1 mg/kg b.w. of histamine was injected via tail vein on the 14th day in all the groups (Sparing the controlH for histamine administration) [12].

2.4. Compound 48/80 challenged mice (n = 6)

Control48/80: Saline water p.o. for 14 days. Disease Control48/80 group: Saline water p.o. for 14 days. HK 48/80 group: 1.38 g/kg/day HK p.o. for 14 days. MMK48/80 group: 0.25 g/kg/day MMK for 14 days. Standard48/80 group: Sodium cromoglycate 80 mg/kg/i.p. for 14 days. 0.1 mL of sterile 1 % EB dye (via tail vein) and 3 μg C 48/80 in 10 μl saline administered intracutaneously on the 14th day in all the groups (Sparing the controlH for C 48/80 administration) [13,14].

2.5. Guineapig model (n = 6)

ControlGH group: Saline water p.o. for 7 days. Disease controlGH group: Saline water p.o. for 7 days. HKGH group: 335 mg/kg/day HK for 7 days. MMKGH group: 32.1 mg/kg/day MMK for 7 days. StandardGH group: 3.25 mg/kg/day i.p. Pheniramine maleate for 7 days. In the Guineapig model, guineapigs belonging to all the groups except controlGH group was sprayed with 0.6 % histamine on the 7th day in the histamine chamber. The animals in the controlGH group was sprayed with water only [15].

2.6. Sneezing rate in guineapig

In a Histamine chamber, male guinea pigs weighing between 350 and 450 g were exposed to a 0.6 % histamine dihydrochloride aerosol created by compressed air at a pressure of 4 atm. For 20 min, the animals were exposed to histamine 10 times or 10 sprays. Sneezing was characterized by an explosive expiration just after a deep inspiration. Sneezing was observed and recorded and number was noted [15].

2.7. Blood collection for eosinophilic count

Blood from guinea-pig belonging to the histamine chamber was collected from the saphenous vein. The quantification of eosinophils was accomplished by combining 10 µl of blood with 90 µl of Discombe's solution, followed by the counting of cells with stained granules using a haemocytometer after a 5 min interval. Formula for counting eosinophils in Haemocytometer: (cell count*dilution factor)/(Area counted*depth). Preparation of Discombe's solution was carried out by mixing 1 % aqueous eosin Y (5 vol) with 5 vol of acetone and distilled water 90 vol [16,17].

2.8. Preparation of dialysate from the crude formulation

Dialysate of MMK and HK was prepared using the dialysis bag method. Samples of 750 mg of MMK and 1000 mg of HK were dissolved in 1500 µl and 2000 µl of water respectively. Samples were added by putting them in a sealed dialysis bag with a molecular weight cutoff (MWCO) of 12–14 kDa. After being submerged in 100 mL of PBS with a pH of 7.4 and subjected to incubation at a temperature of 37 ◦C. The incubation process included placing the bags on a magnetic stirrer set at a speed of 100 rpm. After 24 h, the samples were collected and filtered using 0.22 µm filters [18].

2.9. Preparation and application of Evans blue (Miles assay) in mice

EB dye (0.1 mL of 1 % w/v in 0.9 % saline) was prepared and Sterilized by autoclaving (121 ◦C, 15 psig for 15 min) followed by passing the solutions through a 0.22 µm filter aseptically. 100 µl of EB solution was injected into the tail vein of mice and allowed the dye to properly circulate for 30 min. A cervical dislocation was used to kill the mice 30 min after the challenge, and ear samples were taken. EB was extracted for 24 h at 55 ◦C in 250 µl of formamide, and its concentration was measured in a spectrophotometer at 620 nm [19].

2.10. Preparation and application of histamine and compound 48/80 in mice

Filtered (0.22 µm) histamine solution was injected in mice intravenously in a dose of 1 mg/kg b.w. Each animal was given 100 µl of the histamine solution prepared in 0.9 % saline. We found that administration of 0.2 mmol/kg body weight b.w. of dose was not tolerated in our experimental mice leading to high mortality rate. So we adjusted the dose to 1 mg/kg b.w. where hyperpermeability was observed without animal death [20]. Similarly, C 48/80 was dissolved in 0.9 % saline. The prepared C 48/80 (3 µg in 10 µl saline) was filtered using 0.22 µm filter [13].

2.11. Membrane stability assay

Blood was taken from a healthy goat that had not had any pharmaceutical treatment 14 days prior to this study. Sodium oxalate was used as an anticoagulant in the blood. Furthermore, maintaining all of the blood samples at 4 ◦C for 24 h was ideal for preservation before use. After undergoing a 5 min centrifugation at 2700 rpm, the supernatant was discarded. With an autoclaved isotonic saline solution, the cell suspension was cleaned. The same centrifugation conditions were used again till the supernatant was clear and visually colourless. The cells were suspended again in 20 % (v/v) PBS solution (10 mM, pH 7.4). A solution was prepared by combining 0.5 mL of 20 % RBC suspension, 1 mL of 0.15 M PBS (pH 7.4), and 2 mL of 0.25 % sodium chloride. The mixture was mixed and then allowed to stand at room temperature for 15 min 0.5 mL of crude extracts of formulations with concentrations of 250 µg/mL, 500 µg/mL, and 1000 µg/mL were added. Aspirin was utilised as a standard agent at an equivalent concentration to the extracts. Following incubation at room temperature, all experimental samples were centrifuged at 5000 rpm for 5 min, and absorbance was measured at 530 nm [21].

2.12. Plasma collection and quantification of plasma histamine in compound 48/80 challenged mice

From the retro-orbital plexus of mice, blood samples of less than 1 mL were collected and plasma was extracted by cold centrifugation (4 ◦C) at 3500 rpm for 10 min. Histamine was quantified in the plasma using LC-MS/MS. To prepare a 100-fold concentrated stock solution, 1 mL of deionized water was added to the bottle containing the powdered protease-inhibitor cocktail. 100 mL of (1:100) protease inhibitor solution was prepared by diluting the buffer. After centrifugation, the obtained plasma was rapidly mixed with 10 µL of the protease inhibitor solution per sample and eventually stored at □ 20 ◦C.

3. Methodology for LC-MS/MS study

Utilising LC-MS/MS triple-quadruple quantification facilitates the precise detection of minimal changes in blood histamine levels or other endogenous substances post-test substance administration, enabling data collection with a very low limit of quantification. We utilised a thermostatic auto sampler, on-line degasser, and binary pump all made by Shimadzu with an LC system (LC-20AD). An Ab Sciex QTRAP (API-4000) mass spectrometer with an ESI (electrospray ionization) source was linked to the LC. The data was collected using Analyst Software version 1.6.3. For separating the histamine Phenomenex Kinetex (5µ C18 100A 50 × 3 mm) column was employed. The chromatographic conditions include 10 µl injection volume at a flow rate of 0.5 mL/min and a column temperature of 25 ◦C, a linear gradient was eluted using mobile phases A and B. ESI with both negative and positive ions were used as the ionization source. Nitrogen was used as curtain gas and was kept at a flow of 20.00 psig while GS1 and GS2 gas were kept at 40.00 and 45.00 psig, respectively. Both the temperature of the gas and capillary voltage was set at 400 ◦C and □ 4500.00 V respectively. Similarly, the declustering potential (DP) and the collision energy was applied for parent ionization and product ionization respectively. Multiple reactions monitoring (MRM) was the last step in the mass spectrometry detection response. Liquid-liquid extraction method was used for extracting histamine from plasma matrix where metoprolol was used as an internal standard (IS) [22].

3.1. Quantification of intracellular Ca2+ signaling in CHO cells

Calcium signaling in CHO cells was quantified using Fluo-4 NW Calcium Assay Kit. The calcium response rate was assessed according to the kit manual with minor modifications in the procedure reported earlier [23,24]. In brief, CHO cells were cultured in flat black clear bottom 96 well plates at a density of 2000 cells per well in CD CHO media. All the solutions

were pre-incubated at 37 ◦C before supplementing them to the cells. After reaching 70 % confluence, cells were pre-incubated with vehicle control or at different concentrations of raw and dialysate of MMK and HK, for 16 h and then exposed to Fluo4 NW solution for 60 min at room temperature. The medium was aspirated from the culture and cells and was washed twice with HBSS assay buffer. Cells were cultured in assay buffer and then stimulated with histamine (10 μM) at different incubation durations for a maximum of 120 min. The fluorescence intensity in each well was measured at an excitation wavelength of 494 nm and an emission wavelength of 516 nm. The peak response was recorded right after washing and at 20min intervals up to 120min. The highest intensities of each sample were adjusted in relation to the background and the average reaction of 10 μM histamine (100 %). The values were plotted against the time elapsed between eliminating unbound antihistamines from the cells and activating the cells with histamine. The fluorescence images of cells at different washout durations were documented using a Zeiss LSM 700 Confocal microscope (Carl Zeiss 700, Germany) at 20× magnification after mounting with DAPI solution.

3.2. Statistical analysis

Data analysis was conducted with GraphPad Prism® 9.0 software (GraphPad Software Inc., La Jolla, CA, USA). All the data were expressed as Mean ± SD and graphically represented using bar diagram. The one-way ANOVA test followed by Tukey's test was used to evaluate the statistical comparisons between different groups. A comparison was considered statistically significant when $p \leq 0.05$.

4. Result

4.1. HK and MMK attenuates histamine and compound 48/80 induced EB dye leakage

The intensity of EB dye leakage from mice ears, was assessed through two methods: histamine and compound 48/80 induced EB dye leakage. In the histamine and compound 48/80 induced model significant EB dye leakage was observed in the disease control group compared to the control group (***$p < 0.001$) of both the models. In the histamine model, treatment with HK, MMK and the standard drug significantly attenuated dye leakage (**$p < 0.01$, *$p < 0.05$, and ***$p < 0.001$, respectively) as compared to the disease controlH group. From the observed Fig. 1, it can be derived that HK showed better attenuation of dye leakage than MMK. Similarly, in the Compound 48/80-induced model treatment with HK, MMK and the standard drug significantly attenuated dye leakage (*$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$, respectively) as compared to the disease control48/80 group. From our observation of Fig. 2 it can be

interpreted that MMK prevented the dye leakage more than HK. The extravasation of EB dye from the mice ear, as observed in this specific investigation, is depicted in Fig. 3.

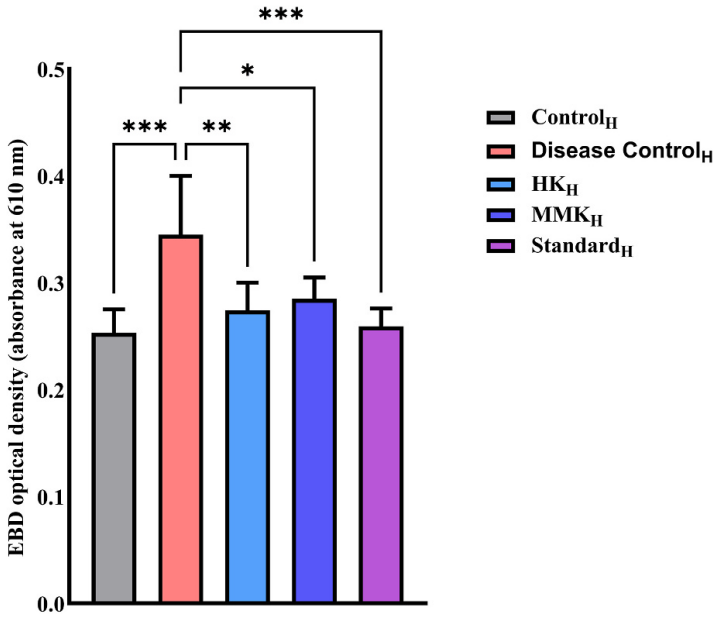

Fig. 1. Represents the optical density of EB dye leakage of controlH, disease controlH, HKH, MMKH and StandardH denoted groups in mice model on administering histamine (i.v.) externally where *p < 0.05, **p < 0.01, ***p < 0.001. The percentage inhibition of HK, MMK and standard (pheneriamine maleate) were found to be 22.81 %, 19.71 % and 27.04 % respectively. Statistical comparisons made are as follows; controlH group vs disease controlH group; disease controlH Vs HKH, MMKH and StandardH.

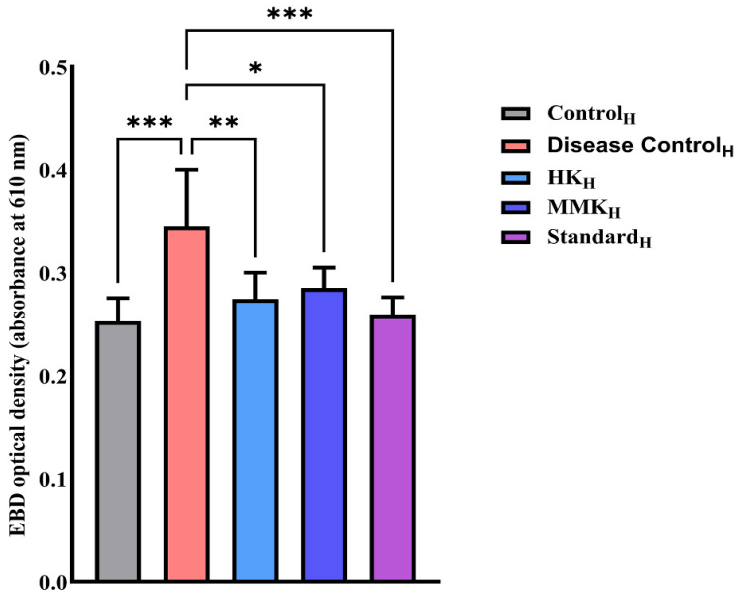

Fig. 2. Represents the optical density of EB dye leakage of control48/80, disease control48/80, HK48/80, MMK48/80 and Standard48/80 denoted groups in mice model on administering C

48/80 where * p < 0.05,**p < 0.01, ***p < 0.001. The percentage inhibition of HK, MMK and standard (sodium chromoglycate) were found to be 14.58 %, 22.40 % and 26.04 % respectively. Statistical comparisons made are as follows Control48/80 vs disease control48/80 and disease control48/80 vs HK48/80, MMK48/80 and Standard48/80.

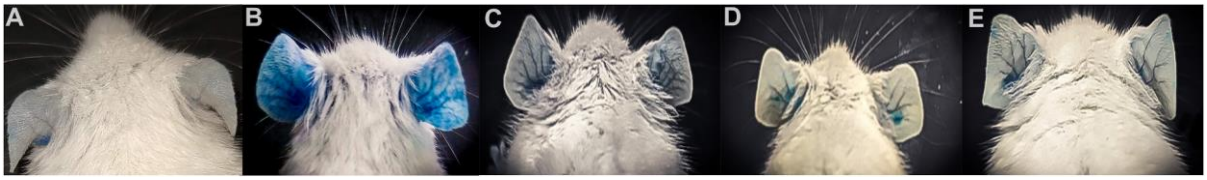

Fig. 3. Represents the image of the two ears of the mice belonging to different groups showing EB dye leakage where (A) controlH (B) disease controlH (C) MMKH (D) HKH, and (E) StandardH groups in mice model on administering histamine externally (i.v.).

4.2. HK and MMK reduces sneezing in guineapigs The number of sneezes per 20 min in guineapig is represented by Fig. 4. There was a highly significant (****p < 0.0001) increase in the disease controlGH as compared to the ControlGH. The number of sneezes in all the three groups namely HKGH (***p < 0.001), MMKGH (**p < 0.01) and standardGH group (***p < 0.001) decreased significantly as compared to the disease controlGH group. Though, in both the HK and MMK group, number of sneezes decreased but the number of sneezes was lesser in the HKGH group than the MMK group animals.

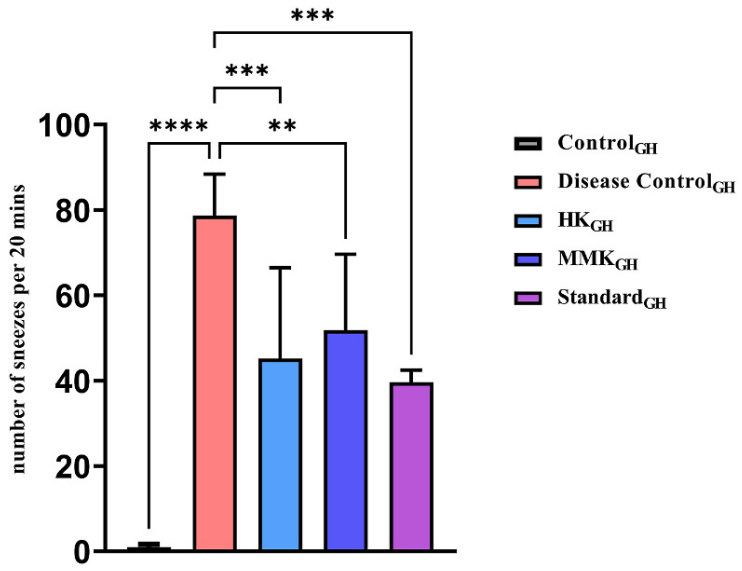

Fig. 4. Represents number of sneezes per 20 min in guineapig evaluated in a histamine chamber. The statistical comparisons were made as follows ControlGH vs disease controlGH and disease controlGH vs HKGH, MMKGH and StandardGH where * p < 0.05, **p < 0.01,

***p < 0.001, ****p < 0.0001. The percentage inhibition of HK, MMK and standard (sodium chromoglycate) were found to be 42.58 %, 34.11 % and 49.6 % respectively.

4.3. HK and MMK attenuates blood eosinophilic count in guineapigs

Fig. 5 represents blood eosinophilic count measured on the 7th day of the experiment in guineapigs. Guineapigs in the disease controlGH group showed significant elevation (***p < 0.001) of blood eosinophil count as compared to the controlGH group. The blood eosinoophilic count in all the groups namely HKGH (**p < 0.01), MMKGH (*p < 0.01) and standardGH group (***p < 0.001) was significantly reduced as compared to the disease controlGH. From our observation of Fig. 5, the HKGH group animals showed more decrease in blood eosinophilic count than the MMKGH group.

4.4. HK and MMK attenuates plasma histamine in compound 48/80 induced mice

Fig. 6 represents the plasma histamine level in C 48/80 induced mice model measured by LC-ESI-MS/MS. The plasma histamine level in the **Disease Control48/80** group significantly increased (***p < 0.001) in comparison to the **Control48/80** group animals. The plasma histamine level significantly decreased in **HK group48/80** (*p < 0.05), **MMK Group48/80** (*p < 0.05) and **Standard group48/80** (**p < 0.01) when compared to the **Disease Control48/80** group. From the observed Fig. 6, the plasma histamine in the **HK group48/80** group was found to be lesser than **MMK Group48/80** group animals.

4.5. Protective effect on the RBC In vitro by HK and MMK

Fig. 8 represents the anti-inflammatory activity of HK and MMK by induction of hypotonicity in goat RBC. The anti-inflammatory activity of HK and MMK is expressed as inhibition of RBC haemolysis in percentage. The anti-inflammatory effect is the extent of giving protection to goat RBC by the different concentrations of HK and MMK in comparison to the standard drug aspirin. At 1000 µg/mL concentration, HK (94.33 %) and MMK (92.67 %) showed the highest % inhibition of RBC membrane rupture.

4.6. HK and MMK inhibits intracellular calcium release in CHO cells

Fig. 9 represents calcium response % vs time after washout (mins) in CHO cells. In Fig. 9A i.e. for HK raw, the calcium response % started to increase steeply after the 40th min but did not change much from the 60th min till the 120th min. HK raw at a concentration of 250 µg/ml showed the best intracellular cytosolic calcium inhibition against histamine challenge in CHO

cells. For HK's dialysate i. e. Fig. 9B, the calcium response % started to increase from the 20th min washout period. Among the different concentrations of HK dialysate, 250 µg/ml equivalent showed the best inhibition of calcium response %. Similarly, In Fig. 9C, the calcium Response % of MMK raw started to increase sharply from the 40th minute of washout period. From all the tested concentrations of MMK raw, 250 µg/ml was best able to prevent the intracellular cytosolic calcium. In Fig. 9D, MMK dialysate, the calcium response % started to increase from the 20th min and continuously increased till the 120th min. The MMK dialysate concentration which best inhibited intracellular cytosolic calcium was 250 µg/ml equivalent. Figs. 10 and 11 represents the fluorescence images of CHO cells at 20× magnification after treatment with 250 µg/ml of HK raw and dialysate (Fig. 10) and MMK raw and dialysate (Fig. 11). The images were captured using confocal microscope.

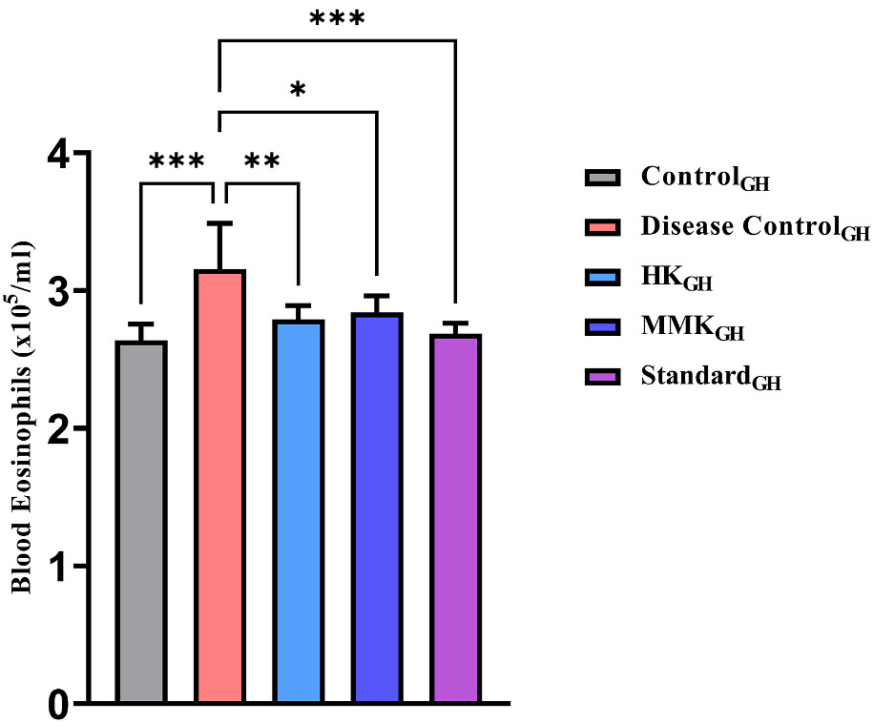

Fig. 5. Represents blood eosinophilic count in guineapigs evaluated in neubauer Chamber. Statistical comparison was made as follows controlGH vs disease controlGH and disease controlGH vs HKGH, MMKGH and standardGH where * $p < 0.05$,**$p < 0.01$, ***$p < 0.001$. The percentage inhibition of HK, MMK and standard (sodium chromoglycate) were found to be 11.56 %, 10.04 % and 14.82 % respectively.

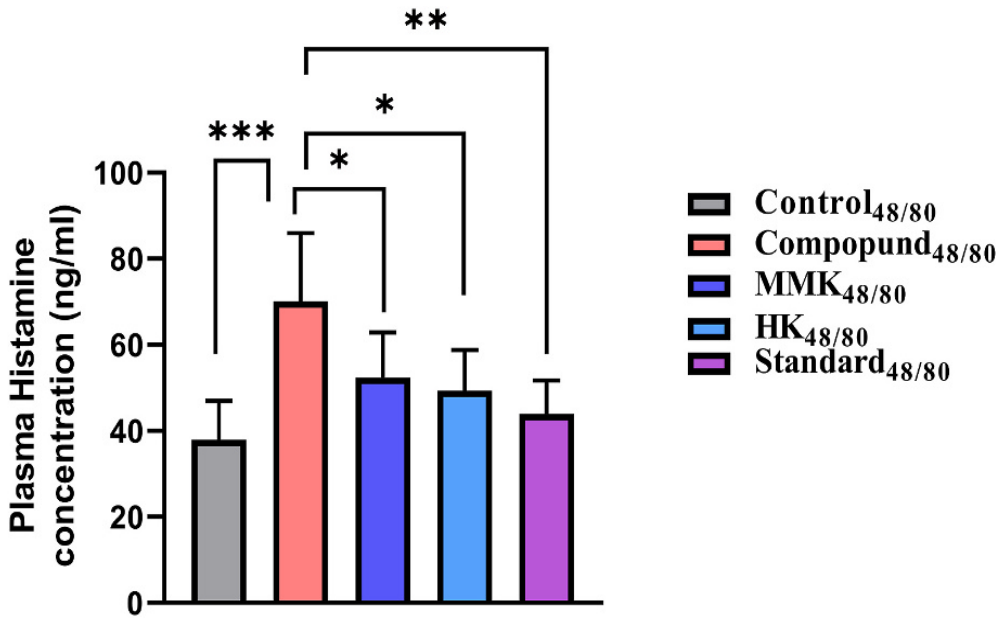

Fig. 6. Represents plasma histamine in C 48/80 challenged mice evaluated using LC-MS/MS. The statistical comparison was made as follows control48/80 vs disease control48/80, disease control48/80 vs HK48/80, MMK48/80 and Standard48/80 where * $p < 0.05$,**$p < 0.01$, ***$p < 0.001$. The percentage inhibition of HK, MMK and standard (sodium chromoglycate) were found to be 29.62 %, 25.37 % and 37.28 % respectively.

5. Discussion

In this present experiment, HK and MMK were tested for anti-allergic activities through H1 receptor. Both the formulations prevented EB dye leakage in histamine and C 48/80 challenged mice. Both the formulations attenuated the number of sneezes and blood eosinophilic count in guineapigs. Further, they also decreased plasma histamine level in C 48/80 challenged mice. A decrease in intracellular cytosolic calcium level was exhibited by both the ayurvedic polyherbal drug formulations (HK and MMK) in CHO cells.

During allergic inflammation activated mast cells release histamine. This causes hyperpermeability in the blood vessels. Vascular hyperpermeability is linked to allergic symptoms like conjunctivitis, nasal congestion and urticaria. Understanding the mechanism behind how histamine causes vascular hyperpermeability might lead to new ways to treat allergic diseases. According to some scientific reports, histamine causes the breakdown of the endothelial cell barrier. Although many of these studies are conducted in *in vitro* systems, the underlying causes or mechanisms involved in *invivo* systems remain unclear. Histamine-induced increased blood flow causes hyperpermeability due to the liberation of NO and endothelial barrier disruption. Nowadays, vasoconstrictors are used in the treatment of rhinitis

[25]. Our findings corroborate the usefulness of HK and MMK and suggest that they might be useful in treating various allergic symptoms.

As per published reports, leukotrienes primarily increased vascular permeability. Eosinophils are the primary originators of the cysteinyl leukotrienes LTC4/D4/E4, which are lipid byproducts that have significant functions in asthma and other types of allergic inflammation [26,27]. The vessel walls and smooth muscle layer of the venule are much more delicate than that of an artery or large vein and are permeable due to structural weakness. Structural weakness is the cause of less expression of adhesion protein. The integrity of the epithelial barrier is important for blood vessel permeability [28]. A Study suggests that the endothelial H1 receptor is functionally important in histamine-induced vascular leakage [29]. Inflammation is a clinical manifestation of an allergic reaction. Histamine causes vasodilation followed by flare. Endothelial cells are mural cells of blood vessels, especially capillary or end vessels. At end vessels, epithelial cells are connected with adhesion molecules like VE-Cadherin [30]. The presence of histamine makes detachment of adhesion molecules and plasma get leaked in extravascular space. In case of allergic conditions end vessel dilation causes redness under the skin.

Fluid accumulation and edema can also result from tissue insult directly or indirectly related to histaminergic response [31]. An *invitro* assay involving hypotonic solution induced goat RBC membrane disruption was conducted to determine the toxicity of HK and MMK on mammalian cell membrane. Both HK and MMK were observed to be nontoxic to mammalian cell membrane as was evident in the findings with the goat RBC experiment [Fig. 8].

The dye leakage test can be related to the membrane stabilization test (goat RBC) and indicates that HK and MMK had no role in plasma extravasation. The dye leakage test was conducted using two distinct principles. Primarily, through external histamine incorporation and secondly through a potent mast cell degranulator i.e. C 48/80 by provoking intrinsic histamine release. In both cases, dye leakage was prominent as described in our result [Figs. 1 and 2].

When HK and MMK were given orally to the mice, a decreased amount of dye leaching out (Figs. 1 and 2) (spectrophotometrically) was observed, which shows that these two formulations might diminish the activity of histamine. Externally [Fig. 1], HK showed greater ability than MMK in preventing the dye leakage while the reverse was observed i.e. dye leakage caused due to degranulation of mast cell by C 48/80 [Fig. 2].

In another study with guineapigs it was found that HK and MMK were able to decrease the number of sneezes [although 50 % of the population showed higher suppression of number of sneezes while others didn't] [Fig. 4]. The histamine chamber experiment (histamine sprayed in histamine chamber) involving guineapigs [Fig. 4] and dye leakage tests [Figs. 1 and 2] indicate the presence of histamine externally, extrinsicly (histamine administration) and intrinsicly (C 48/80) does not promote histaminergic responses when treated with HK and MMK indicating anti-allergic effect.

Furthermore, a study was conducted to measure the number of eosinophils in guinea pig's blood where before exposure to external histamine the test agents were administered. Treatment with both HK and MMK significantly decreased ($p < 0.05$) the blood eosinophil count. Histamine being a potent mediator of inflammation can stimulate the activation and production of eosinophils through H1 receptor mediated superoxide ions generation [32]. Eosinophilia is a hallmark of allergic inflammation. The sprayed histamine on guineapigs might have triggered inflammation which further released more histamine from mast cells, causing eosinophil activation and proliferation. Further, the activated eosinophils can attract more mast cells to the site of inflammation, generating a vicious cycle that amplifies the allergic response. Based on our experiment, it is evident that HK and MMK might have inactivated the H1 receptor in histamine exposed mice. Moreover, these ayurvedic formulations may also have stabilized mast cells in compound 48/80 induced mice. Therefore, it is quite reasonable to believe that these ayurvedic formulations might have played an important role in reducing the number of blood eosinophils in histamine sprayed guineapigs which can be co-related with the decreased sneezing rate in the same [Fig. 5].

The observed reduced plasma histamine (measured using LC-ESI-MS/MS) after C 48/80 challenge in the HK48/80 and MMK48/80 groups [Figs. 6 and 7] and the decrease in EB dye leakage in the HK48/80 and MMK48/80 group [Fig. 2] indicate that the HK and MMK may probably prevent mast cell degranulation. Mast cell degranulation is a key step in the release of histamine [33]. From the above-mentioned observations, HK and MMK appear to prevent direct histamine release in the plasma as well as stabilize the mast cell from undergoing degranulation and subsequently releasing histamine. From the observed results, HK and MMK activity may be related to their mast cell stabilising effect.

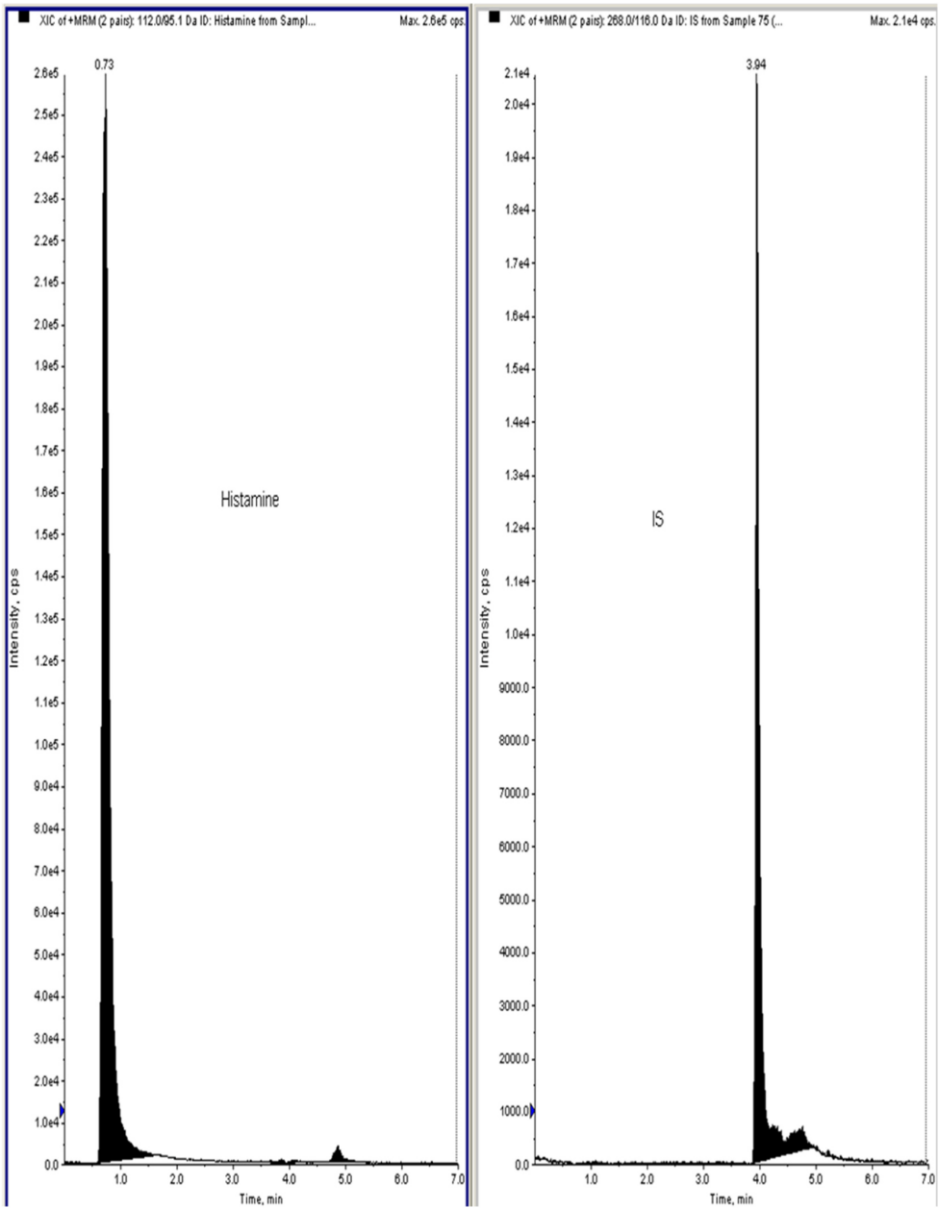

Fig. 7. Peaks of raw data obtained from an LC-MS/MS based MRM of plasma Histamine with metoprolol as an internal standard (IS).

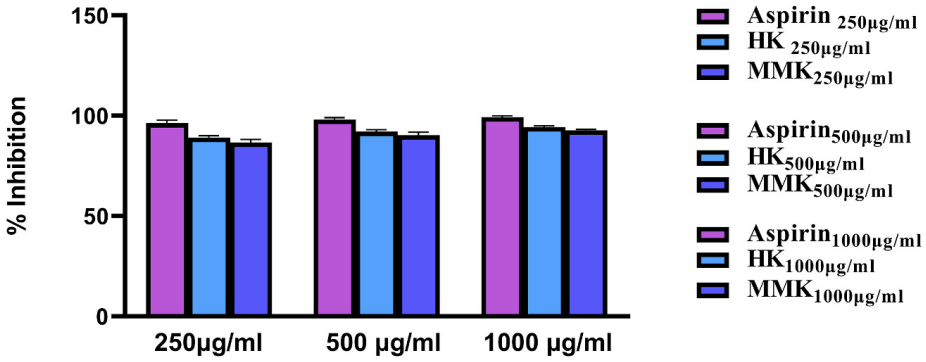

Fig. 8. represents protection of RBC in terms of % inhibition of standard drug (aspirin), HK and MMK's (ability to prevent RBC rupture) at concentrations of 250,500 and 1000 µg/mL.

Among the many factors that govern histamine release calcium-mediated calcium release from ER where Calcium is the second messenger, is the most important event which ultimately culminates in exocytosis. Finally, this exocytosis is responsible for histamine release from mast cells [34,35].

Thus, intracellular cytosolic calcium seems to play a significant part in ensuring either histamine release or its inhibition. Moreover, the histamine release process is primarily mediated through calcium involving phospholipase C and phospholipase A2 pathways activation [36,37]. Initially released Ca2+ from ER binds to the ryanodine receptors (RyRs) on the ER which again triggers more release of Ca2+ from it. HK and MMK both in raw formulation and their dialysate, were studied for intracellular calcium release in histamine-challenged CHO cells [Fig. 9].

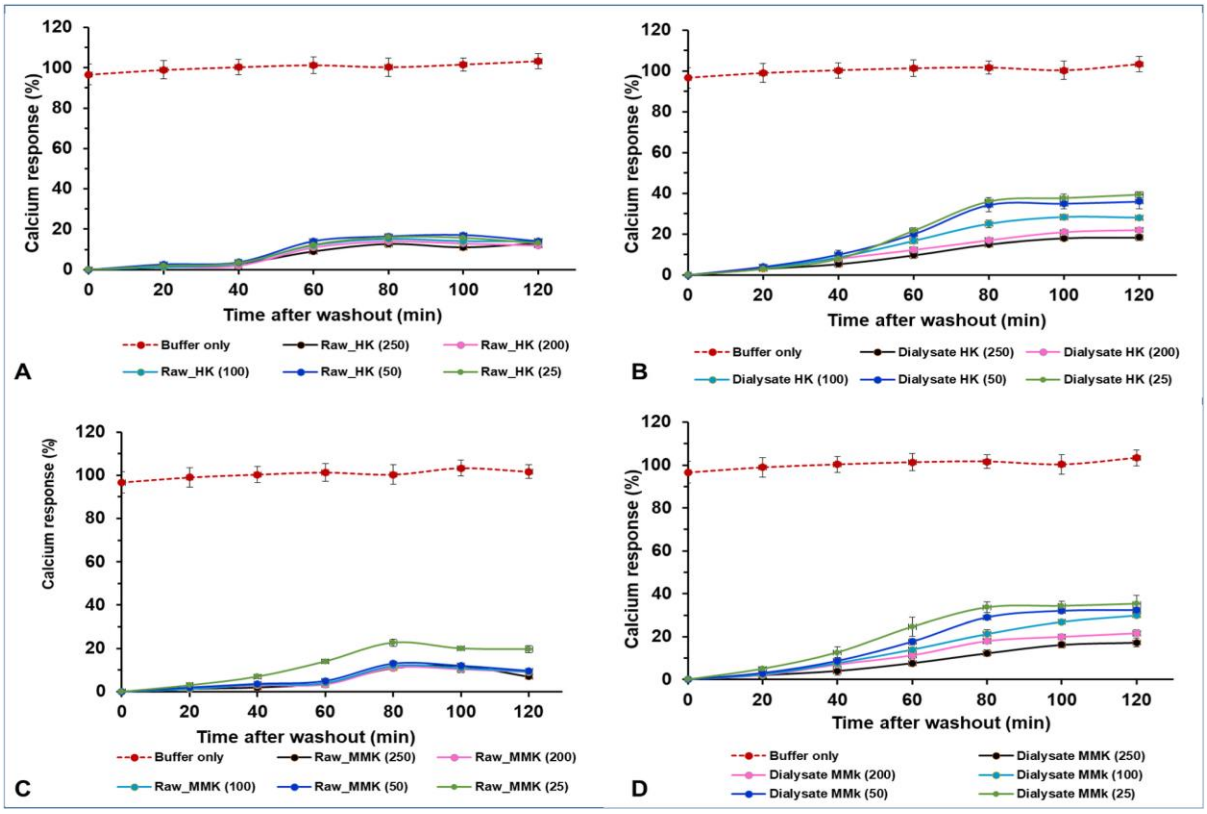

Fig. 9. Represents calcium response % vs Time after washout (mins) for different concentrations of HK raw, HK dialysate, MMK raw and MMK dialysate in CHO cell line. The red colored line denotes the calcium response % elicited by buffer. All concentrations are mentioned in µg/ml in case of raw HK and MMK while their dialysates are in µg/ml equivalent. The varying color indicates different concentrations of HK and MMK in their raw and dialysate forms.

The intracellular calcium assay in CHO cells is presented in Fig. 9. In this study, the histaminergic response has been presented as a percentage abundance of calcium ions present within the cell. This finding can also be correlated with histamine binding to H1 receptors resulting in subsequent liberation of Ca2+ from ER. The presence of HK [Fig. 9A and B], decreased the appearance of intracellular cytosolic calcium, even after the addition of histamine at all-time points with subsequent washouts up to 40th min (for HK raw) and 20th mins (for HK dialysate) indicating non-responsiveness of H1 receptor-mediated Ca2+ release. This probably indicates HK both raw and in dialysate form, hindered histamine to release intracellular cytosolic calcium via H1 receptor in CHO cells. Similar findings were observed in the MMK both raw up to 40th minute [Fig. 9C] and in dialysate form up to 20th minute [Fig. 9D].

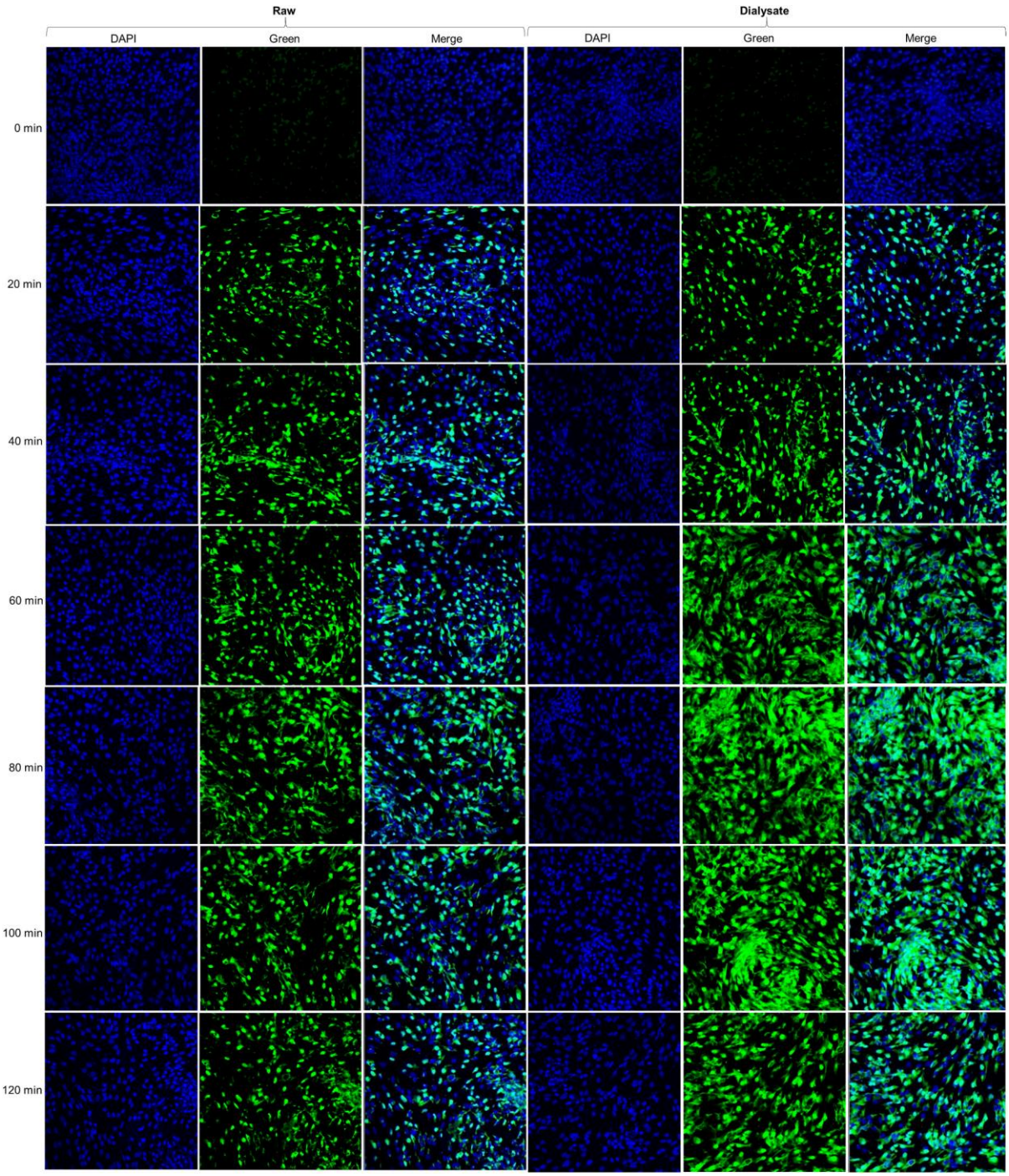

Fig. 10. Represents the fluorescence images of CHO cells at different washout durations for 250 µg/mL of HK raw and 250 µg/mL equivalent of HK dialysate is shown using Confocal microscopy at 20× magnification.

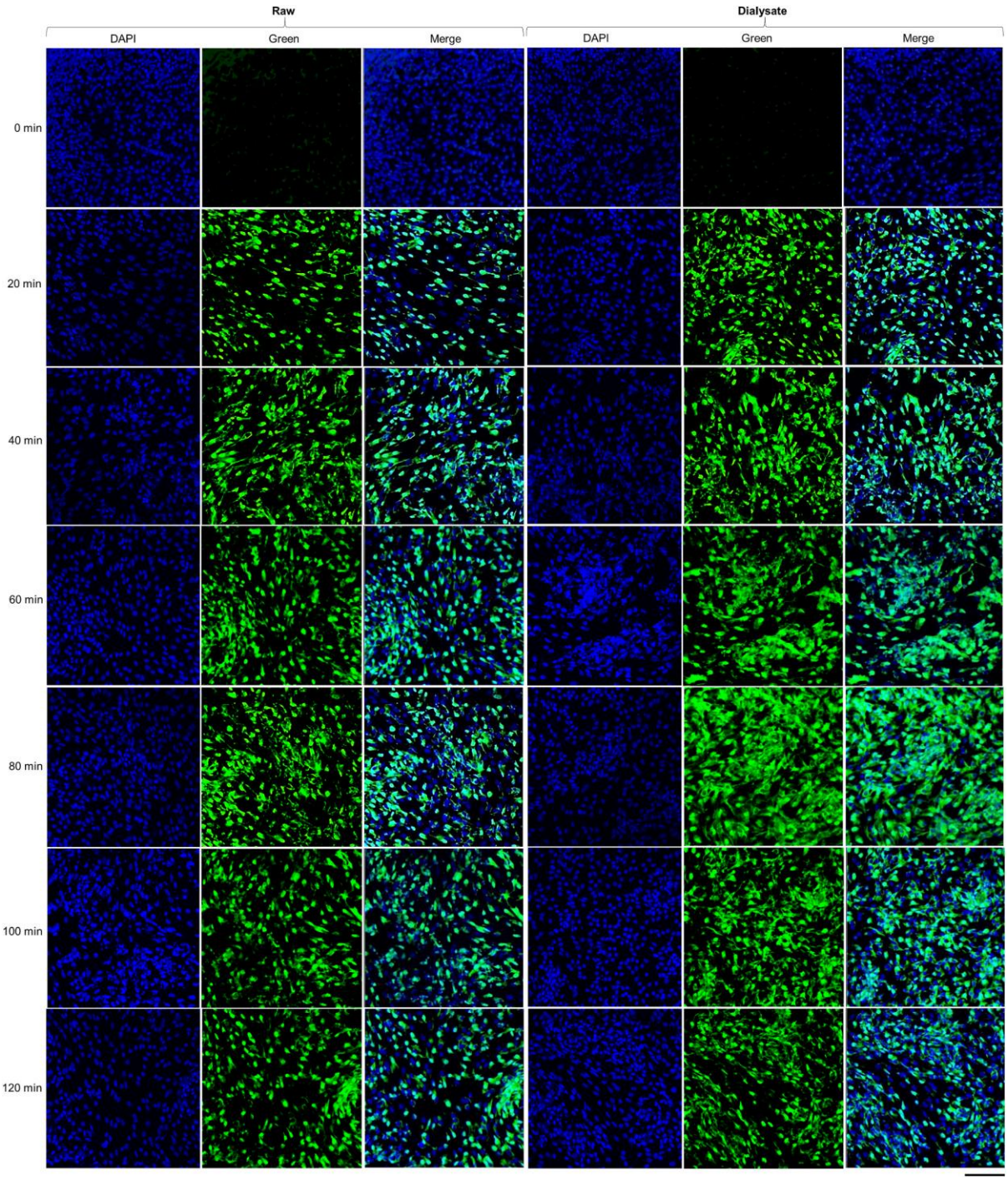

Fig. 11. Represents the fluorescence images of CHO cells at different washout durations for 250 µg/mL of MMK raw and 250 µg/mL equivalent of MMK dialysate is shown using Confocal microscopy at 20× magnification.

Further, in the same study after the 60th minute histamine-mediated Ca2+ release was observed to be almost saturated till the end point. This might be due to the desensitization of histamine receptors owing to the presence of HK and MMK both in raw and in dialysate form.

As per our observation, both HK and MMK in raw form showed more inhibition for histamine-mediated Ca2+ release as compared to their dialysate form. Among many probable reasons behind this phenomenon, it can be due to the synergistic/holistic effect of multiple components of these clinically used ayurvedic polyherbal drug formulations. However, the dialysate form of both HK and MMK, showed comparatively better dose-related response. It will be important to mention that the dialysate form (involving 12-14kD membrane) of these preparations corresponds to the behaviour of these formulations in plasma following oral administration of HK and MMK.

Therefore, HK and MMK both raw/dialysate indicate H1 receptor inactivation resulting in decreased intracellular cytosolic Ca2+ appearance indicating prevention of exocytosis of various cells like mast cells. It will be relevant to mention here that these two formulations significantly inhibited C 48/80 mediated dye leakage with a simultaneous reduction in plasma histamine in mice, which has already been mentioned earlier in this text.

The traditional use of HK and MMK has been mentioned above [9,10]. There are many plant components present in the two ayurvedic formulations. These include *Curcuma longa, Zingiber officinale, and Piper nigrum* in HK, and *Rubia cordifolia*, *Piper longum*, and *Tinospora cordifolia* in MMK, as well as additional plant components. The scientific reports indicated a substantial anti-allergic effect of the plant components stated above [38–43].

The aforementioned investigations demonstrated that the experimental ayurvedic formulations provided protection against histamine-induced allergy manifestation by decreasing capillary permeability, stabilising mast cells, and alleviating allergic rhinitis. The aforementioned observations further motivated the utilisation of a Ca2+ release assay. HK and MMK exhibited a dose-dependent suppression of histamine-induced calcium release in CHO cells. HK and MMK appears to protect against histamine mediated allergy modulating intracellular cytosolic calcium.

In Ayurveda, HK has been used to manage Vataja Pratishyaya, a terminology used in their system which can be co-related to allergic rhinitis in modern medicine [10,44]. MMK has also been reported to act against some skin diseases as mentioned in ayurvedic literature [11]. The above mentioned ayurvedic literature led us to form a hypothesis that both HK and MMK might

play a role in alleviating histamine mediated allergic rhinitis. Thus, based on our experiment, this reverse pharmacological study appears to validate the rationality and conceived hypothesis for the clinical use of HK and MMK in histamine mediated allergic rhinitis, within the ambit of our study's limitations.

6. Conclusion

In our experiment, we have introduced administration of histamine externally mimicking sudden clinical anaphylactic condition. We have also arranged for the intrinsic release of mast cell histamine with compound 48/80. During the course of the investigation guineapig sneezing in histamine chamber was induced to mimic rhinitis. In the experiment, the standard drug pheniramine maleate was used for the suppression of histamine-mediated H1 receptor activity. However, sodium chromoglycate was used as a standard mast cell stabilizer. In all the above-mentioned experiments investigational ayurvedic preparation showed protection against histamine-induced allergic manifestation which involved increased capillary permeability, mast cell stabilization, and allergic rhinitis as compared to allopathic medicine pheneramine. Observations mentioned so far prompted to involve Ca2+ release assay. Interestingly, enough HK & MMK showed dose-dependant inhibition of histamine-induced Ca2+ release in CHO cells. Finally, besides antiallergic activity of these two clinically used ayurvedic preparations it was also found to be nontoxic to mammalian cell membrane as was evident in the findings with the goat RBC experiment. The component plants of these two ayurvedic formulations e.g. *Curcuma longa, Zingiber officinale* and *Piper nigrum* component of HK and *Rubia Cordifolia, Piper longum* and *Tinospora cordifolia* component of MMK along with other plant components have been reported earlier. Report revealed significant anti-allergic action/potential of the above-mentioned plant components. It will be important to mention that report suggests that polyherbal combinations are often beneficial from the therapeutic point of view as compared to that of the individual plant component [8]. Therefore, this reverse pharmacological investigation probably justifies the clinical use of HK and MMK within the limitation of our experiment.

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CHAPTER V

Phytochemical analysis, probable molecule prediction and receptor interaction through molecular docking method.

1.Introduction

In prior experiments involving two classical preparations, Haridra Khanda (HK) demonstrated greater efficacy in our experimental setup compared to Maha Manjiasthadi Kwath (MMK). According to our previous experiments we found that Hridra Khanda showed better activity in our experimental setup. This chapter focuses on the metabolite profiling of HK, conducted using LC-qTOF-MS, to identify tentative metabolites present for the first time. This experiment confirmed and identified the preliminarily recognised bioactive metabolites in HK.

HK has demonstrated antihistaminic activity. This chapter details the molecular docking conducted on histamine receptor-1 (H1R) and Fc epsilon receptor (FcεR), a receptor on immune cells that interacts with IgE, an antibody associated with allergies.

2. Experimental Section

2.1. Chemicals

Acetonitrile (Mass grade) (Cas no - 75-05-8), Phosphate buffer (10x) (SRL Cat no. 78529), Methanol (Mass grade) (Cas no.- 67-56-1), Ethyl acetate (Cas no.- 141-78-6), Folin-Ciocalteu, Gallic acid (Cas no.- 5995-86-8), Sodium Carbonate (Cas no.- 497-19-8), Formic acid (Cas no.- 64-18-6).

2.2. Development of Solvent Systems and Preparation of HK extract

In this study, we systematically selected different solvent systems to optimize the extraction of HK so as to obtain a high percentage yield. 100 g of HK was subjected to maceration in 5 different solvent systems, namely Ethanol (Et), Methanol (MeOH), Et: Water (1:1), MeOH: water (1:1) and only aqueous, kept for 24 hr with continuous shaking (Mini Rotary Shaker RS-12R Remi). The different fractions were filtered with the help of Whatman filter paper No.1. The collected filtrates were concentrated by using a rotary evaporator (CCA-1110, EYELA, Japan(1). The concentrated crude extracts thus obtained were stored at -20°C. The % yield of HK in different fractions was estimated by utilizing the formula yield (%) = (weight of extraction/weight of dry sample) × 100.

2.3. Estimation of TPC

The TPC in HK extracts was estimated by utilising the Folin-Ciocalteu spectrophotometric method, in which gallic acid acted as the reference standard. A 20 µL aliquot of HK extract (10 mg/mL) was thoroughly mixed with 100 µL of Folin-Ciocalteu reagent. Following the mixing

of 80 µL of 7.5% Na_2CO_3, the resulting mixture was vortexed again and eventually incubated for 30 mins/45°C. The absorbance of the resulting mixture was measured at 750 nm (Spectramax M5 spectrophotometer, Molecular Devices). The TPC was calculated as gallic acid (mg) equivalents (GAE) per gm of HK(2).

2.4. Condition of Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (LC-qTOF-MS)

Methanolic extract of HK (2 µL injection volume) was analysed using an Agilent 1290 Infinity LC system paired with an Agilent 6530C Q-TOF mass spectrometer consisting of a dual Agilent Jet Stream (AJS) and ESI source. Chromatography-based separation was conducted on a column set at 40 °C, utilizing a gradient of aqueous solution (0.1% formic acid) and organic phase (methanol). The gradient started with 95% aqueous having 0.3 mL/min of flow rate, progressing to 70% organic from 8 min to 14 min, then returning to initial conditions i.e. 95% aqueous in the 15 min. The MS was conducted in negative ionization mode, covering a mass range of 100–3000 m/z and 1 spectrum/second of the acquisition rate. Source parameters included a gas temperature of 300 °C, gas flow rate of 8 L/min, sheath gas temperature of 350 °C, sheath gas flow of 11 L/min, nebulizer pressure of 35 psi, and a capillary voltage of 3500 V. Skimmer, fragmentor and octopole RF peak voltages were fixed at 65 V, 175 V, and 750 V, respectively. The real-time mass calibration was achieved with reference masses enabled at 112.9855, 966.0007, and 1033.9881 m/z. The detected masses were matched with Metline, which is an Agilent database. For the targeted MS/MS experiment, the mass range covered was 100-1000 m/z at a fixed collision energy of 10.00, 20.00 and 40.00 eV with an acquisition rate of 12 spectra/s. Analysis of peaks and spectra was conducted with Mass Hunter Workstation B.06.00 (Agilent Technologies, 2012), and tentative compounds were identified.

2.5. Molecular docking studies

To investigate the binding interactions between the identified phytocompounds from HK and the active sites of Histamine H1 receptor (H1R) and IgE receptor (FcεR) molecular docking simulations were performed utilizing the Libdock module of Discovery Studio 3.0 software, Dassault Systèmes, San Diego, USA (3). After identifying and cleaning the phytocompounds, the Discovery Studio 3.0 workspace was utilized to decrease energy through the "Prepare Ligands" tool. The X-ray crystallographic structures of H1R (PDB ID: 3RZE) and FcεR (PDB ID: 2Y7Q) were obtained from the Protein Data Bank (http://www.rcsb.org/pdb) and optimized for docking analysis(4,5). The optimization strategy entails the incorporation of hydrogen

atoms, the removal of water molecules, and the elimination of other non-interacting heteroatoms (6). The CHARMm force field was utilized to model the structures of the protein and ligand. Docking studies were performed with the standard LibDock module. Optimal ligand configurations are maintained after a final phase of energy minimization, allowing for flexibility in ligand positioning. A LibDockScore of ≥100 is commonly seen as indicative of a higher affinity between small molecule ligands and the receptor, suggesting enhanced binding efficiency(3).

2.6. Cell viability assay

2.6.1. Cell culture

RAW 264.7 cells were obtained from the National Centre for Cell Science (NCCS) in Pune, India. They were cultured in DMEM supplemented with 10% FBS and antibiotics (100 U/mL penicillin and 100 U/mL streptomycin) at 37°C in a humidified incubator with 5% CO_2. The cells were then harvested using 0.05% trypsin.

2.6.2. Evaluation of cell viability

To evaluate the cytotoxic effect of the dialysed HK the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl tetrazolium bromide (MTT) assay was performed. Briefly, 5×10^3 RAW 264.7 cells per well cultured and treated with 15.625, 31.25, 62.5, 125.0, and 250.0 μg ml^{-1} of the dialysed HK the control were incubated for 24 hours. Post incubation, the cells were further treated with MTT solution and incubated with 5% CO_2 at 37°C for 3 hours. The supernatants were removed, and DMSO was added to solubilize the crystals of formazan forme d. The supernatants were then withdrawn, and their absorbances were evaluated using a SpectraMax-M5 (Molecular Devices, USA) at 570 nm. The cell viability was determined using the following equation.

Cell viability (%) = (OD of experimental group / OD of control group) x 100

2.7.CYP inhibition assay

CYP (1A2, 2C9, 2D6, 3A4, 2B6, and 2C19) Inhibition Assay Using HLM

2.7.1.Preparation of Reagents and Buffer

A stock solution of 1 M potassium phosphate buffer by titrating 1 M K_2HPO_4 (Sigma, Cat: 60356) with 1 M KH_2PO_4 (Sigma, Cat: P0662) to reach a pH of 7.4 was prepared. This stock solution was diluted by adding 30 mL of the 1 M buffer solution to 270 mL of deionised water to achieve a final concentration of 100 mM. The pH was adjusted to precisely 7.4, and this

buffer was used throughout the CYP inhibition experiment. $MgCl_2$ (Sigma, Cat: M2670, FW: 203.3) was dissolved in deionised water to reach a concentration of 33.23 mM to achieve the required $MgCl_2$ concentration in the assay mix.

2.7.2.Preparation of HK and Positive Control

HK was dissolved in DMSO at 0.2,1 and 5 μg/mL concentration. The final concentration of the organic solvent in the assay did not exceed 1% (v/v). Miconazole nitrate (Sigma, Cat: M3512) was taken as a positive control inhibitor for the CYP enzymes and was used in the concentrations at IC_{50} points (0.2, 1, and 5 μg/mL) for validation.

2.7.3.Preparation of HLM Solution

Human Liver Microsomes (HLM) (Xenotech LLC, Mixed Gender, Cat No: H2610, Lot No: 2310132) stock was prepared at 20 mg/mL. For the pre-incubation phase, HLM stock was diluted to achieve 1 mg/mL for CYP1A2, 2C9, 2D6, 3A4, 2B6, and 2 mg/mL for CYP2C19 in the final assay volume. After pre-incubation, for the 4 CYPs cocktail assay (CYP1A2, 2C9, 2D6, 3A4), HLM concentration was adjusted to 0.10 mg/mL, while for the CYP2C19 assay, it was adjusted to 0.20 mg/mL

2.7.4.CYP Enzyme Substrates and Cocktail Preparation

Individual substrates were prepared at the following concentrations CYP1A2: 10 μM Tacrine HCl (Sigma, Cat: A3773), CYP2C9: 35 μM Diclofenac Sodium (Sigma, Cat: D6899), CYP2D6: 6 μM Dextromethorphan HBr (Sigma, Cat: D9684), CYP3A4: 8 μM Midazolam HCl (Dormicum , Roche Molecular Biochemicals (Basel, Switzerland), CYP2B6: 20 μM Bupropion, CYP2C19: 200 μM S-Mephenytoin (Cayman, Cat: 11913). A cocktail of substrates for CYP1A2, 2C9, 2D6, 3A4, and 2B6 and a separate solution of S-Mephenytoin for CYP2C19 were prepared, maintaining the specified concentrations.

2.7.5.Pre-Incubation Step

Two sets of reactions were prepared for each test condition, i.e., one with NADPH (1.2 mM final concentration) and one without NADPH, to assess time-dependent inhibition. HLM was added (1 mg/mL for CYP1A2, 2C9, 2D6, 3A4, and 2B6; 2 mg/mL for CYP2C19), NADPH, and HK (10 μg/ml) in a 20 μL reaction volume to initiate the pre-incubation. The mixture was incubated for 30 minutes at 37°C with gentle shaking to allow for any potential enzyme-inhibitor interactions.

2.7.6. Dilution and Incubation with Substrate

Following the 30-minute pre-incubation, the cocktail was diluted in each reaction mixture 10-fold with 100 mM potassium phosphate buffer (pH 7.4) containing the appropriate cocktail of substrates and $MgCl_2$ (3.3 mM). Further, the diluted reaction mixture was incubated at 37°C for an additional 10 minutes for CYP1A2, 2C9, 2D6, 3A4, and 2B6. For CYP2C19, the incubation period was extended to 20 minutes. This step enables enzyme-substrate interactions, allowing for product formation in the presence of the test inhibitor.

2.7.7. Reaction Termination and Sample collection for LC-MS analysis

The reaction was stopped by adding ice-cold acetonitrile with 0.1% formic acid to each sample in a ratio of 3:1 (acetonitrile volume). An internal standard, bucetin at 459.2 > 322.2 was added to each sample. The samples were cooled to 4°C and centrifuged at 2500 g for 15 minutes to precipitate proteins. Further, the supernatant was collected for LC-MS analysis.

2.7.8.LC-MS/MS Analysis

For this study, the Agilent 6495, coupled with Rapidfire and the Agilent LC 1260 Infinity II, was used. The mobile phases were as follows: Phase A consisted of water with 0.1% formic acid, while Phase B/C for four CYP enzymes (CYP1A2, 2C9, 2D6, 3A4) was composed of 80:20 MeCN: water with 0.1% formic acid. For CYP2C19, Phase B/C was 80:20 MeCN/MeOH: water with 0.1% formic acid. A Rapidfire C18 cartridge with a 4 μL volume was employed, operating in trap-and-elute mode. The flow rate was set at 1.0 mL/min, with a cycle time of 20 seconds. Transition monitoring was performed for each CYP enzyme product by monitoring specific Q1/Q3 transitions in multiple reaction monitoring (MRM) mode: For CYP1A2, the transition monitored was OH-Tacrine (215.1 > 197); for CYP2C9, it was OH-Diclofenac (312 > 229.9); for CYP2D6, Dextrophane (258.1 > 156.9); for CYP3A4, OH-Midazolam (342.2 > 202.8); for CYP2B6, OH-Bupropion (256.1 > 237.9); and for CYP2C19, OH-Mephenytoin (235.1 > 150.1). Finally, the IC50 values were determined using GraphPad Prism based on Dose-response inhibition (Nonlinear regression, 4 parameters) curve fitting.

2.8. Bioassay of Histamine

2.8.1. Preparation of Tyrode solution or Physiological salt solution

1 litre of tyrode solution was prepared by dissolving Nacl (8.0 g), Kcl (0.2 g), Magnesium sulphate heptahydrate (0.1 g), Sodium dihydrogen Phosphate dehydrate (0.5 g), glucose (1 g), (1 g), Calcium chloride (0.2 g) in distilled water. Sodium bicarbonate (1 g) was dissolved

separately in distilled water and added to the final solution to prevent chances of salt precipitation.

2.8.2. Bioassay of Guineapig ileum

From the euthanized guineapig few centimeters of ileal portion was rapidly cut, removed and rinsed. The ileal portion was placed in a watch glass containing atropinised and cold tyrode solution. Further, the ileal portion was cut about 2 cm long segments. The segment was mounted in jacketed 20 mL organ bath filled with of Biopac ITBS100 (Biopac systems,Inc, USA) containing tyrode solution maintained at 37°C and aerated (95 % O_2/5 % CO_2). Tyrode's solution was supplemented with atropine at a concentration not affecting H_1 receptors (0.05 μM), to block cholinergic muscarinic receptors. The tissue was mounted isotonically with a preload of 0.5 g (about 5mN) was applied and the tissue was allowed to attain equilibrium for 30 mins before adding any test substances. During an equilibration period tissues were stimulated thrice with histamine (2×1 μM, 1×10 μM) followed by wash-out.

After achieving equilibrium, tissue was exposed to different concentrations of dialysed HK at concentrations of 250 and 125 μg/ml. Dialysate was introduced in organ bath and histamine (1.4 μM) was introduced after 5 minutes of incubation. MP36 (Biopac systems, Inc, USA) was used for data acquisition.

3. Result:

3.1. Percentage Yield and TPC of HK

The % yield (w/w) obtained after extracting HK in 5 different solvent systems are given in Table 1. It was found that HK methanolic extract showed a higher % yield of 5.8±0.81 (w/w) and TPC of 147.68±2.05 mg gallic acid/g HK extract in comparison to other solvent systems.

Table. 1: Represents the %yield (w/w) and TPC of HK

Sl.NO	Name of the Solvent System	Ratio	% Yield (w/w)	TPC as mg gallic acid per gm of HK extract
1	Ethanol	1	2.8 ± 0.29	89.46±1.71
2	Methanol	1	6.7±0.81	147.68±2.05
3	Ethanol + Water	1:1	2.9±0.74	94.67±1.24
4	Methanol + Water	1:1	4.4±0.43	133.07±1.94
5	Water	1	2.8±0.66	39.64±1.07

3.2. LC-qTOF-MS-based metabolic profiling of HK

The metabolites present in the HK methanolic extract were analysed with the help of LC-qTOF-MS in negative mode as shown in Table 2 and positive mode as shown in Table 3. Our analysis revealed that the metabolites detected in the methanolic extract of HK were primarily phenolic acids, flavonoids, non-reducing sugars, fatty acids and organic acids. A list of 34 identified metabolites present in HK has been mentioned in table 2 for negative mode, and about 23 metabolites were identified in positive mode mentioned in Table 3, along with its chromatogram in Figures 1 and 2, respectively. The obtained MS/MS fragmented masses of each metabolite obtained at a collision energy of 10.0, 20.00 and 40.00 eV are also mentioned in table 2 and 3.

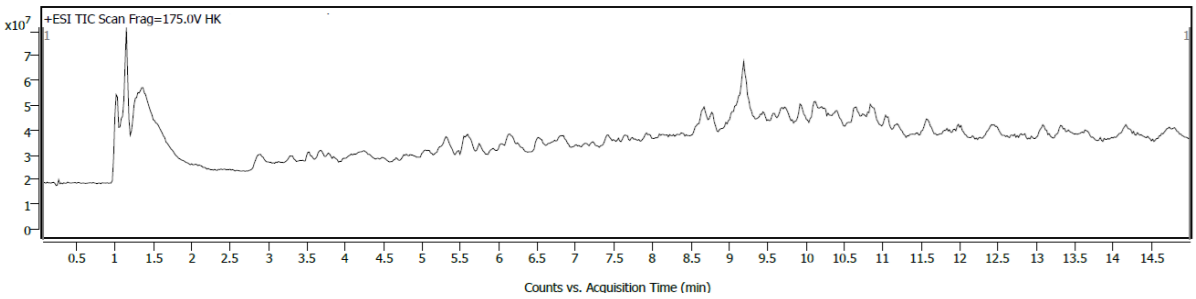


Fig. 1: The figure represents the Total Ion chromatogram (TIC) from the LC-qTOF-MS-based metabolite profiling of HK methanolic extract for negative mode.

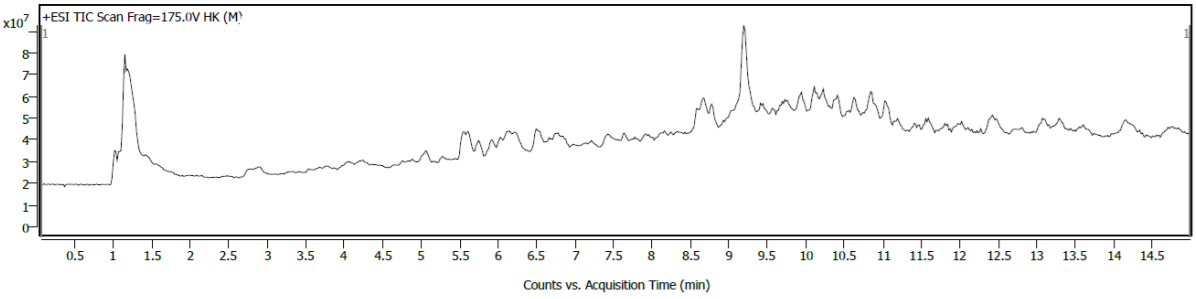


Fig. 2: The figure represents the Total Ion chromatogram (TIC) from the LC-qTOF-MS-based metabolite profiling of HK methanolic extract for positive mode.

Table 2: Represents the LC-qTOF-MS based metabolite profiling of methanolic extract of HK in negative mode.

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
Citric acid	$C_6H_8O_7$	192.0271	1.011	191.0197	143.5324 125.7252 109.8627	171.2448 153.4311 125.7218 109.8624	107.9023
alpha-L-Arabinofuranose	$C_5H_{10}O_5$	150.0524	1.077	149.0455	132.6486 119.7743		
Inositol	$C_6H_{12}O_6$	180.0632	1.127	179.0559	171.1912 159.3686 147.4733 133.6495		131.6466 121.0476
Caffeic acid	$C_9H_8O_4$	180.0427	2.526	179.0351	159.335 133.6476	133.6441	105.9066
Hesperetin 7-O-glucoside	$C_{22}H_{24}O_{11}$	464.1321	4.474	509.1302	330.6464	503.7151 424.6998	389.0616 239.5393 235.6597 207.9021 137.6082 122.7579
Cinnamic acid	$C_9H_8O_2$	148.0522	4.574	193.0503	191.0402 176.1789 132.6478	191.0515 132.6494	131.6012
Hesperetin	$C_{16}H_{14}O_6$	302.0785	4.59	347.0766	300.0012 255.3795 164.3344	343.447 300.0005 272.2614 256.4683 240.4914 213.6674	236.5473 199.9605 191.0033 132.6338 107.9052

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
						191.0039 180.2265 186.2665 123.7216 107.9037	
Chebulinic acid	$C_{41}H_{32}O_{27}$	956.1103	6.272	955.1047	945.0563	945.0596	
1,2,3,4,6-Penta-O-galloyl-beta-D-glucose	$C_{41}H_{32}O_{26}$	940.1162	6.488	939.1092	929.231 761.018	929.2153 761.0274	929.2107 761.0106 505.7179 442.3786 214.7876 202.8823 165.2621
syringic acid	$C_9H_{10}O_5$	198.0525	6.521	197.0453	194.8532 177.0303 167.257 123.727	194.8513 177.0307 123.7314 115.7196	139.5737 113.7199
8-Hydroxyluteolin 4'-methyl ether 8-glucoside	$C_{22}H_{22}O_{12}$	478.1103	6.921	477.1021	472.1387 450.2924 397.0264 341.5191 311.8171 159.3507 129.5869	472.1402 381.993 311.8106 278.1234 159.3544 129.5894	203.912 177.1735 159.3531 133.6469 117.2166
o-Methoxycinnamaldehyde	$C_{10}H_{10}O_2$	162.0672	7.387	161.0598	159.389 144.5231 129.6924 117.8184	159.3908 144.5226 117.8224 102.449	115.8244 101.9895

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
					101.942		
Dehydrozingerone;	$C_{11}H_{12}O_3$	192.0784	7.52	191.0713	189.0964 174.2178 169.1805 131.6871 103.9598	169.1826 157.2883 113.8256	119.6957
Cosmosiin	$C_{21}H_{20}O_{10}$	432.1058	7.72	431.0982	426.7169 383.0774 337.499 325.7363 258.4205 185.151	426.4677 394.8685 266.2293 249.3124 185.1533 169.327 141.6243 115.7204	338.532 275.5017 265.2393 226.6596 185.1477 137.8329 123.9829
Rhamnetin 3-rhamnosyl-(1->4)-rhamnoside	$C_{28}H_{32}O_{15}$	608.1735	7.753	607.1658	604.025 600.7973 481.9538 395.7593 325.5439 295.9341 182.1591 167.2379	600.7859 515.0711 333.5879 295.9371 281.0639 185.9309	316.7394 298.4153 295.9374 293.373 282.0762 281.058
(-)-Secoisolariciresinol	$C_{20}H_{26}O_6$	362.1716	7.803	361.1647	357.3527 321.8025 313.8256 234.4996 101.976	357.3466 294.8395 240.6046 101.9714	311.6828 122.73
(+)-Dihydrokaempferol	$C_{15}H_{12}O_6$	288.0638	8.02	287.0565	284.1948	229.0987	133.6463

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
					240.6024 222.8411 173.1493 125.6981	222.8416 181.1064	127.7325 113.8898
Homoplantaginin	$C_{22}H_{22}O_{11}$	462.1161	8.136	461.1088	456.2818 438.4621 420.6447 396.8868 309.7862 286.1193 250.3982 222.6941 208.8306 187.0899 167.2596 159.3889 149.437 133.6453 121.735 101.9938	456.2818 438.4621 420.6447 396.8868 309.7862 286.1193 250.3982 222.6941 208.8306 187.0899 167.2596 159.3889 149.437 133.6453 121.735 101.9938	450.2528 250.3983 224.6994 198.9585 181.1251 167.2604 159.3919 145.5208 135.6044 121.7364 105.9083
Calebin A	$C_{21}H_{20}O_{7}$	384.1207	8.469	383.1132	379.1092 364.2285 347.4163 244.4879 258.352 147.5137	379.1008 364.2351 258.356 244.4865 229.6215 185.0922	277.1552 229.9189 185.0959 173.2207 148.4766 132.648
Naringenin	$C_{15}H_{12}O_{5}$	272.069	8.636	271.0617	268.2382 250.426 206.8531	268.233 250.4309 240.4643	154.5222 141.5293 137.4807

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
					170.4056 143.5888	181.1079 159.4412	133.559
[6]-Dehydroshogaol	$C_{17}H_{22}O_3$	274.1563	9.202	333.1702	329.6881 242.6374 228.5773 122.7346 107.8638	311.8639 286.1863 268.331 257.387 252.4452 169.2414	193.0389 117.2434
Apigenin	$C_{15}H_{10}O_5$	270.0526	9.285	269.0452	266.4007 248.5487 222.8405 198.9437 153.2918 141.5686 100.0197	248.416 181.1083	251.4307 108.9494
6-Gingerol	$C_{17}H_{26}O_4$	294.182	9.601	293.1747	290.1758 246.588 233.6386 217.8539 205.4455 185.1305 159.3906 141.5644	289.9257 276.3654 244.5923 240.4539 233.6421 220.1865 204.9464 196.4488 185.1295 159.3905 144.5165 129.7156 117.8166	194.9778 183.1384 175.2514 157.3984 141.565 130.6604 115.8238

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
3-Hydroxy-1,7-bis(4-hydroxyphenyl)-6-heptene-1,5-dione	$C_{19}H_{18}O_5$	326.1151	9.685	325.1077	321.714 306.84552 216.8228 187.128 133.6459	715.9352 330.5835 111.8143	305.8652 246.4663 236.581 218.7595 181.1106
1-(4-Hydroxy-3-methoxyphenyl)-7-octen-3-one	$C_{15}H_{20}O_3$	248.1408	9.718	247.1336	244.44 147.8154 133.6848 109.898 106.1177	187.1224 172.262 133.6858 109.8983	131.69 118.8181
3,4-Dimethyl styrene	$C_{10}H_{12}$	132.0935	9.768	191.1074	189.1209 131.6828 105.9484	186.0286 105.9439	112.7531
Germacr-4-en-12-oic acid, 6-alpha-hydroxy-, gamma-lactone	$C_{15}H_{24}O_3$	252.1727	9.868	251.1656	248.527 204.9947 177.2457		
Dihydrocurcumin	$C_{21}H_{22}O_6$	370.1411	10.517	369.1338	365.2674 215.7852 201.9107 172.2572 156.3968 147.5152 132.6487	365.2605 316.2876 216.7764 200.919 171.2638 156.3986 147.5164 132.649 105.9462	199.9213 175.2095 156.3978 147.5159 132.6501 112.8698 100.9946
4'-Methoxyacetophenone	$C_9H_{10}O_2$	150.0679	10.65	149.0606	132.6479 103.8753		
bis-(4-hydroxycinnamoyl)methane	$C_{19}H_{16}O_4$	308.1049	10.65	307.0976	303.8961 260.4555	303.704 258.4579	175.2602 156.3929

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
					210.8398 158.0962 159.352 141.5681 117.819	242.598 185.0941 141.5679 117.8202	141.5671 134.6014 117.8159 103.9809
3,12-Dihydroxyhexadecanoic acid	$C_{16}H_{32}O_4$	288.2297	10.85	287.2225	284.2258 238.7019 153.5351 111.8936	284.2256 238.7029 209.0029 181.2416 163.4166 153.5358 139.5929 113.8869	183.0791 139.5475
(5S,6R)-5-Hydroxy-2-methyl-6-(4-methylphenyl)-2-hepten-4-one	$C_{15}H_{20}O_2$	232.1461	10.95	231.1388	198.7683 185.0817 162.2267 116.8224	210.7357	
Curcumanolide B	$C_{15}H_{22}O_2$	234.1609	13.514	233.1543	230.7247 161.4208	212.6725 161.4199	131.6889

Table 3: Represents the LC-qTOF-MS based metabolite profiling of methanolic extract of HK in Positive mode.

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
Punicalagin	$C_{48}H_{28}O_{30}$	1084.065	3.281	1102.0983	765.0603 621.0202	765.0548 621.0072 603.006	621.0101 602.9872 557.0145
Methyl 2-(methylamino)benzoate	$C_9H_{11}NO_2$	165.0795	3.581	166.0868	149.9905 131.0443 120.0809 103.0576	151.1001 120.0806 103.052	
Cinnamic acid	$C_9H_8O_2$	148.0529	3.581	166.0868	149.9905 131.0443 120.0809 130.0576	151.1001 103.052	103.0534
Casuarinin	$C_{41}H_{28}O_{26}$	936.0854	4.647	954.1186	937.097 919.092	767.0619 599.0457 484.7271 435.045 345.0215 255.0336	369.046 345.0243 277.0198 154.0168
Corilagin	$C_{27}H_{22}O_{18}$	634.0799	4.896	652.1136	635.0923 617.0745 405.0524 153.0155	635.0932 429.0422 387.0332 303.0118 277.031 153.0173	387.0294 303.0093 277.0332 268.99 206.9388 153.0166
1,6-Di-O-galloyl-beta-glucose	$C_{20}H_{20}O_{14}$	484.085	5.063	507.0744	507.075 477.4528 449.6337	394.0316 337.054 295.0267	395.9962 153.0173

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
					405.0222 337.0537	237.0774 153.0128	
Piperine	$C_{17}H_{19}NO_3$	285.1363	5.462	286.1439	143.0492 135.044 171.0439	135.044 201.0545 115.0546	115.0546 135.044
Chebulagic acid	$C_{41}H_{30}O_{27}$	954.096	6.128	977.0856	938.0796 803.0569 785.0861	807.0682 787.0931 487.0486 132.0169	807.0652 786.0642 505.1828 487.0533 403.0202 361.0156 267.0473 249.0454 153.0173
Chebulinic acid	$C_{41}H_{32}O_{27}$	956.1116	6.828	979.1008	903.0475 894.0778 809.0642	903.5558 809.0779 657.0698. 571.0871 487.0609 471.0436 360.994 301.0438 153.0156	829.0301 829.0751 787.0925 657.0736 639.0631 571.5598 487.0433 471.0375 453.0501 437.042 425.0553 379.0304 361.0175 315.0099 301.0361

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
							283.0174 242.9843 153.0177 127.0383
Quercetin-3-O-glucoside (Isoquercetin)	$C_{21}H_{20}O_{12}$	464.0966	7.427	487.0849	469.0902 440.7768 422.9308 317.981 290.1059 205.0793 163.04	429.1185 379.1202 351.0528 303.0043 175.0385 146.0571 109.0615	377.0639 370.9812 362.9176 315.0424 187.0595 177.1004 146.063 131.0457 103.0975
Rhamnetin 3-rhamnosyl-(1-4)-rhamnoside	$C_{28}H_{32}O_{15}$	608.1736	7.777	631.1625	415.1614 405.218 207.064	291.1447 250.0472	550.1012 415.0349 379.0639 309.0957 239.0263 153.0182
3,4,5-Trimethoxydihydrocinnamic acid	$C_{12}H_{16}O_5$	240.1	7.826	241.107	223.0974 181.0856 166.0631 136.0528 109.0655	166.0632 148.0525 137.0627	181.0832 165.051 148.052 137.061 123.0438 107.049
Homoplantaginin	$C_{22}H_{22}O_{11}$	462.1163	8.143	485.1052	467.1454 315.0836 257.1124	453.0977 315.0846 297.0766 193.0072 163.037	448.1738 302.1864 251.125 184.1679 131.0456

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
						147.0437 102.0903	123.1149 102.0923
7-Methoxycoumarin	$C_{10}H_8O_3$	176.0471	9.575	177.0543	156.0249 145.028 130.9962 119.085 107.0845	149.0585 134.0359 117.0344 105.0354	115.0477 106.0417
Tridecanoic acid	$C_{13}H_{26}O_2$	214.1924	10.307	237.1821	177.9556 163.1134 149.0241 121.0994 111.0825	205.1774 121.1004 105.0693	105.0679
Demethoxycurcumin	$C_{20}H_{18}O_5$	338.1153	10.757	339.1226	321.2498 303.1024 281.1356 275.0462 256.3317 245.101 223.0647 177.0543 147.0322 121.0982 117.0326	307.0778 293.1571 255.0941 209.8442 177.0509 145.027 117.0342	245.0444 233.0856 177.0519 147.0395 119.0484
Curcumin	$C_{21}H_{20}O_6$	368.38	10.817	391.112	368.572 350.849	177.999 190.864	191.537
Palmitic acid	$C_{16}H_{32}O_2$	256.2399	11.406	274.2738	201.0571 106.0825	212.9282 175.0753 121.1007	277.1186 215.0542 174.0182 128.1422

Name of the molecules	Formula of the Compound	Mass	RT	m/z	10 V	20 V	40 V
1-[7-(1,3-Benzodioxol-5-yl)-1-oxo-6-heptenyl]pyrrolidine	$C_{18}H_{23}NO_3$	301.1674	11.789	302.1744	284.0359 261.0471 220.8209 202.1816 123.1156 102.0905	225.9266 193.0191 123.1123 102.0868	167.8781 102.0908
2-Pentadecanone	$C_{15}H_{30}O$	226.229	12.888	244.2629	226.9076 172.1304 153.1373 140.1426 126.0862	212.0725 185.0488	200.1379 180.8969
Gingerenone A	$C_{21}H_{24}O_5$	356.1622	13.287	379.1514	227.1044	311.1514 265.9416	174.0624
N-Isobutyldeca-trans-2-trans-4-dienamide	$C_{14}H_{25}NO$	223.1933	14.136	224.2006	192.1015 150.0271	164.9271 150.1301 106.0665	148.0765
[12]-Gingerdione	$C_{23}H_{36}O_4$	376.2604	14.186	394.2952	356.8552 212.9155 188.1024	359.2472 238.133	190.9438 115.0578

3.3. Ligand-Receptor Interaction analysis of bioactive metabolites in HK

It is widely acknowledged that diminished binding between small molecule ligands and receptors correlates with elevated LibDock scores, signifying enhanced interactions and more potential activity of the components(3). The docking outcomes were assessed utilizing the LibDock score as the selection criterion. The LibDock scores for H1R (3RZE) and FcεR (2Y7Q) and the five most effective active components in HK are illustrated in Figures 1 and 2, respectively. The docking data indicate that the LibDock score of Curcumin in positive ionization and Dihydrocurcumin in negative ionization with H1R was markedly superior to those of other active compounds. The interacting amino acids of the macromolecules, together with their interactions such as conventional hydrogen bonds and hydrophobic interactions, are presented below. In addition to [12]-Gingerdione, Gingerenone A, 1-[7-(1,3-Benzodioxol-5-yl)-1-oxo-6-heptenyl] pyrrolidine and Demethoxycurcumin, in positive ionization and 3-Hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione, Hesperetin, bis-(4-hydroxycinnamoyl) methane and (+)-Dihydrokaempferol, in negative ionization, were identified as the most stable molecules among the phytoconstituents derived from HK. However, in case of FcεR, Gingerenone A in positive ionization and 1,2,3,4,6-Penta-O-galloyl-beta-D-glucose, in negative ionization, had superior stability and exhibited a more favorable Libdock score compared to other components. Regarding the other macromolecule, FcεR, in addition to Homoplantaginin, curcumin, [12]-Gingerdione and Demethoxycurcumin, in positive ionization and Hesperetin 7-O-glucoside, Homoplantaginin and Cosmosiin, Dihydrocurcumin, in negative ionization have notable docking scores relative to other chemical constituents. The docking outcomes are presented in Tables 4 and 5.

Table 4: Docking analysis of the most active components with H1R (3RZE)

Positive Ionization			
Phyto- Compound	**Lib Dock score**	**Hydrogen Bond**	**Hydrophobic Interaction**
Curcumin	153.558	THR A:112, ASN A:198	PHE A:424, ILE A:115, PHE A:432 (2), TRP A:428, LYS A:179, ILE A:454, MET A:451, HIS

Positive Ionization			
Phyto- Compound	**Lib Dock score**	**Hydrogen Bond**	**Hydrophobic Interaction**
			A:450, PHE A:199
[12]-Gingerdione	146.526	ASP A:178	TRP A:158, PHE A:432(2), TYR A:108, PHE A:435, TYR A:431, ILE A:454(2)
Gingerenone-A	141.753	HIS A:450, LYS A:191, TYR A:431	ILE A:454(2), TYR A:458, TRP A:428, PHE A:432(2), LYS A:179, PHE A:435(2), TYR A:108
1-[7-(1,3-Benzodioxol-5-yl)-1-oxo-6-heptenyl] pyrrolidine	139.328	-	PHE A:424, PHE A:199, ILE A:115, PHE A:432(2), SER A:111, TRP A:428(3), TYR A:431, HIS A:450, ILE A:454, LYS A:179
Demethoxycurcumin	132.199	-	PHE A:435, PHE A:432 (2), ALA A:195, HIS A:450, LYS A:179, ILE A:454

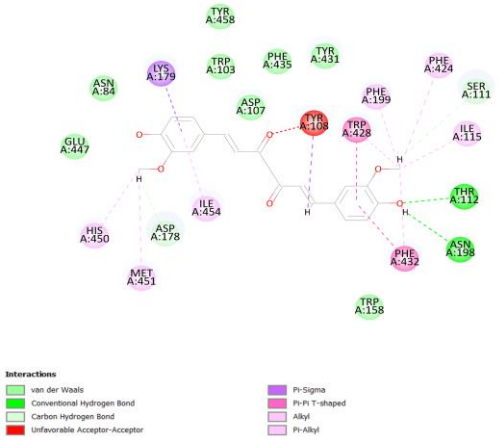
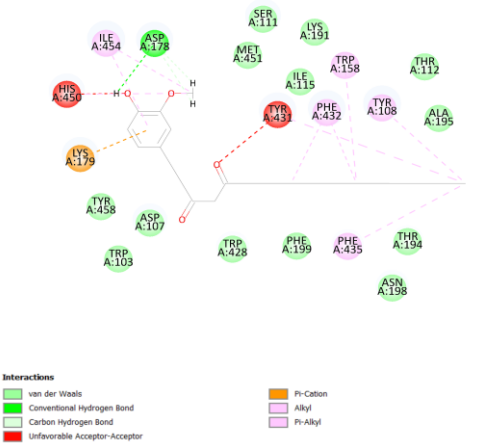
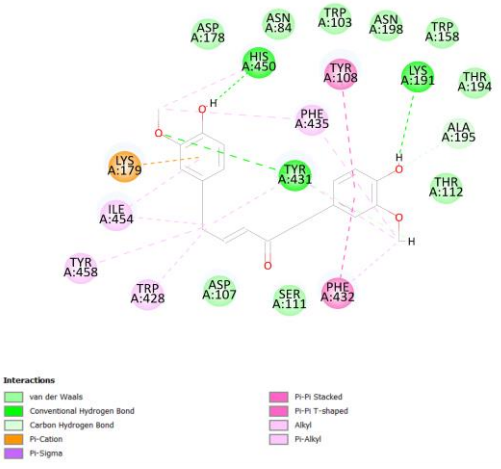
Negative Ionization			
Phyto- Compound	**Lib Dock score**	**Hydrogen Bond**	**Hydrophobic Interaction**
Dihydrocurcumin	141.706	THR A:112	ILE A:454, LYS A:179, PHE A:184, PHE A:453, LYS A:191, TRP A:158, TYR A:108(3)
3-Hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione	140.987	THR A:194	ILE A:454, ASP A:107, TYE A:108
Hesperetin	139.085	THR A:112	ILE A:115, SER A:111, PHE A:432, TRP A:428, LYS A:179, ILE A:454, ASP A:107, TYR A:108
bis-(4-hydroxycinnamoyl) methane	139.01	THR A:112, TYR A:431, THR A:194	TYR A:108, ALA A:195, TYR A:431
(+)-Dihydrokaempferol	137.39	-	PHE A:435, TRP A:428, ILE A:115

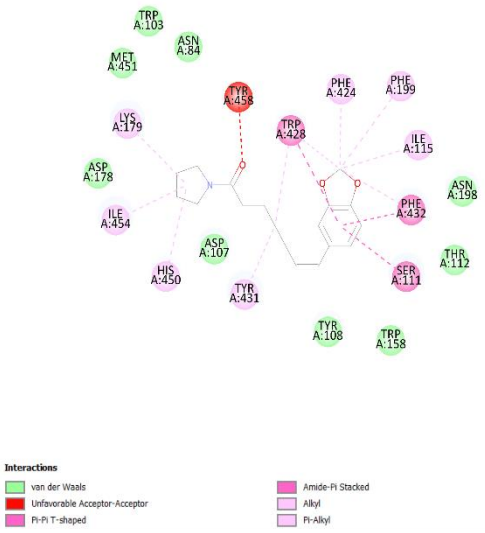
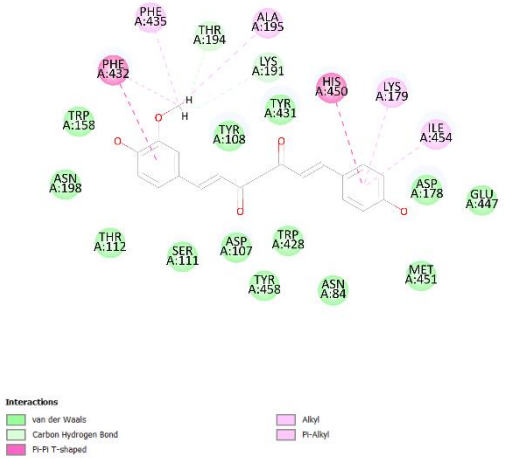
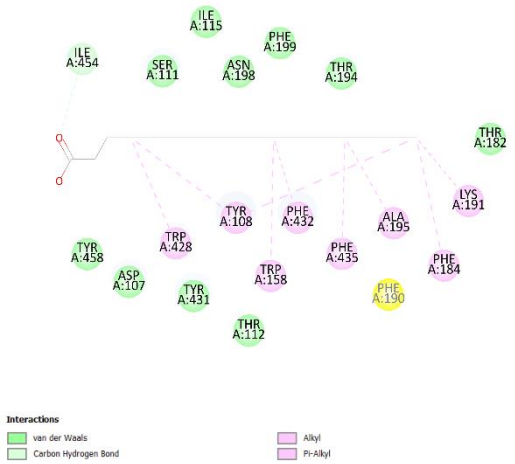
Table 5: Docking analysis of the most active components with FcεR (2Y7Q)

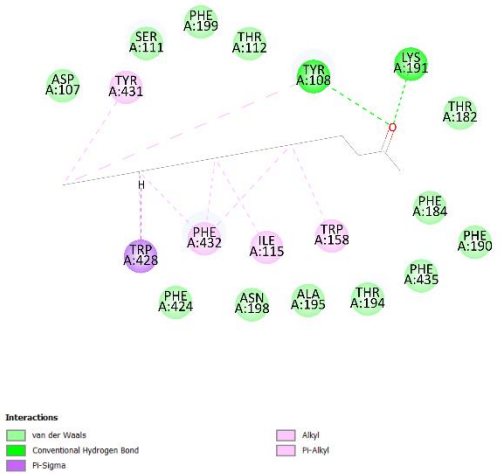
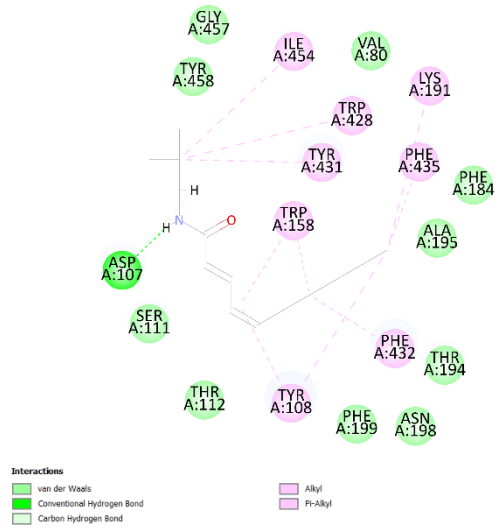
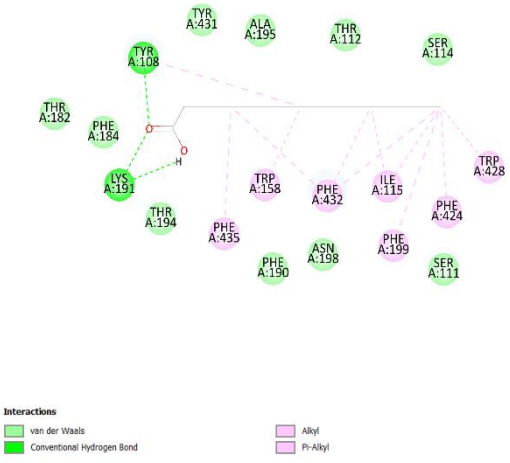
Positive Ionization			
Phyto- Compound	**Lib Dock score**	**Hydrogen Bond**	**Hydrophobic Interaction**
Gingerenone A	112.96	-	ILE A:119, TYR A:129, LYS A:117(3), LYS A:154(2), TYR A:116
Homoplantaginin	108.293	LYS A:117	LYS A:145(2), LYS A:117 (2), TYR A:116, ARG B:334
curcumin	107.761	ARG B:334, GLU A:132	ARG B:334, TYR A:116, LYS A:117(3), LYS A:154(2),
[12]-Gingerdione	107.013	ARG B:334, LYS A:117	ILE A:119, TYR A:129, LYS A:117(2), LYS A:154, TYR A:116(2)
Demethoxycurcumin	100.324	GLU A:132	LYS A:117(2), TYR A:129, ILE A:119, TYR A:116

Negative Ionization			
Phyto-Compound	**Lib Dock score**	**Hydrogen Bond**	**Hydrophobic Interaction**
1,2,3,4,6-Penta-O-galloyl-beta-D-glucose	141.569	TYR A:129, GLY B:395, ASP A:123	LEU A:127(2), LYS A:122, LYS A:128, PRO B:365, ASP A:145
Hesperetin 7-O-glucoside	113.225	ARG B:334, LYS A;117, ASN B:394, ASP B:362, ASP A:159	LYS A;117(3), TYR A:11692), LYS A:154
Homoplantaginin	108.293	LYS A:117	LYS A:117(2), LYS A:154(2), TYR A:116, ARG B:334
Cosmosiin	107.664	LYS A:117, ASP B:362	LYS A:154(2), LYS A:117(3), TYR A:116, ARG B:334
Dihydrocurcumin	107.595	TYR A:129, GLY B:395, ASP A:123	LEU A:127(2), LYS A:128, PRO B:365, LYS A:122

Table 6: 2D docking representation of H1R (3RZE) with tentative phytomolecules present in HK positive ionisation

Phytocompound	
curcumin	
[12]-Gingerdione	
Gingerenone A	

1-[7-(1,3-Benzodioxol-5-yl)-1-oxo-6-heptenyl]pyrrolidine	
Demethoxycurcumin	
Palmitic acid	

2-Pentadecanone	
N-Isobutyldeca-trans-2-trans-4-dienamide	
Tridecanoic acid	

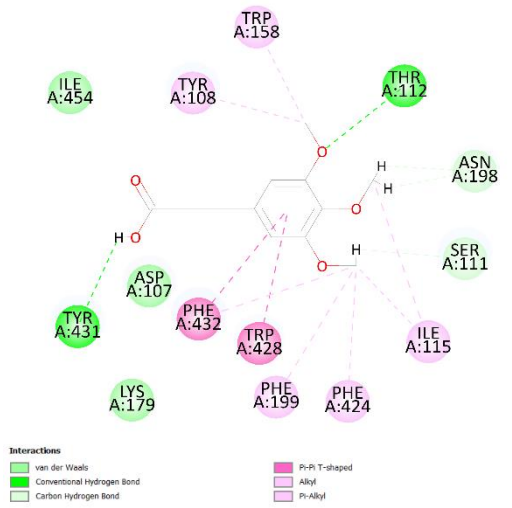
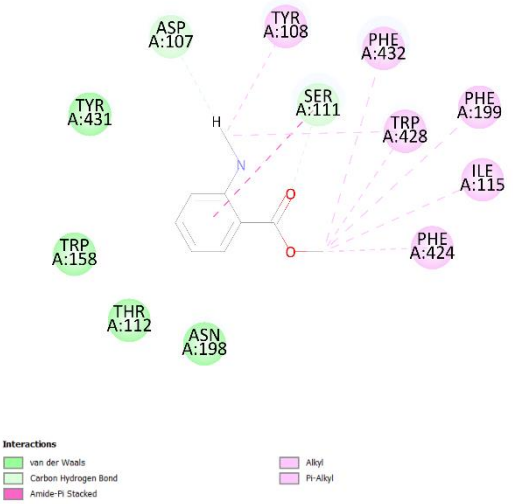
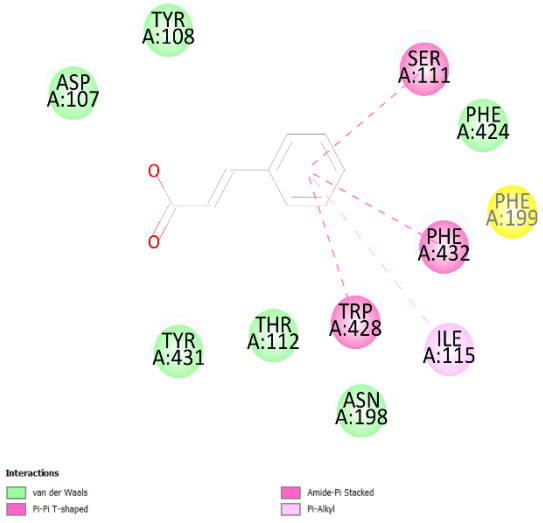
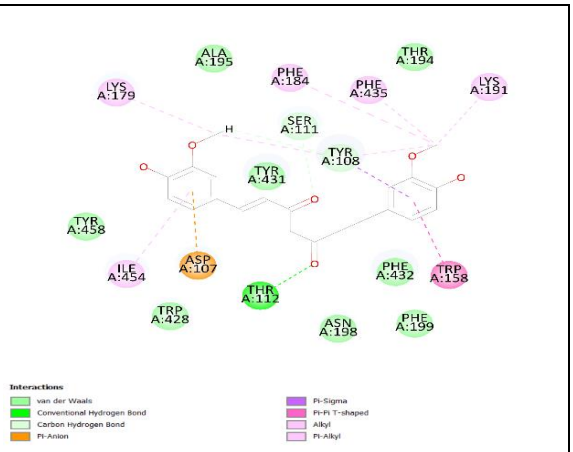
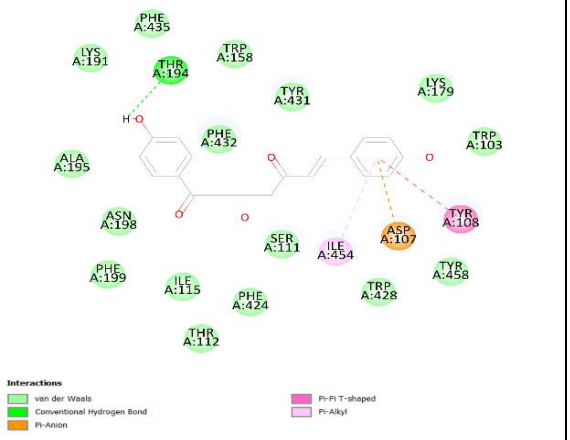
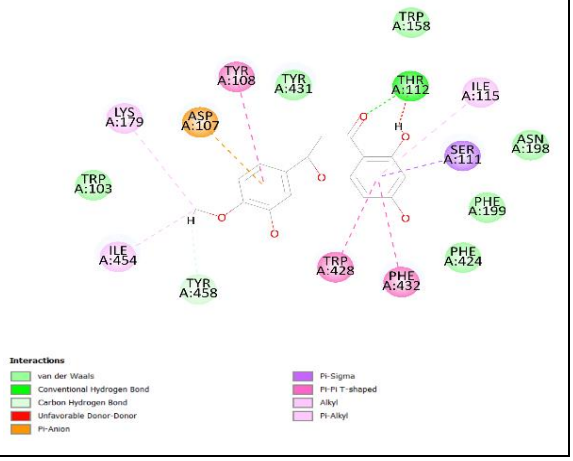
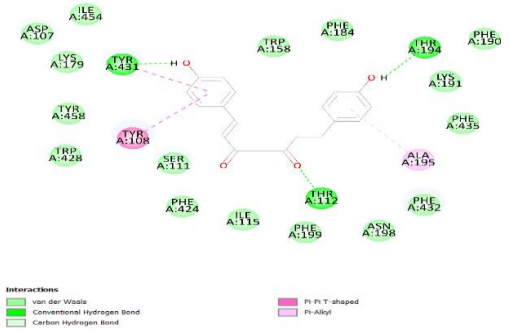
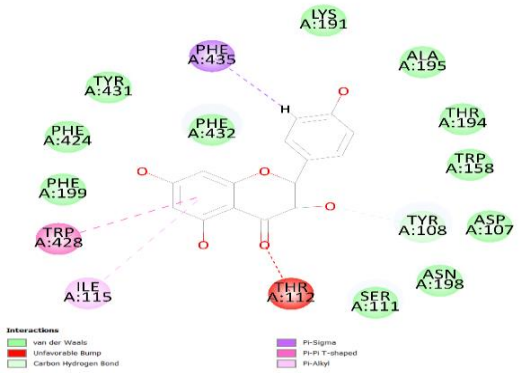
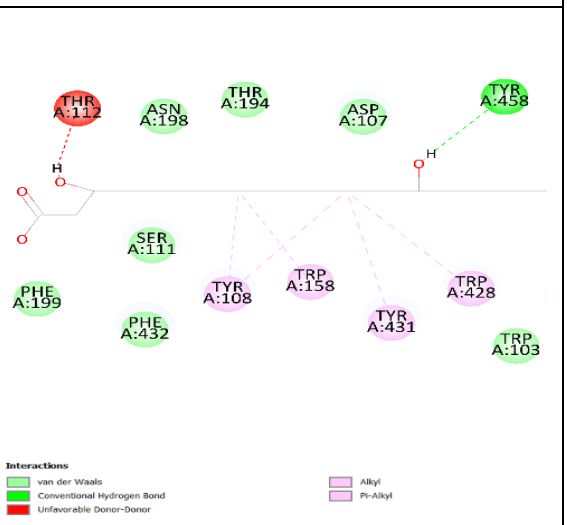
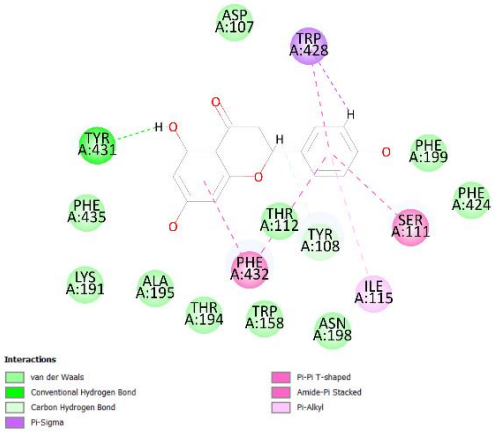
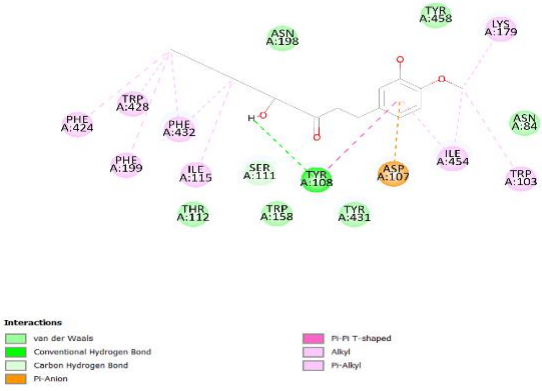
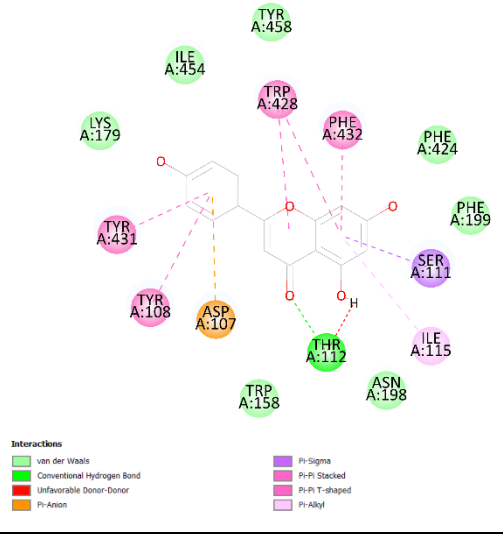
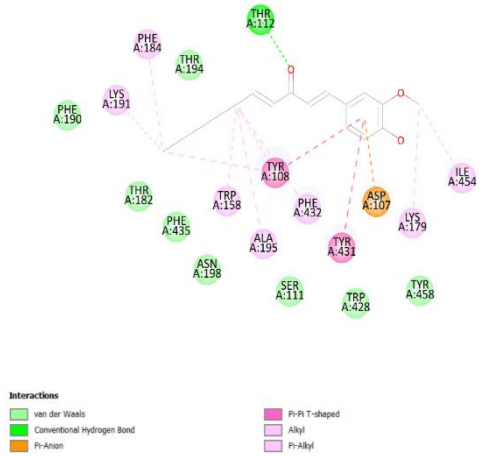
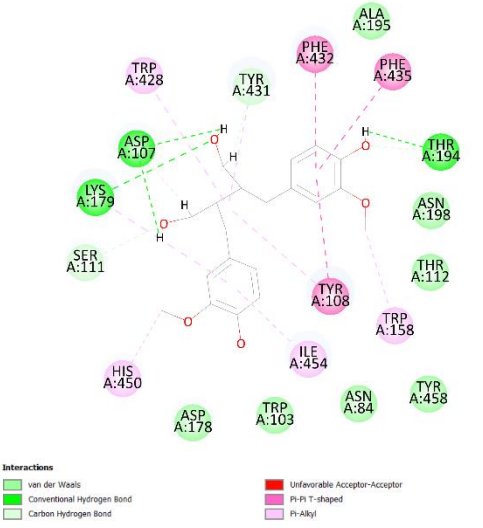
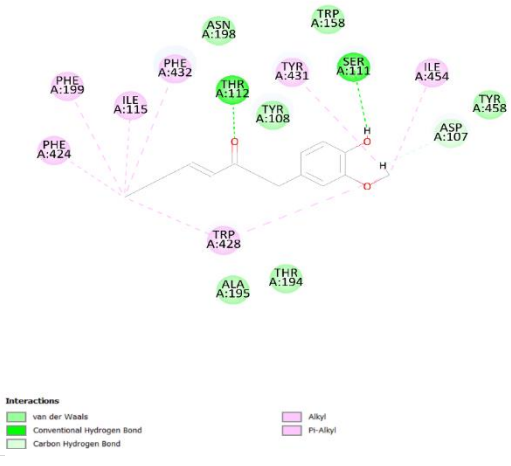
3,4,5-Trimethoxydihydrocinnamic acid	
Methyl 2-(methylamino)benzoate	
Cinnamic acid	

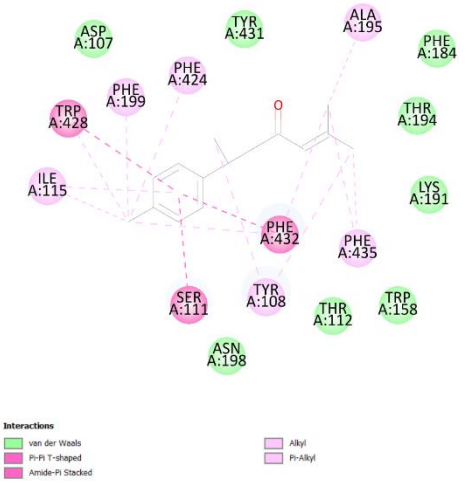
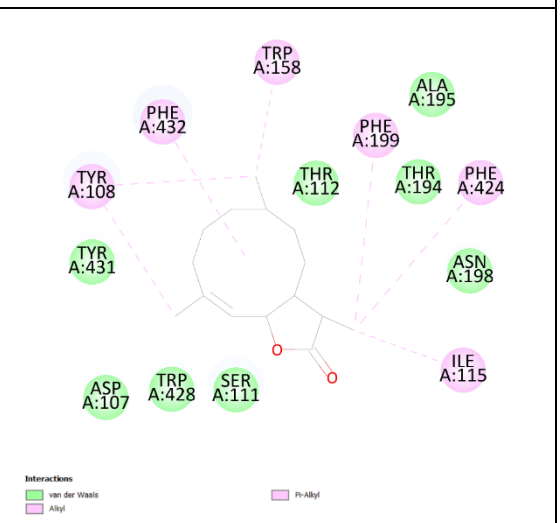
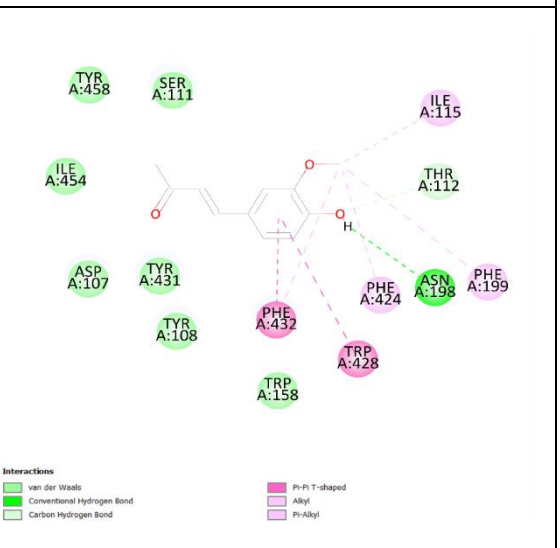
Table 7: 2D docking representation of H1R (3RZE) with tentative phytomolecules present in HK negative ionisation

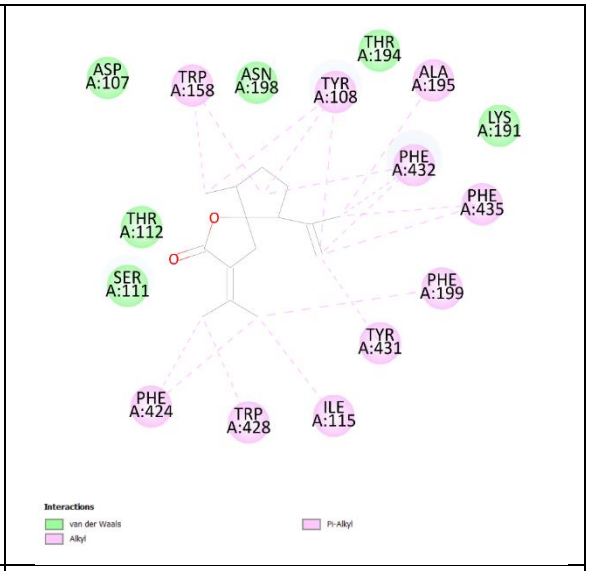
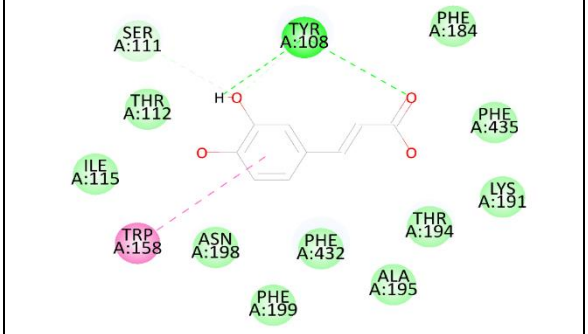
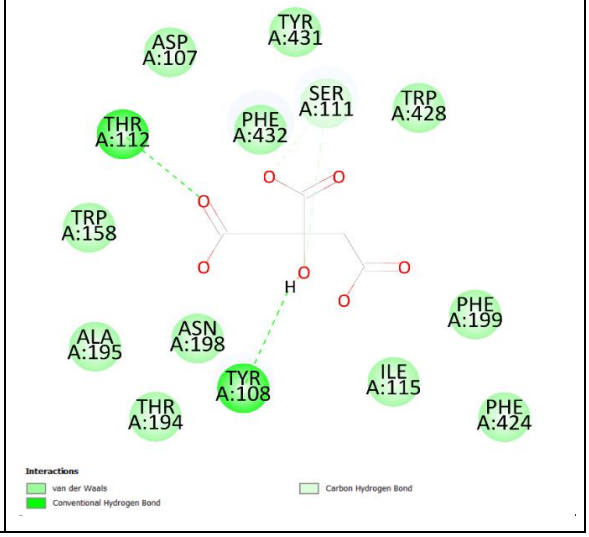
Dihydrocurcumin	
3-Hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione	
Hesperetin	

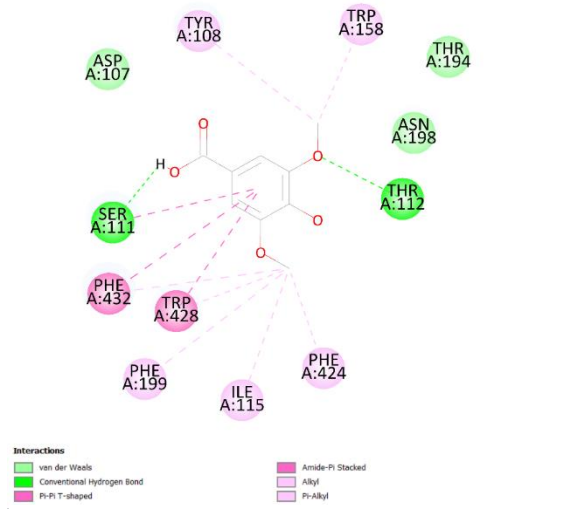
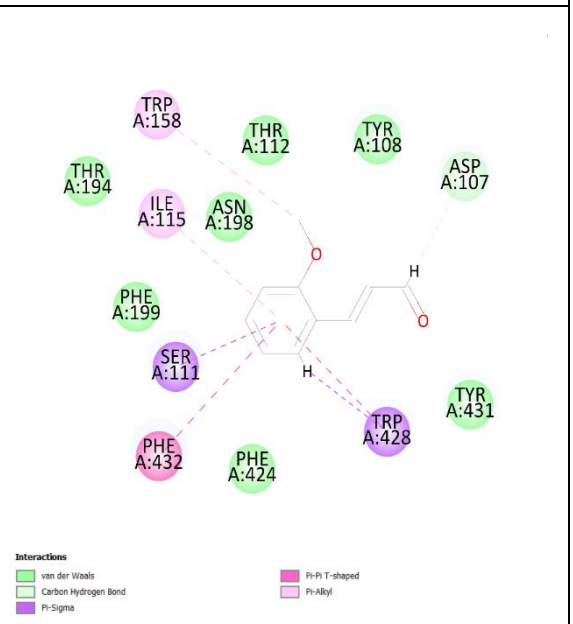
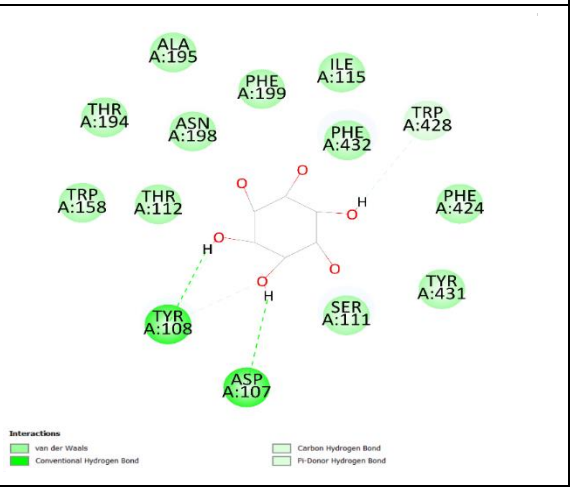
bis-(4-hydroxycinnamoyl)methane	
(+)-Dihydrokaempferol	
3,12-Dihydroxyhexadecanoic acid	

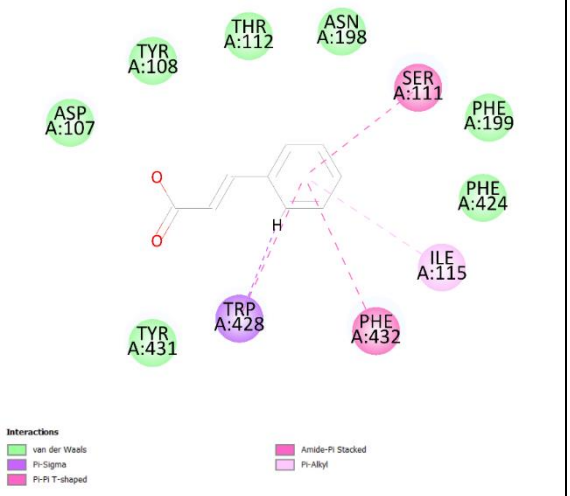
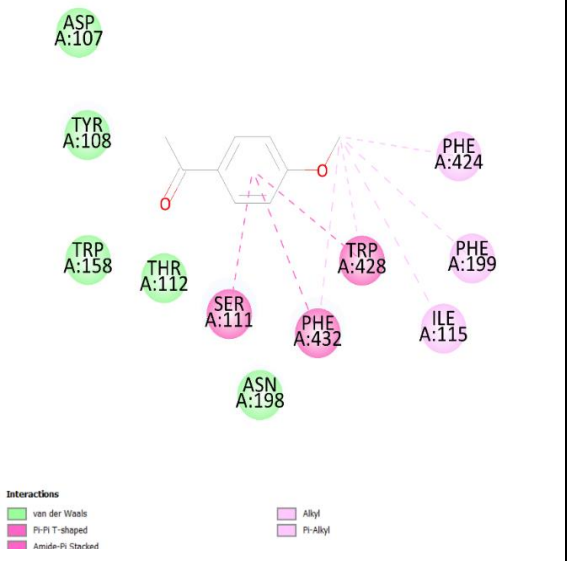
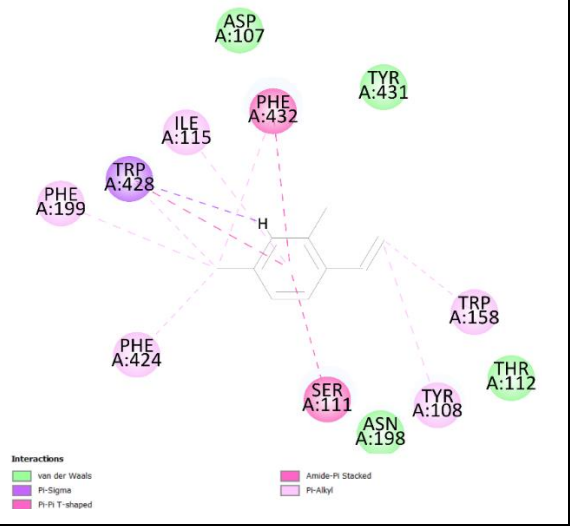
Naringenin	
(S)-6-Gingerol	
Apigenin	

[6]-Dehydroshogaol	
(-)-Secoisolariciresinol	
1-(4-Hydroxy-3-methoxyphenyl)oct-4-en-3-one	

2-Methyl-6-(4-methylphenyl)hept-2-en-4-one	
Germacr-4-en-12-oic acid, 6-alpha-hydroxy-, gamma-lactone	
Dehydrozingerone	

Curcumanolide B	
Caffeic acid	
Citric acid	

syringic acid	
o-Methoxycinnamaldehyde	
Inositol	

Cinnamic acid	
4'-Methoxyacetophenone	
3,4-dimethyl styrene	

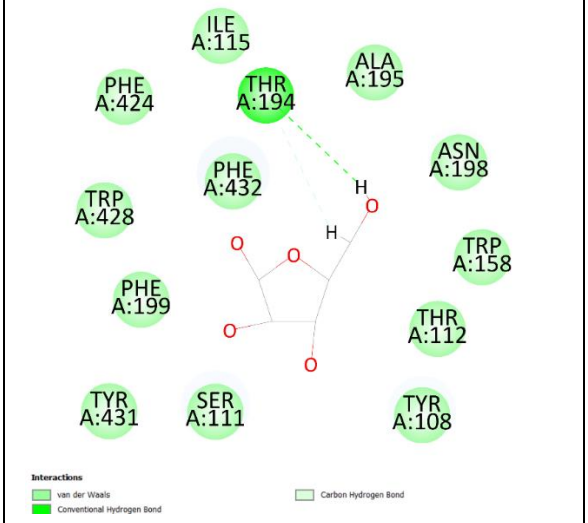
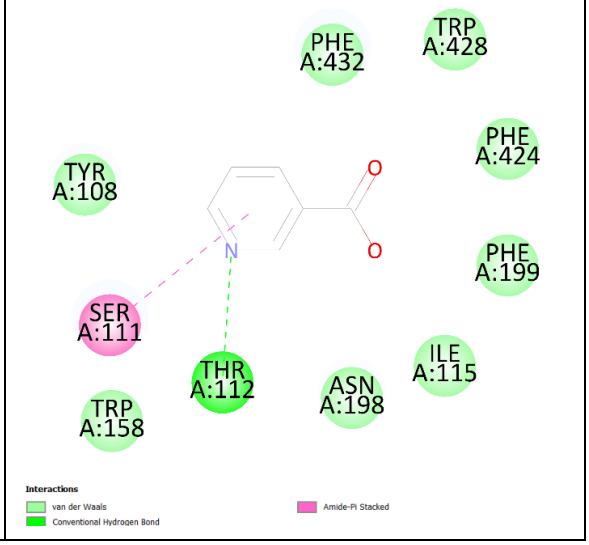
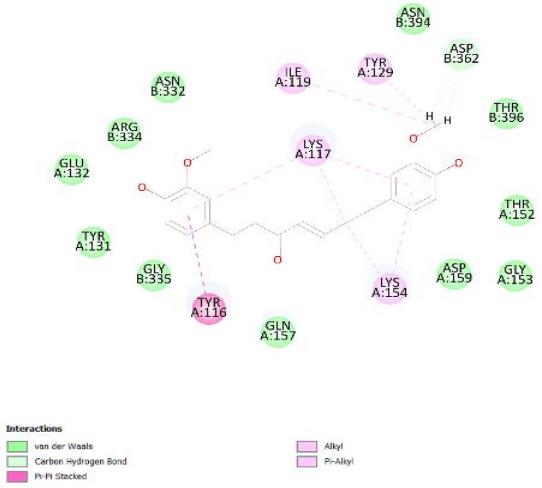
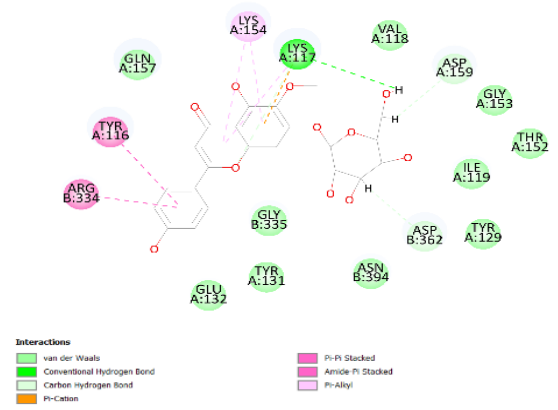
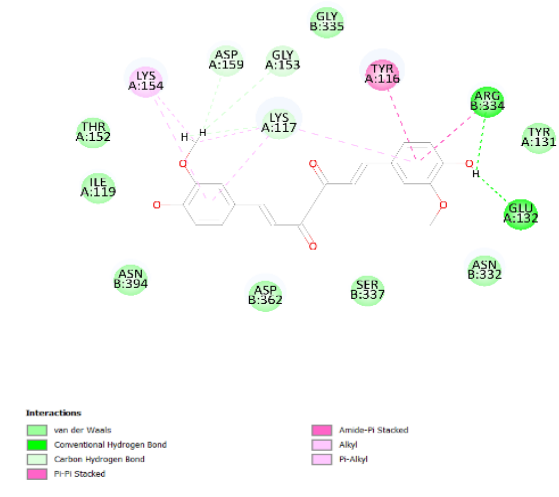
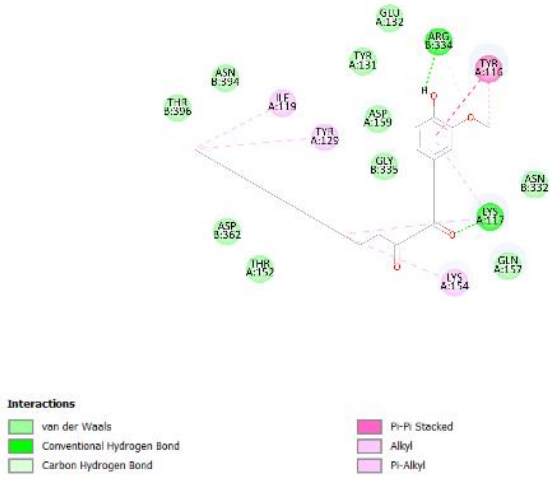
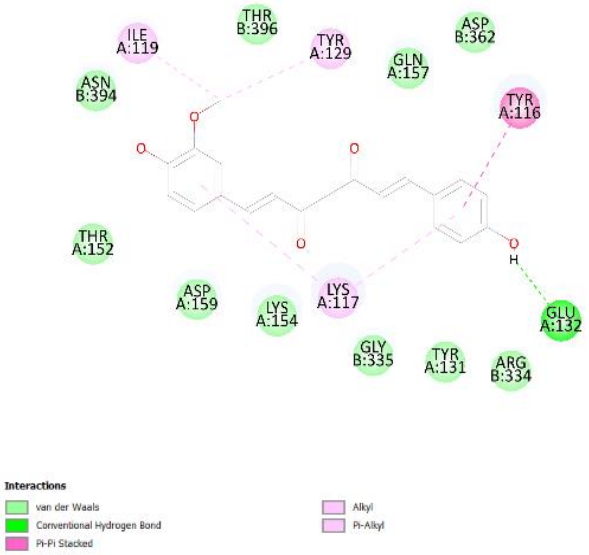
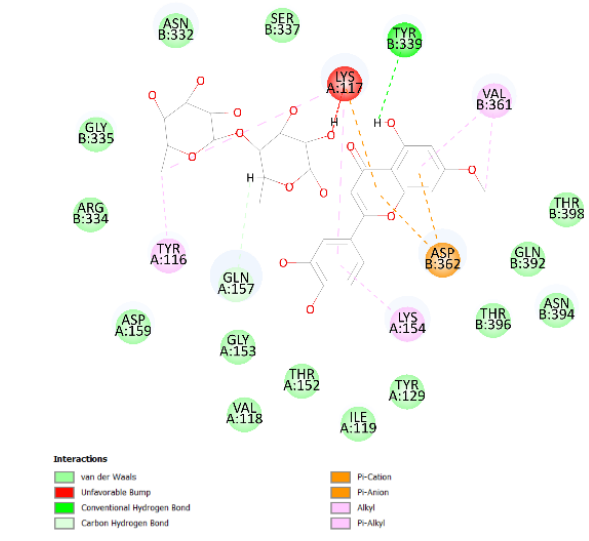
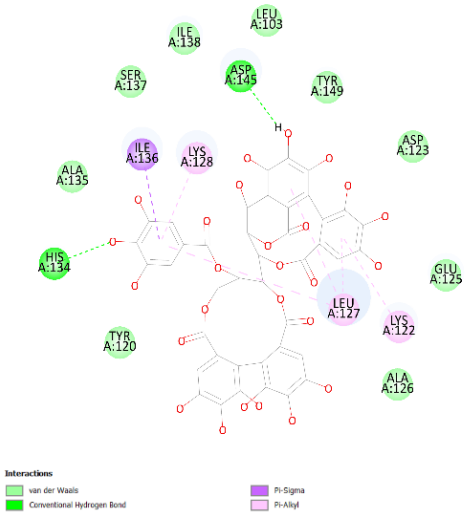
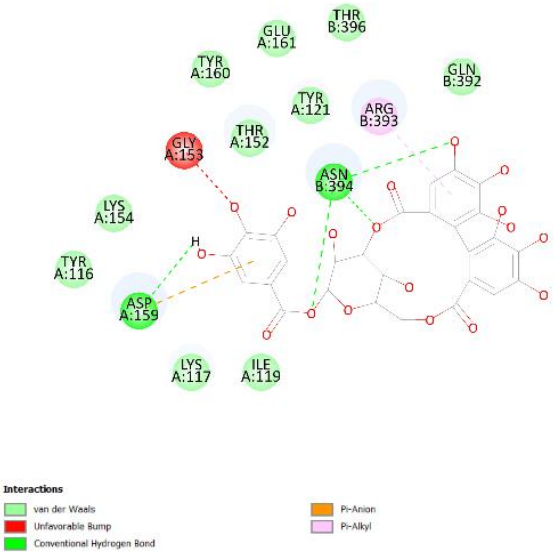
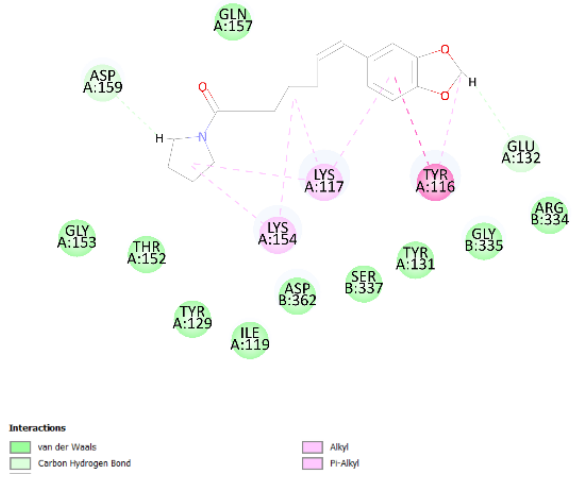
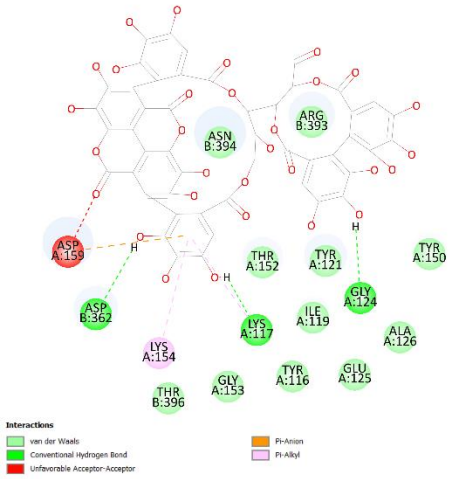
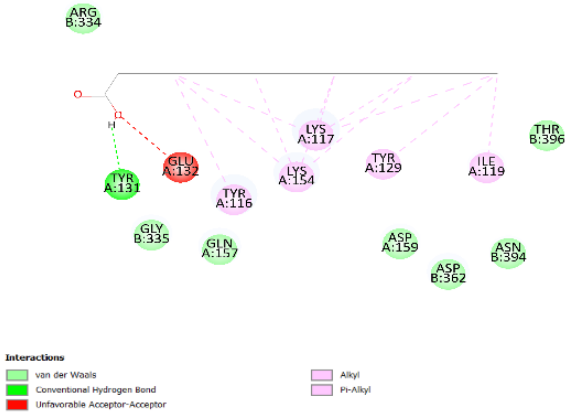
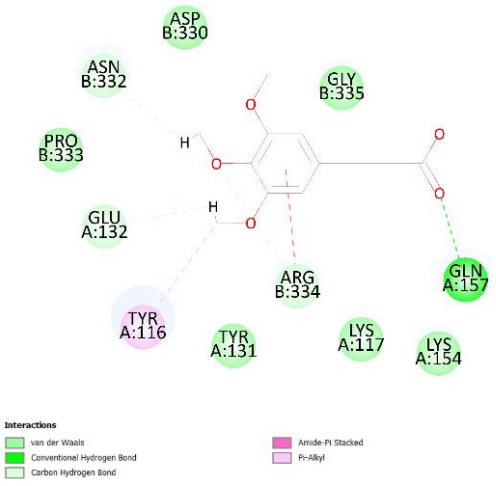
alpha-L-Arabinofuranose	
Nicotinic acid	

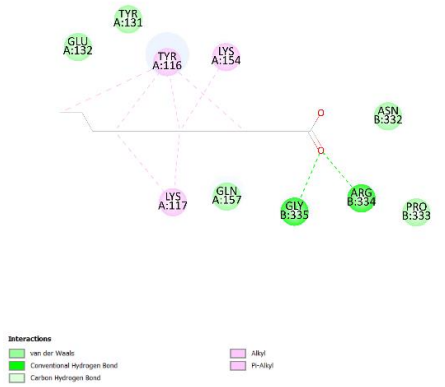
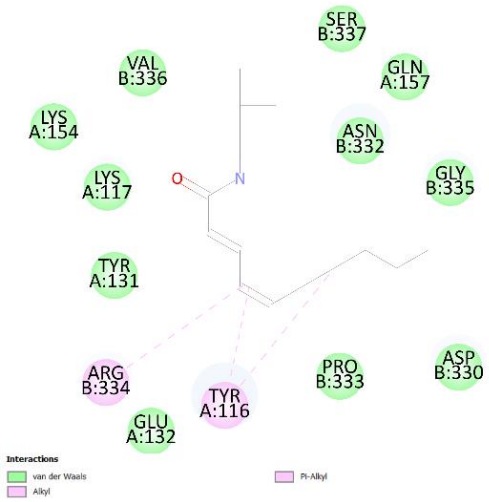
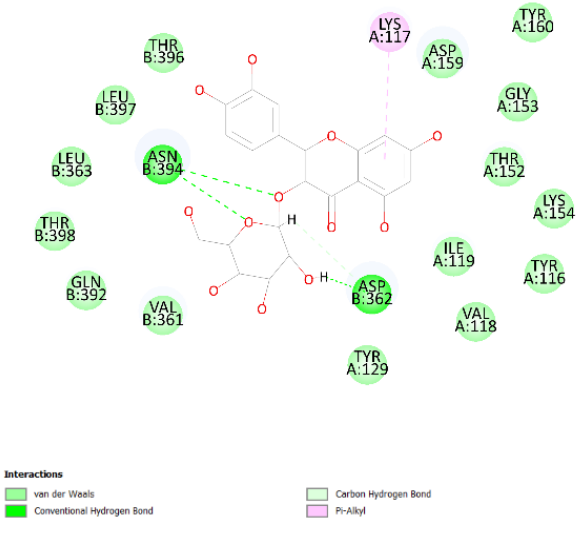
Table 8: 2D docking representation of FcεR (2Y7Q) with tentative phytomolecules present in HK positive ionisation

Gingerenone A	
Homoplantaginin	
curcumin	

[12]-Gingerdione	
Demethoxycurcumin	
Rhamnetin 3-rhamnosyl-(1-4)-rhamnoside	

Casuarinin	
Corilagin	
1-[7-(1,3-Benzodioxol-5-yl)-1-oxo-6-heptenyl]pyrrolidine	

Punicalagin	
Palmitic acid	
3,4,5-Trimethoxydihydrocinnamic acid	

Tridecanoic acid	
N-Isobutyldeca-trans-2-trans-4-dienamide	
Quercetin-3-O-glucoside (Isoquercetin)	

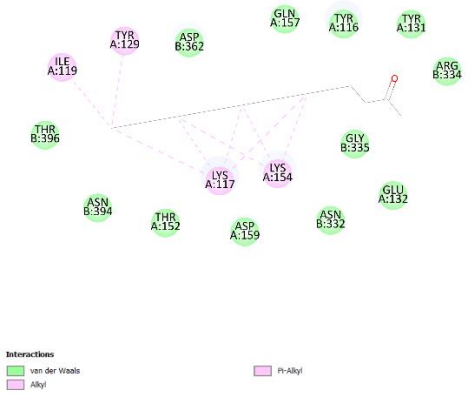
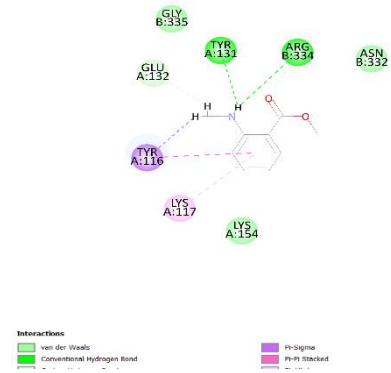
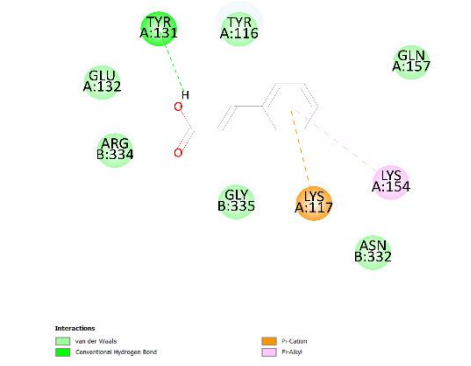
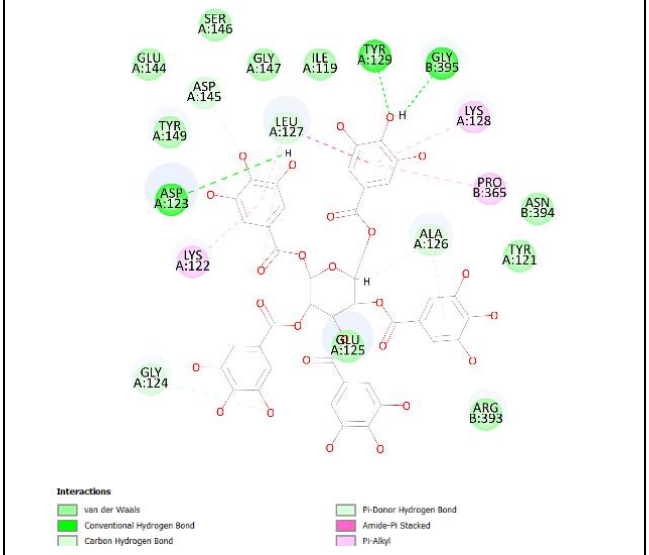
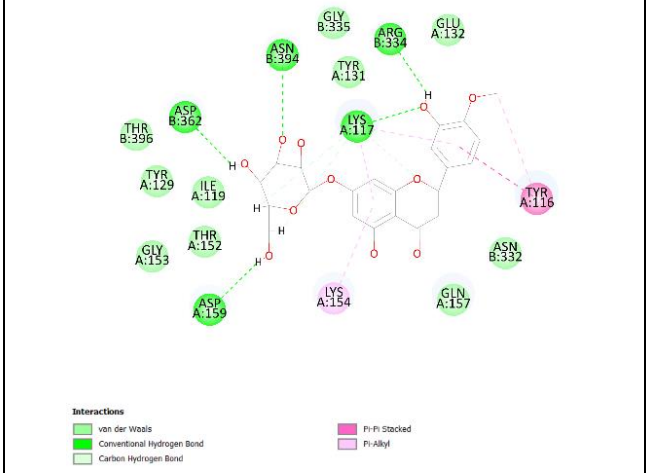
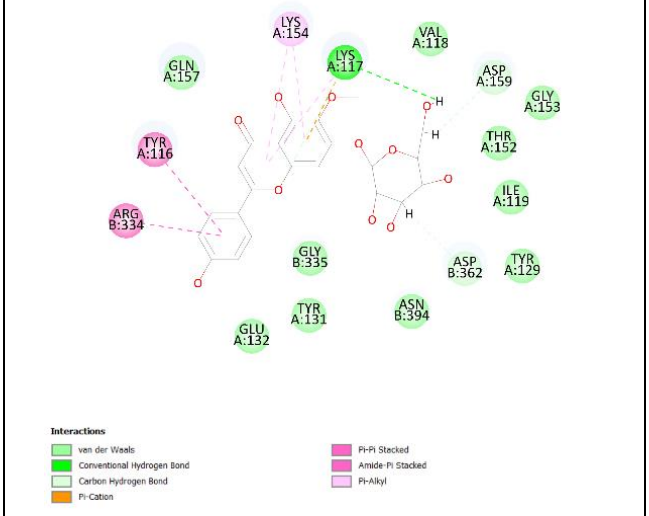
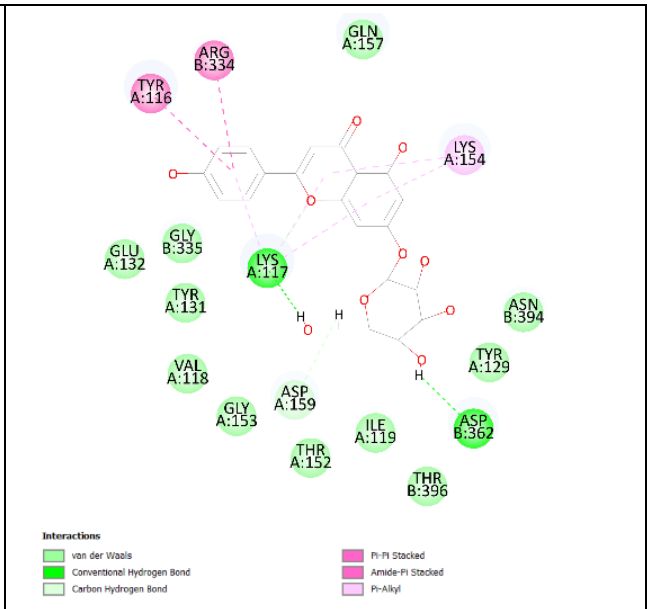
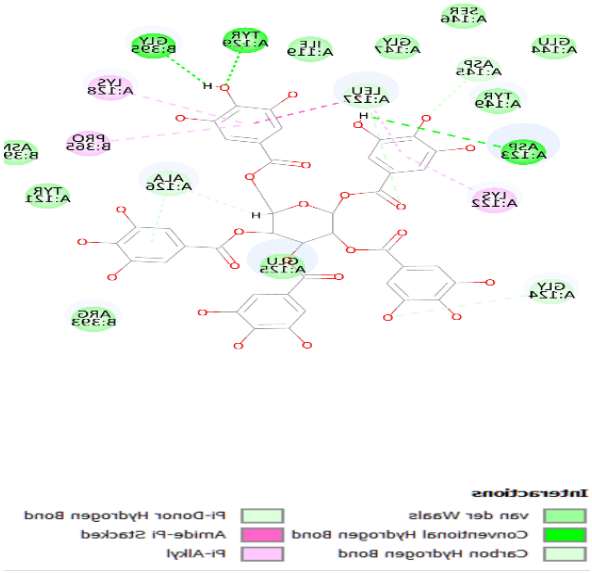
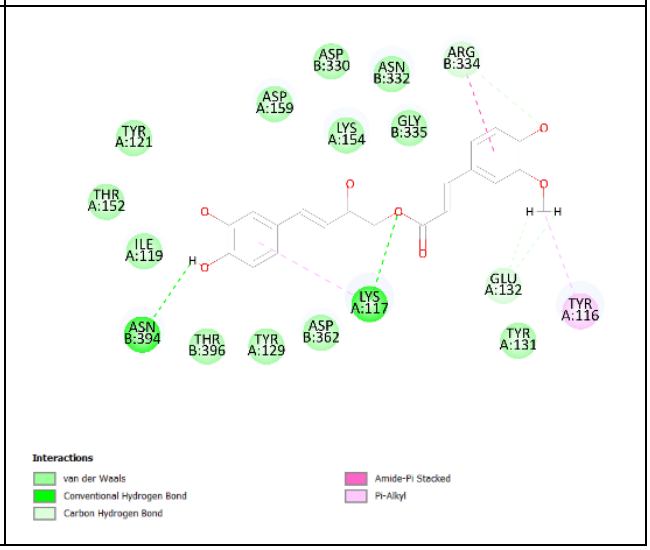
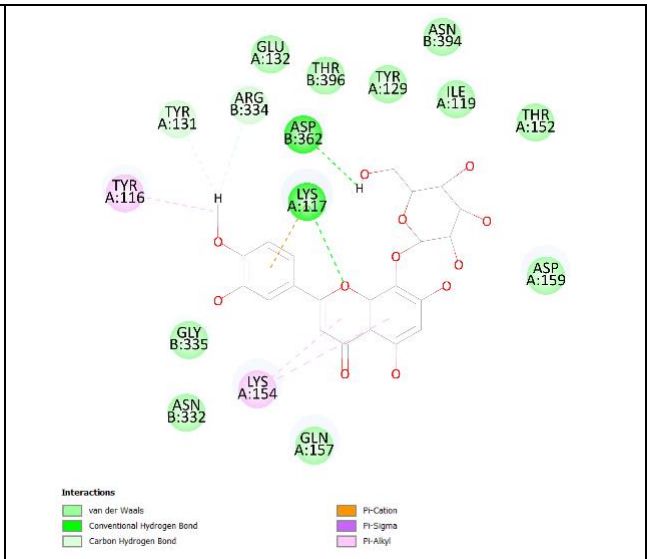
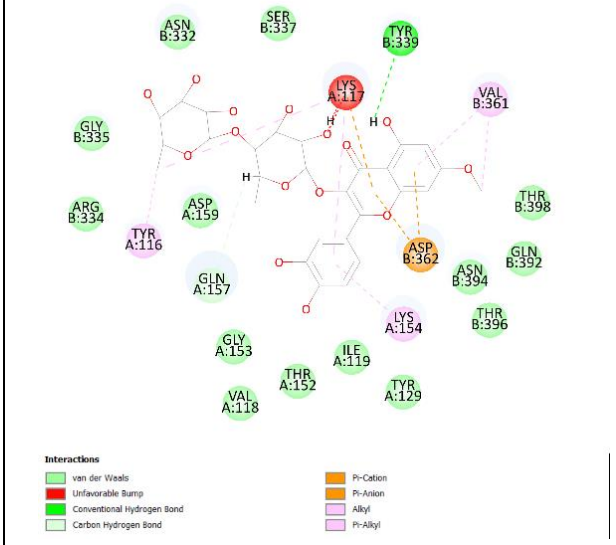
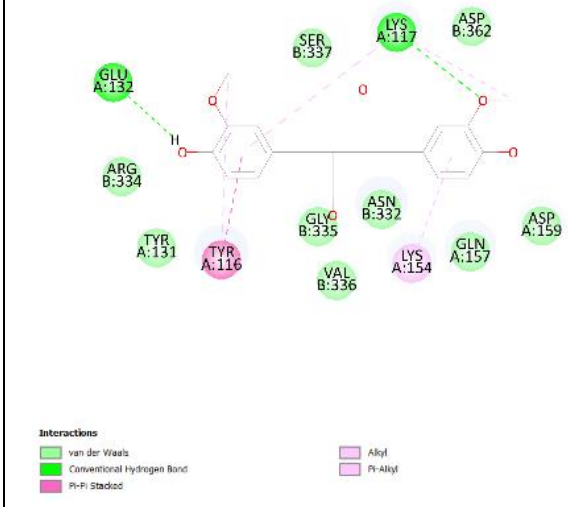
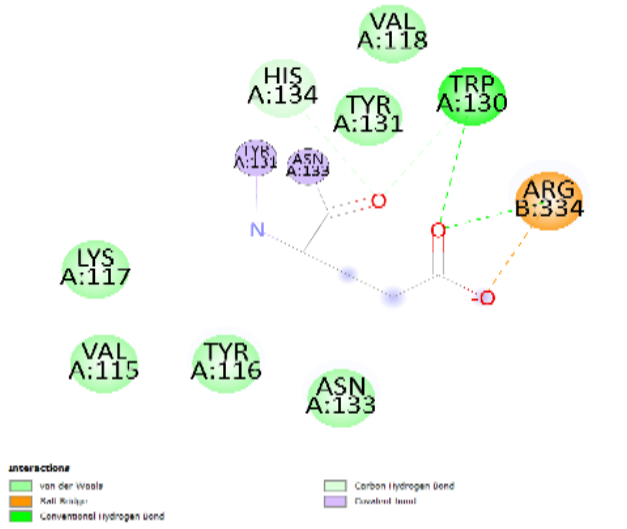
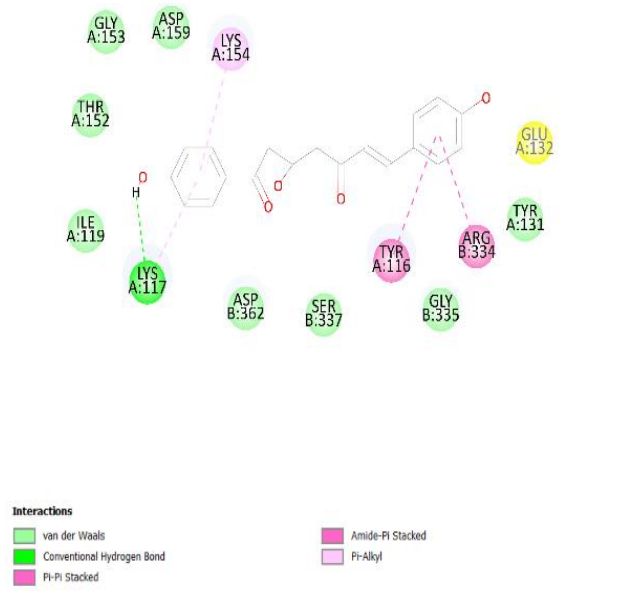
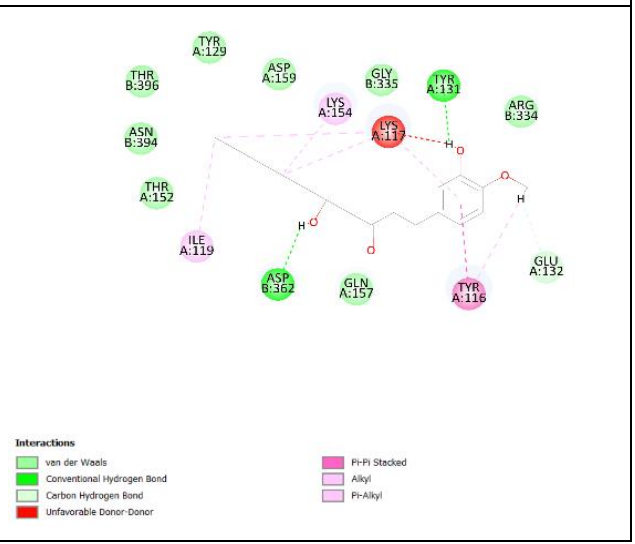
2-Pentadecanone	
Methyl 2-(methylamino)benzoate	
Cinnamic acid	

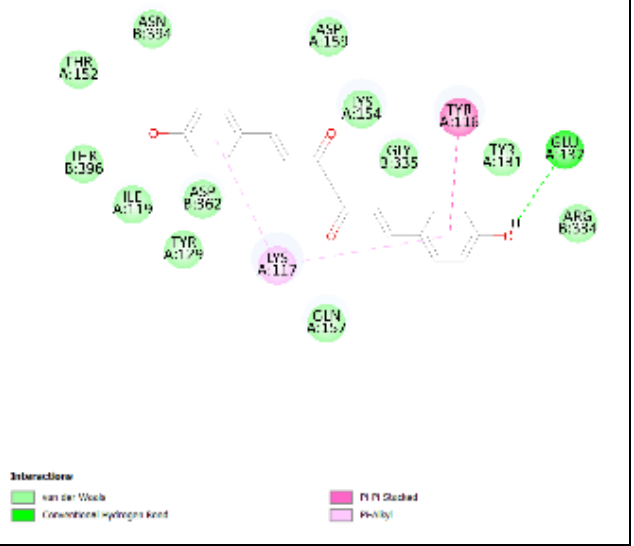
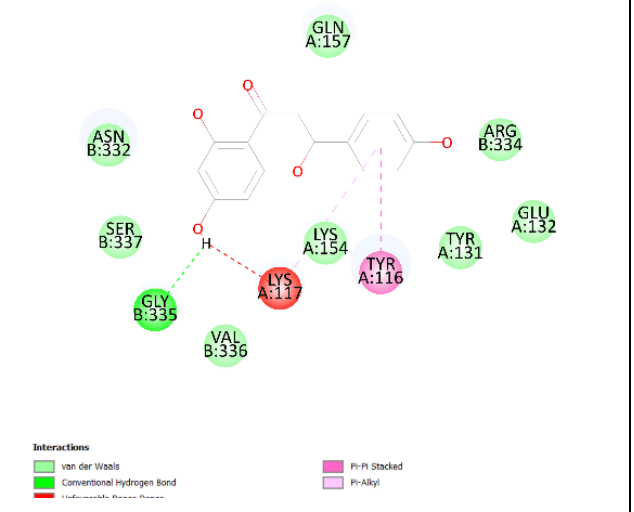
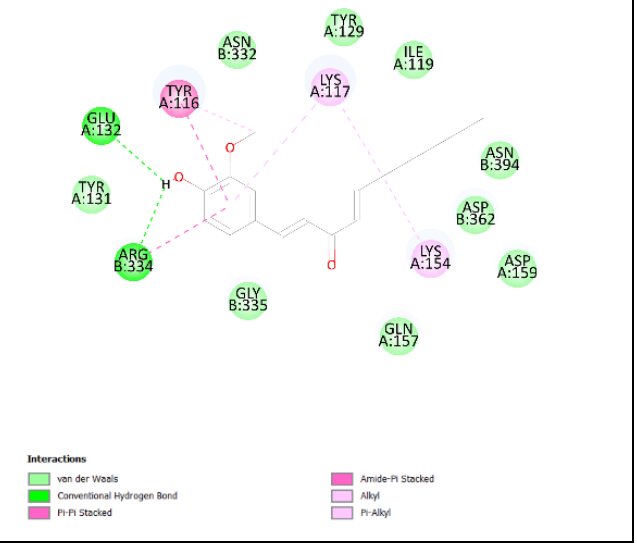
Table 9: 2D docking representation of FcεR (2Y7Q) with tentative phytomolecules present in HK negative ionisation.

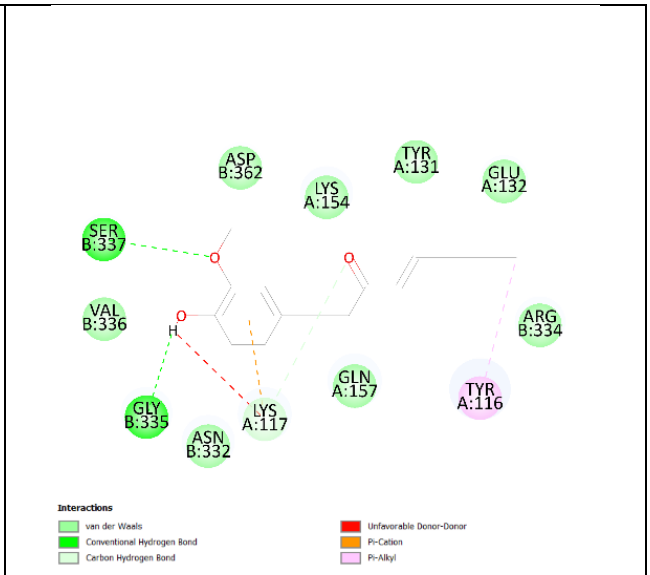
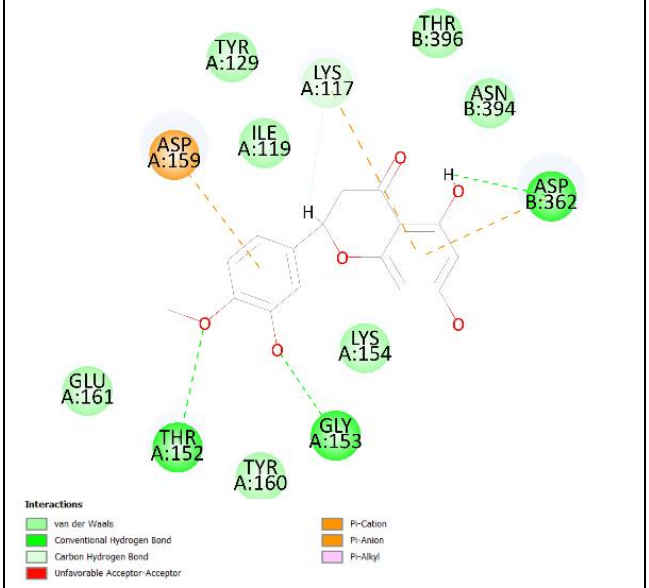
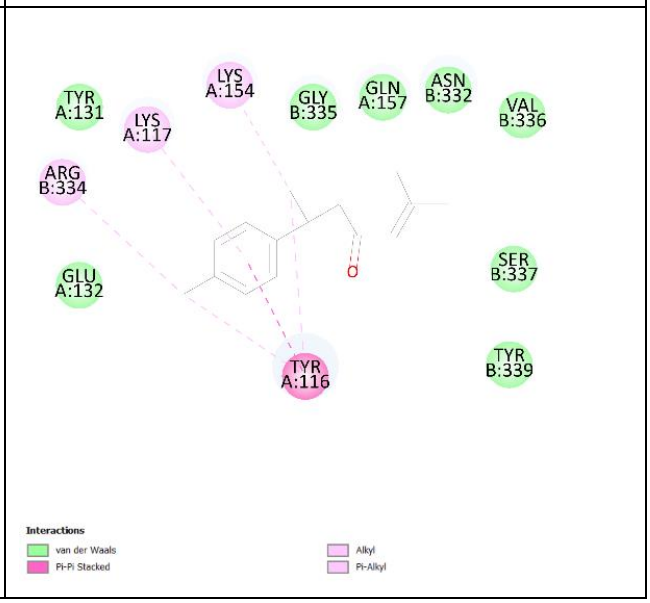
1,2,3,4,6-Penta-O-galloyl-beta-D-glucose	
Hesperetin 7-O-glucoside	
Homoplantaginin	

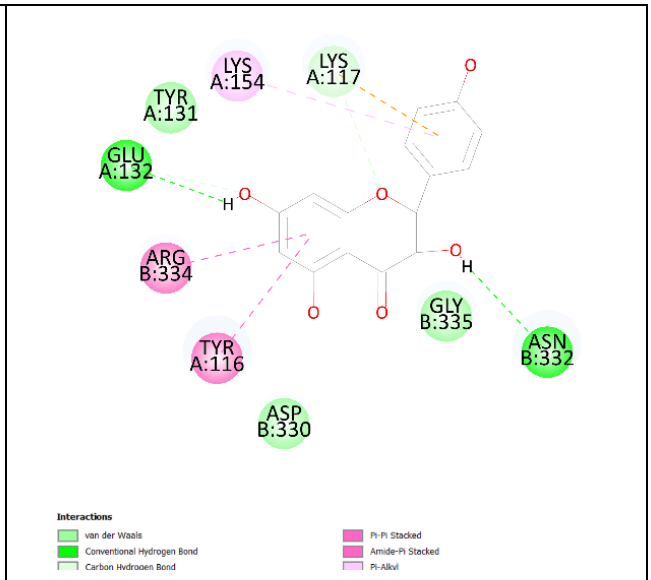
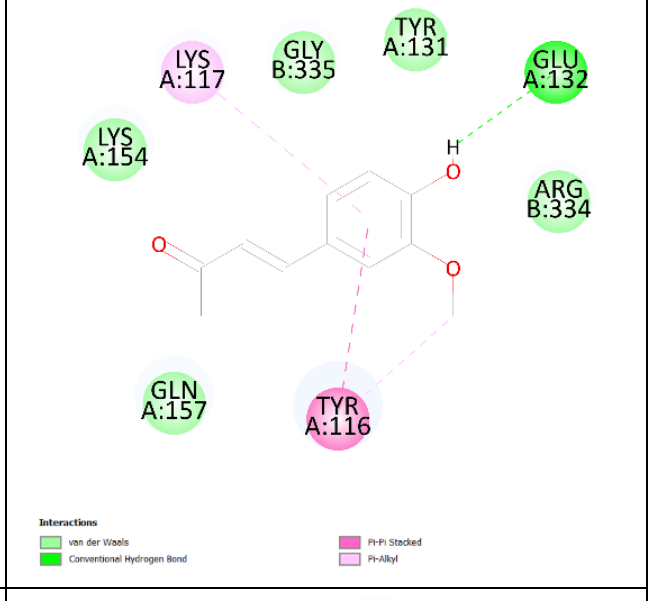
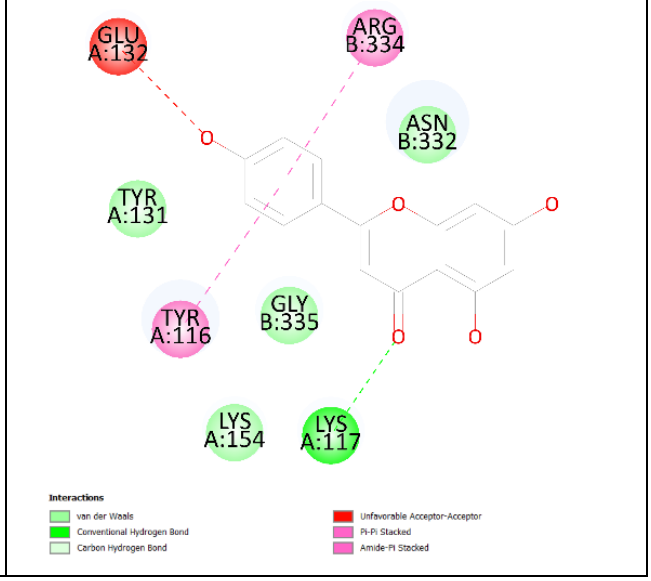
Cosmosiin	
Dihydrocurcumin	
Calebin A	

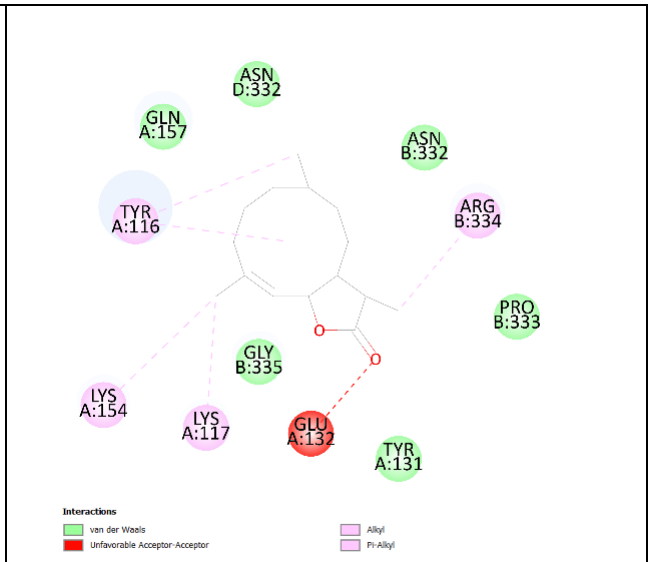
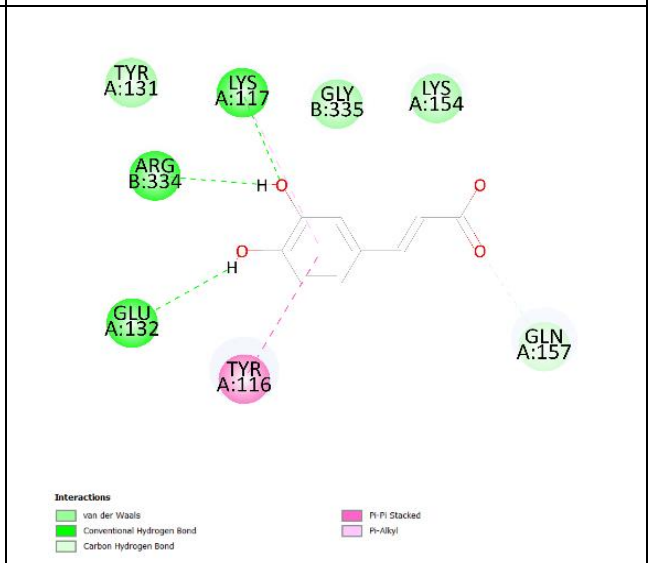
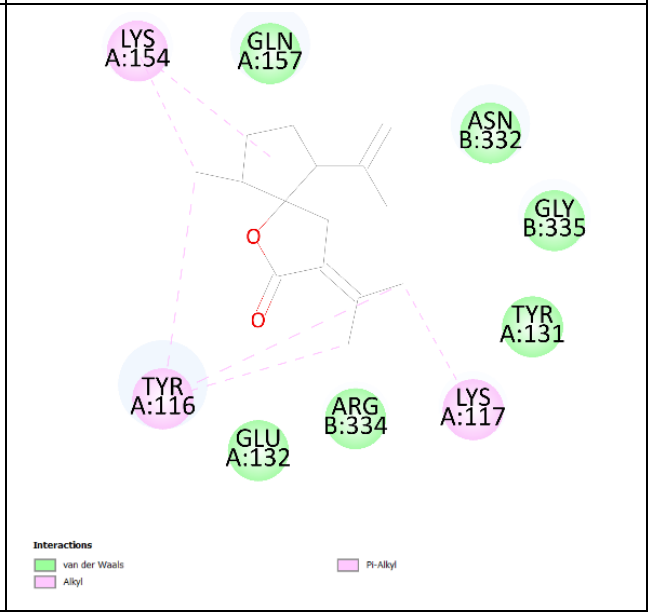
8-Hydroxyluteolin 4'-methyl ether 8-glucoside	
Rhamnetin 3-rhamnosyl-(1-4)-rhamnoside	
(-)-Secoisolariciresinol	

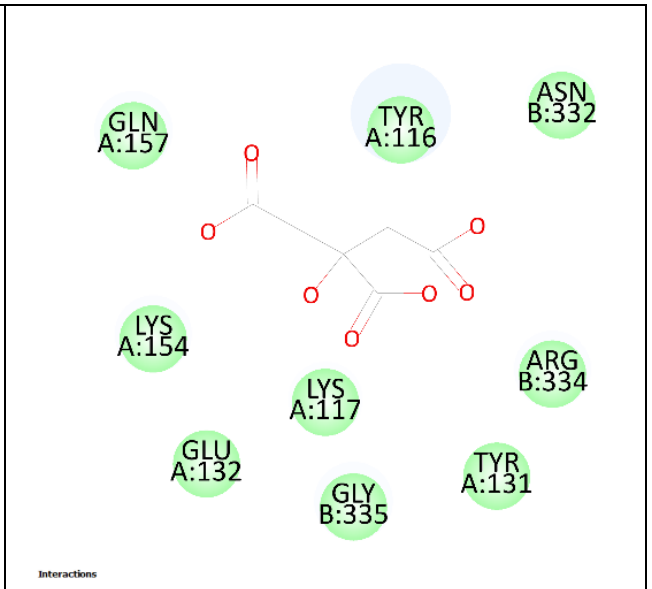
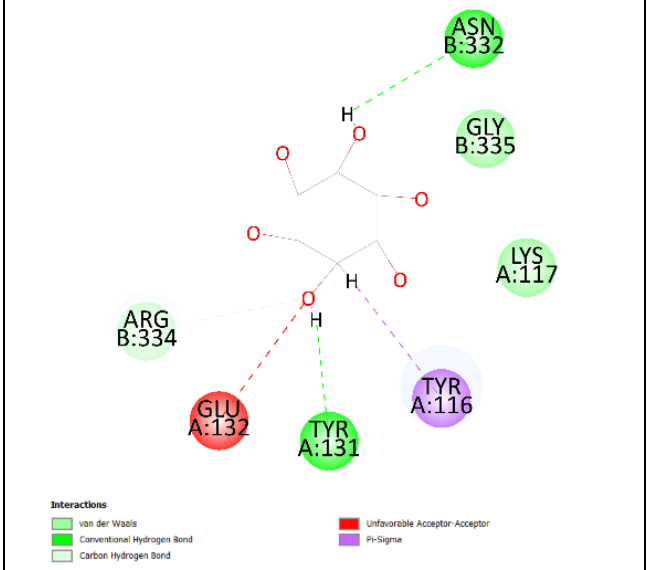
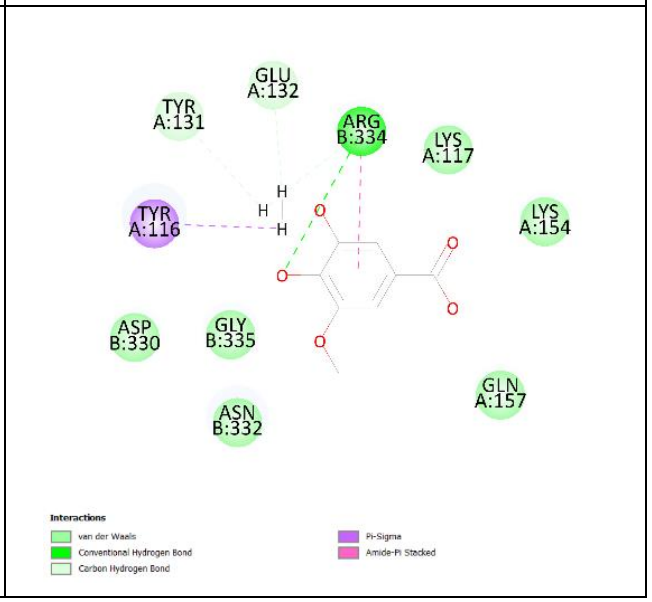
3,12-Dihydroxyhexadecanoic acid	
3-Hydroxy-1,7-bis-(4-hydroxyphenyl)-6-heptene-1,5-dione	
(S)-6-Gingerol	

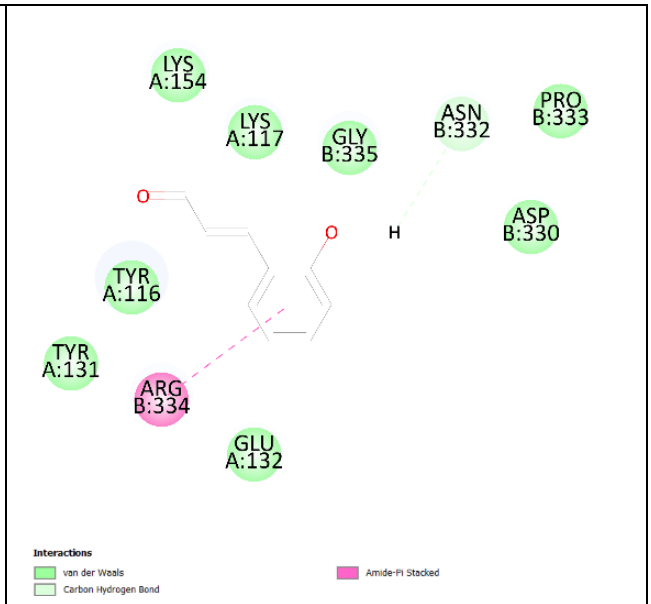
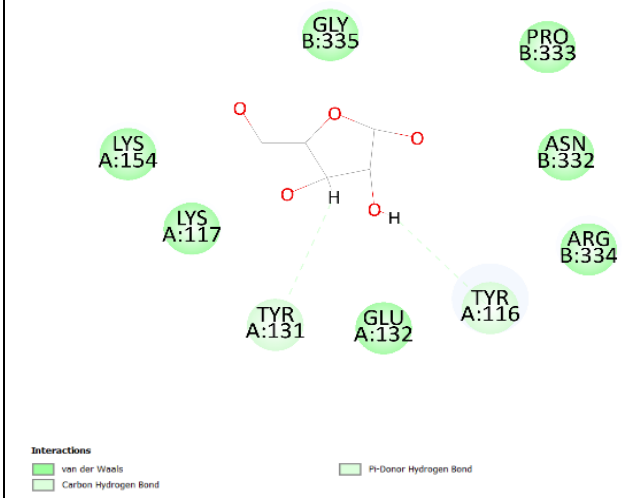
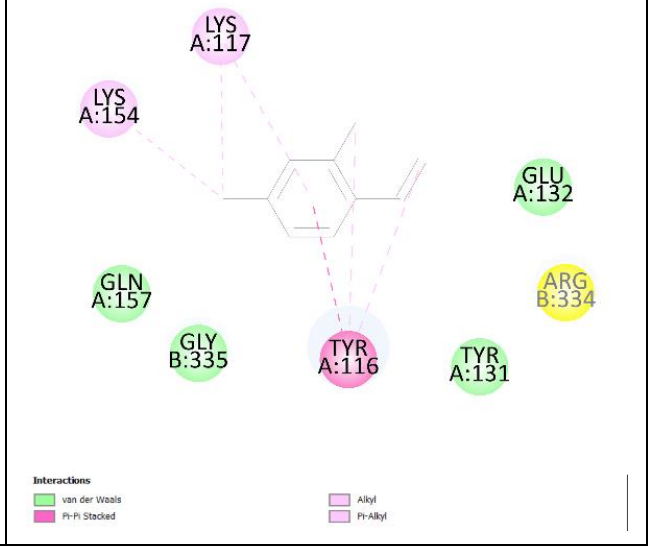
bis-(4-hydroxycinnamoyl)methane	
Naringenin	
[6]-Dehydroshogaol	

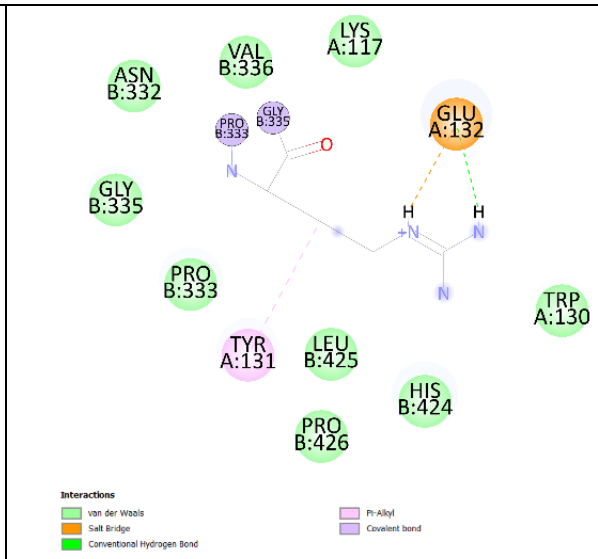
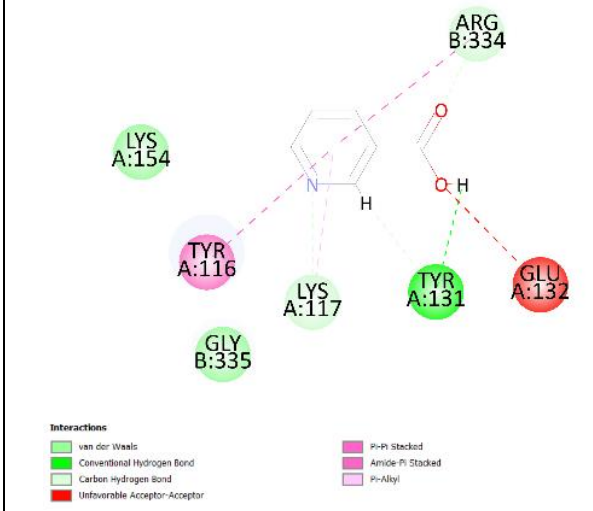
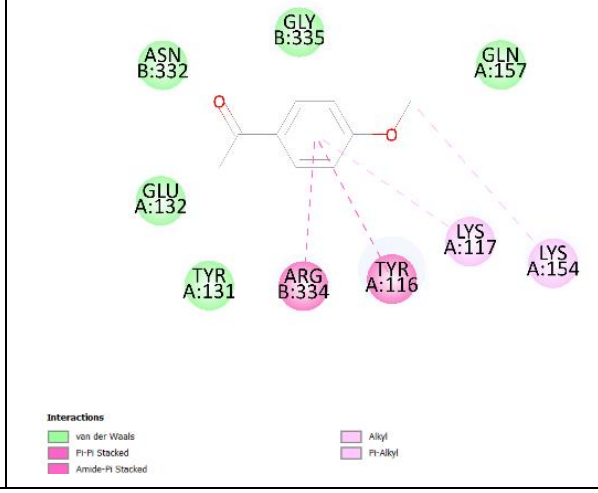
	
Hesperetin	
2-Methyl-6-(4-methylphenyl)hept-2-en-4-one	

(+)-Dihydrokaempferol	
Dehydrozingerone	
Apigenin	

Germacr-4-en-12-oic acid, 6-alpha-hydroxy-, gamma-lactone	
Caffeic acid	
Curcumanolide B	

Citric acid	
Inositol	
syringic acid	

o-Methoxycinnamaldehyde	
alpha-L-Arabinofuranose	
3,4-dimethyl styrene	

Cinnamic acid	
Nicotinic acid	
4'-Methoxyacetophenone	

3.4. Cell viability assay

The cytotoxicity of HK was assessed in RAW 264.7 cells using the MTT assay. As shown in Figure 3, HK at concentrations ranging from 15.625 to 250 µg/mL exhibited no significant difference in cytotoxicity compared to the control group (100 ± 0.011%). The cell viability remained between 99.22 ± 0.56% and 97.89 ± 0.56%, indicating minimal cytotoxic effects.

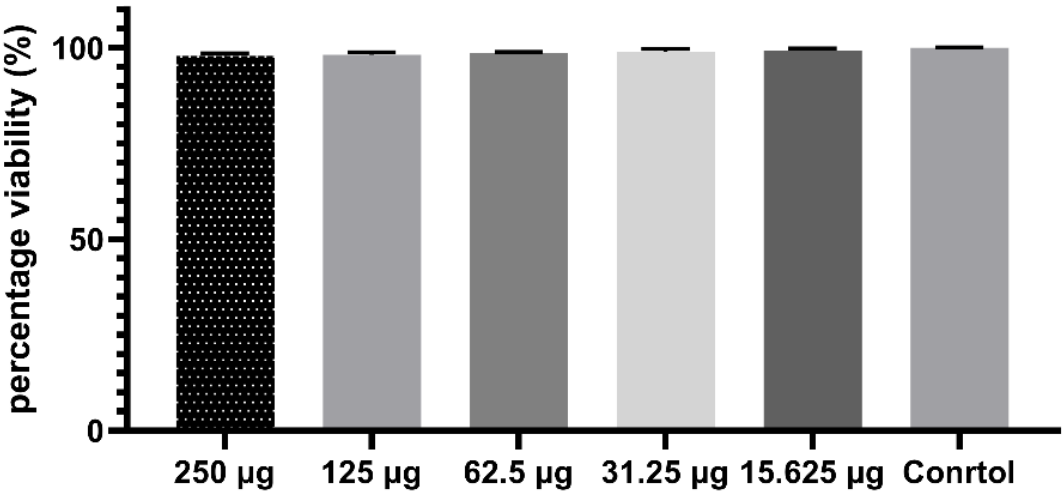


Fig 3. The percentage viability of the RAW 264.7 cells upon treatment with HK shows it does not produces cytotoxicity.

3.5. CYP inhibition assay in Human Liver Microsomes

Table 10: CYP inhibition assay in Human Liver Microsomes

Compound	**CYP1A2**	**CYP2B6**	**CYP2C9**	**CYP2D6**	**CYP3A4**	**CYP2C19**
HK	>5.00	>5.00	>5.00	>5.00	>5.00	>5.00
Miconazole	0.79	<0.20	<0.20	0.64	<0.20	<0.20

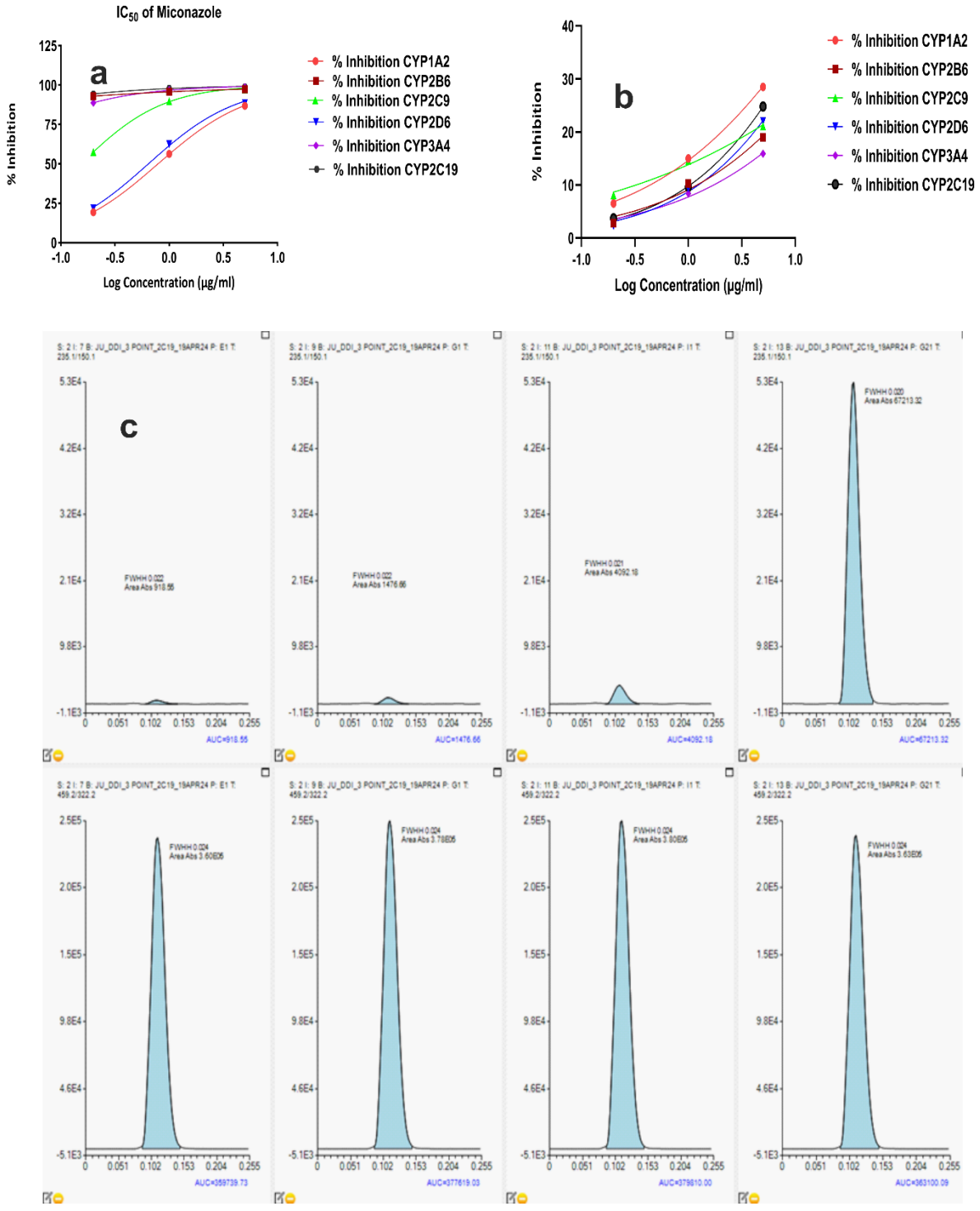


Fig. 4: (a) and (b) shows the % inhibition of CYP450 enzymes i.e. CYP1A2, CYP2B6, CYP2C9, CYP2D6, CYP3A4, CYP2C19 where the IC50 (µg/ml) was estimated. The IC50 (µg/ml) for the (a) positive control Miconazole for inhibiting CYP1A2, CYP2B6, CYP2C9, CYP2D6, CYP3A4, CYP2C19 were 0.79, <0.20, <0.20 , 0.64 , <0.20 , <0.20 while for (b) HK it was found to be more than 5 µg/ml for all the CYPs. (c) Represents the Q1 and Q3 MRM of CYP2C19 substrate OH-Mephynotoin: 235.1> 150.1;Internal standard: 459.2>322.2

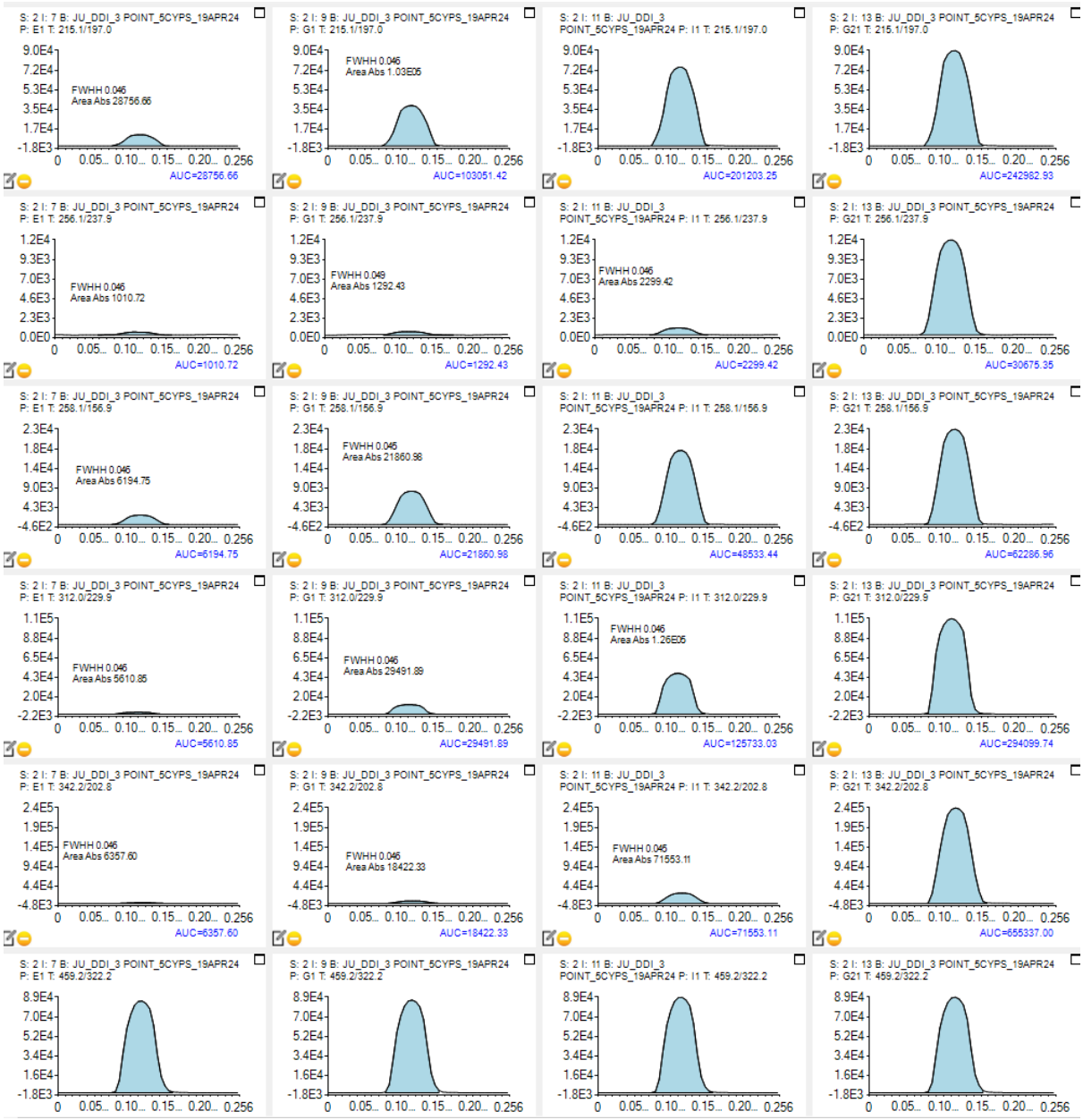


Fig. 5: Represents the Q1 and Q3 MRM of different CYP substrates and internal standards. For 5CYPS-: OH-Tacrine: 215.1> 197.0; OH-Bupropion: 256.1>237.9; Dextrophane: 258.1> 156.9; OH-Diclofenac: 312.0>229.9; OH-Midazolam: 342.2> 202.8; Internal standard: 459.2>322.2.

3.6. Bioassay of Histamine

Figure 6 shows tissue response in presence and absence of test subject figure 6a shows initial response of histamine. Figure 6b and 6c shows response of 250 and 125 µg/ml HK dialysate in the presence of Histamine. Figure 6d showed response of histamine alone. From this study it has been observed that both concentratin inhibit histaminic activity by preventing tissue contraction.

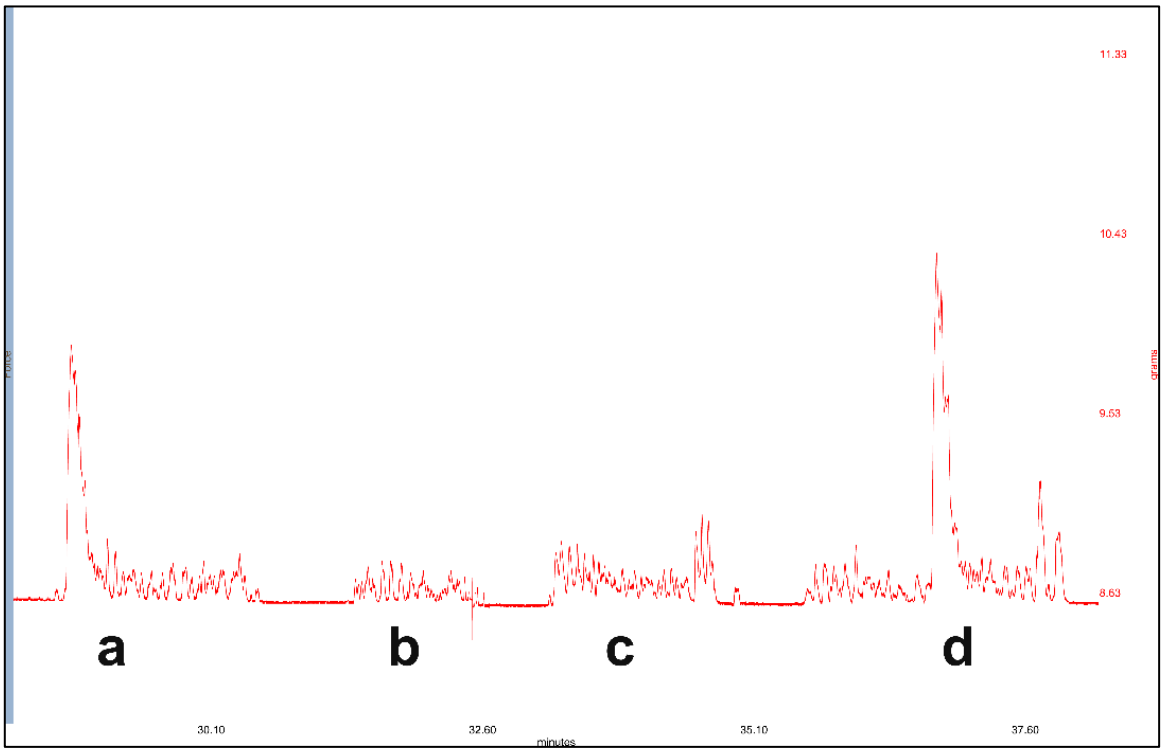


Fig. 6: bio assay of histamine (a) response histamine before treatment, (b) response of histamine with 250 µg/ml in presence of histamine, (c) response of histamine with 125 µg/ml in presence of histamine, (d) response histamine after treatment stating contractility of the tissue remains.

4. Discussion

HK showed more % yield and TPC in the methanolic extract as compared to others (table 1) indicating presence of phenolic compounds. From the metabolite profiling study, 57 metabolites tentatively present in the methanolic extract of HK were identified. Out of these 57 metabolites, 34 were identified in negative mode (table 2) and 23 molecules were identified in positive mode (table 3), we conducted molecular docking studies.

The H1 receptor (H1R) is pivotal in allergic reactions by mediating the effects of histamine, a substance released by mast cells during an allergic response, resulting in symptoms such as sneezing, rhinorrhoea, pruritic eyes, and dermal reactions; consequently, targeting H1 receptors

with antihistamines constitutes a principal therapeutic approach for allergies (11). Histamine stimulates H1R via Gαq/11, subsequently activating phospholipase C and elevating intracellular Ca^{2+} concentrations. Consequently, histamine triggers the contraction of smooth muscle in the respiratory tract, enhances vascular permeability, and stimulates the synthesis of prostacyclin and platelet-activating factor through H1R activation. Consequently, nearly all acute hypersensitivity reactions, including cutaneous symptoms like erythema, pruritus, and edema, may be triggered by the activation of H1R (11). However, computational chemistry has significantly contributed in finding newer leads from various sources in the past decade (12). Molecular docking simulation is one such tool that can be beneficial in understanding ligand-macromolecule interaction (13).

The molecular docking studies on the H1R (PDB ID: 3RZE) revealed that the Libdock score of curcumin and Dihydrocurcumin were the most potent compound among all the identified compounds of positive and negative ionization of mass spectra, respectively. Several studies have also stated Curcumin and its analogue significantly has anti-histaminic properties (14). It is also noted that curcumin exhibits anti-allergic properties by inhibiting histamine release, TNF-α, and IL-4 from activated mast cells, as well as in vivo in type I hypersensitivity animal models (15). The advantageous activity may result from the establishment of many hydrogen and hydrophobic bonds with various amino acids in the active site of H1R. HK may be advantageous because to the existence of Gingerdione.

Gingerenone A, Hesperetin which demonstrated a favorable docking score in H1R. According to previous studies, Gingerenone analogues, obtained from *Zingiber officinal*, significantly reduced histamine release in activated rat peritoneal mast cells (16). However, another study stated that hesperetin has a relaxing effect on histamine-induced tonic contraction in non-sensitized guinea pig tracheas(17).

The FcεR is a tetrameric structure consisting of one α-chain, one β-chain, and two disulfide-linked identical γ-chains. The α-chain extends into the extracellular space and binds with the Fc portion of IgE antibodies. The α- and γ-chains have a transmembrane domain that separates the amino terminal tail in extracellular space and the carboxyterminal tail in the cytoplasm. The α-subunit interacts with β- and γ-chains through charged amino acid residues on single transmembrane domains and hydrophobic contacts (18). Moreover, FcεR, a receptor on mast cells and basophils, triggers allergic reactions when IgE antibodies bind to it (19). As a result, FcεR is considered a possible target for downregulating allergic disorders. Several synthetic

compounds have been reported to suppress FcεR expression and IgE interaction. Meanwhile, natural products have gained popularity due to their efficacy and safety.

The molecular docking experiments on this receptor revealed that the Libdock score of Gingerenone A and 1,2,3,4,6-Penta-O-galloyl-beta-D-glucose were the most effective molecules among all the identified compounds of positive and negative ionization of mass spectra, respectively.

The activity of Curcumin, Demethoxycurcumin, 1,2,3,4,6-Penta-O-galloyl-beta-D-glucose, Hesperetin 7-O-glucoside, Homoplantaginin could be due to the multiple hydrogen bonds with LYS A:117, GLU A:132, GLY B:395, ASP A:123 amino acids of the macromolecule. These amino acids have previously been associated with several other small molecules and played a vital role in their interaction with the active site of FcεR(20). However, Studies have shown that gingerol analouges can treat allergic rhinitis by decreasing cytokine production for T cell activation and preventing the activation of B cells and mast cells (21). Similar to the previous phytocompounds, showed potent activity against FcεR due to the presence of vital hydrogen bond of Homoplantaginin, curcumin, [12]-Gingerdione and Demethoxycurcumin, in positive ionization and Hesperetin 7-O-glucoside, Homoplantaginin and Cosmosiin, Dihydrocurcumin, in negative ionization and hydrophobic interactions with the amino acid backbone of FcεR. However, studies found that curcumin may suppress downstream cascades, including the activation and degranulation of basophils, resulting in the subduing of some pro-inflammatory cytokines, histamine by blocking this key receptor and interaction with IgE (22). Moreover, it is also noted that hesperetin potentially reduced allergic and inflammatory effect in rat basophilic leukaemia RBL-2H3 cells (23).

As a result, docking studies of Phyto molecules obtained from HK, ayurvedic polyherbal formulation indicated that the obtained compounds, integratively, may have potential effect on management of allergic and related condition by regulation histaminic receptor with immune modulatory effect.

Cell viability assays are essential for assessing cellular responses to specific test substances. In this study, the viability of the RAW 264.7 cell line was evaluated to determine the effects of HK. In vitro analysis confirmed that the formulation does not disrupt normal cellular functions or induce cytotoxicity. The RAW 264.7 cell line was selected due to its relevance as a murine macrophage model commonly used to investigate inflammatory responses. Given that the formulation modulates inflammatory cytokines, its impact on this cell line was assessed. The

results demonstrated that HK exhibited comparable cell viability to the control, indicating that the formulation is safe for use within the tested concentration range. (24-27)

In another invitro study, HK exhibited minimum inhibition of all tested CYP isoforms (CYP1A2, CYP2B6, CYP2C9, CYP2D6, CYP3A4, CYP2C19) with IC50 value >5 μg/mL indicating less interaction with these enzymes. From a drug interaction perspective, HK is unlikely to cause clinically relevant inhibition of these CYP enzymes, reducing the risk of drug-drug interactions (DDIs). Moreover, the lesser CYP inhibition indicates that HK does not require significant metabolism through these enzymes for its pharmacological effect. Its efficacy *in vivo/in vitro* is likely mediated by its direct action on the immunological system. The minimum level of CYP interaction also suggests HK might not have interfered with the metabolism of co-administered test substances, i.e., sodium cromoglycate, pheniramine maleate, compound 48/80 and histsamine in *in vivo* experiments.

Guinea pigs are very sensitive to histamine. The contraction of the ileum to histamine is triggered by H1 receptors. These receptors are mainly located in the ileum, bronchi (airways), and capillaries (small blood vessels). The ileum is chosen for studies because it has fewer nerve connections to the surrounding tissue and most of the receptors are found there. From our study, it can be concluded that HK produces some degree of H1 receptor antagonism and, to an extent, a dose-dependent manner.

5. Conclusion

Haridra Khanda (HK) contains several phyto-molecules. Among those metabolites, fifty seven (57) molecules were tentatively identified in LC-MS/qTOF analysis. In the previous chapter, and through bio assay of histamine, Haridra Khanda showed antihistaminic activity. In this chapter, we have showed the probable phytomolecules responsible for antihistaminic activity. During the Moleculer docking study, we also found out that it could have some antiallergic activity, too, while we performed a molecular docking study in the Fcε receptor. In the next chapter, we tried to explore the anti-allergic activity of this classical Ayurvedic formulation.

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CHAPTER VI

Reduction of allergy severity by Haridra Khanda by modulating cytokines and plasma IgE

1.Introduction

Human allergic asthma is a chronic inflammatory condition of the airways, characterized by inflammation, persistent airway hyperresponsiveness (AHR), and intermittent, reversible airway obstruction. Structural changes in the airways, such as subepithelial and airway wall fibrosis, goblet cell hyperplasia/metaplasia, smooth muscle thickening, and increased vascularity, are also common. These changes, collectively known as 'airway remodeling,' are believed to result from repeated allergen exposure, leading to ongoing airway inflammation. Chronic inflammation and structural alterations are thought to have functional consequences, which contribute to the symptoms of asthma(1,2).

Asthma is a significant global non-communicable disease affecting both children and adults, and it is the most prevalent chronic disease in children. According to the World Health Organization, asthma affected approximately 262 million people worldwide in 2019, with another estimate suggesting 358 million cases in 2015. Regardless of the variations in data, there is a clear trend of rising asthma incidence and prevalence over the past five decades. Recent studies indicate that in some countries, up to 15–20% of the general population is diagnosed with asthma, a concerning statistic(3,4).

The Indian Study on Epidemiology of Asthma, Respiratory Symptoms, and Chronic Bronchitis in Adults (INSEARCH) reported an asthma prevalence of 2.05%, with 17.23 million affected individuals in India. The Global Burden of Disease (GBD) study from 1990 to 2019 estimated the total asthma burden in India at 34.3 million, representing 13.09% of the global burden. The study also highlighted that asthma contributed to 13.2 deaths per thousand people in India, and accounted for 27.9% of disability-adjusted life years (DALYs) in the country. India's asthma mortality rate is three times higher, and its DALYs are more than twice the global average for asthma(5,6).

A more recent estimate, as of June 20, 2022, reaffirmed that the asthma burden in India remains at 34.3 million, accounting for 12.9% of the global burden(7).

Medicinal plants used to treat asthma should possess properties such as anti-inflammatory, immunomodulatory, antihistaminic, smooth muscle relaxant, and anti-allergic effects. According to Ayurveda, effective anti-asthmatic drugs should have anti-kapha and anti-vata properties. Antioxidant supplements are also helpful in reducing bronchoconstriction by inhibiting pro-inflammatory processes, neutralizing excess reactive oxygen species and reactive nitrogen species. Despite existing asthma treatments, which often have unsatisfactory

outcomes due to side effects, many patients are turning to complementary and alternative medicine for asthma management(8). Haridra Khanda is indicated in inflammatory disorders related to skin allergic conditions. Various ingredients of Haridra Khand (HK) possess around 34% Vata-Kapha Shamaka (balancing Vata and Kapha Doshas), 33% Tridoshashamaka (balancing all three Doshas) properties, which help to bring the affected Doshas in normal level [9]. In view of this background, we investigated its pharmacological properties in *ovalbumin-induced* allergic asthma experiment. The potential phytomolecules present in HK responsible for the above mentioned therapeutic activities were qualitatively analysed using qToF in previous chapter, which was further confirmed using computational biology through receptor-ligand interactions.

2. Methods

2.1. Animals

The experiment used Swiss albino mice with a weight range of 22.2 to 24.6 grams. The mice were kept in rooms with regulated lighting (12 hours of light followed by 12 hours of darkness) and temperature (maintained at 22 ± 3 °C). During the studies, the subjects were given unrestricted access to regular laboratory meals and were supplied with drinking water at all times. The mouse models were used following an 8-day period of acclimatization, during which no negative clinical symptoms were seen and normal weight increase occurred. The tests were conducted by the procedures authorized by the Institutional Animal Ethics Committee (IAEC) of Jadavpur University, with the reference number JU/IAEC-22/21. The research had five groups, with each group consisting of six animals: a negative control group, a disease control group, a HK high dosage group, a HK low dose group, and a standard group.

2.2. Sensitization and challenge protocol for allergic asthma

The mice in the negative control group were not subjected to any treatment during the whole course of the experiment. The mice in the disease control group (OVA treated) were sensitized intraperitoneally on days 1 and 8 with 20 µg of OVA emulsified in 1 mg of aluminum hydroxide, in a total volume of 200 µl. On days 15, 16, and 17 after the first sensitization with OVA, a dose of 20 µg OVA was administered via the nose. The challenge dosage (20 µg OVA diluted in 40 µl saline) was administered intranasally to the mice by pipetting onto the outside border of their noses. For the nasal OVA installation, mice were anesthetized using an appropriate amount of phenobarbital injection. Following the installation process, the mice were relocated, completely recuperated, and then transported back to their cage. The same

methods were followed for the treatment groups, which included the HK high dose group, HK low dosage group, sodium cromoglycate group (standard group). The HK high dose group received an oral dosage of 1.5 mg/kg/day of HK, whereas the HK low dose group received an oral dose of 1 mg/kg/day of HK. These doses were administered after the first sensitization and continued until day 17 of the final challenge. The other HK-treated groups were administered sodium cromoglycate or standard at a dosage of 80 mg/kg (i.p.). After 48 hours after the last exposure to OVA, the mice were euthanized using a lethal dosage of phenobarbital sodium. The specimens used for analysis were obtained from euthanized animals. Lung inflammation, serum IgE production, and protein levels of inflammatory and epithelial cell derived cytokines were assessed.

2.3. Measurement of body and organ weights

The body weight of the mice was recorded on days 1, 3, 8, 10, 15, 17, and 19 following the initial sensitization with OVA. On day 19, the mice were euthanized, and the weights of the left lung and spleen were measured.

2.4. Bronchoalveolar lavage fluid (BALF) preparation

Forty-eight hours after the final OVA challenge, the mice were anesthetized. The left lung was ligated, and the right lung was lavaged three times via the tracheal tube with 0.7 ml of phosphate-buffered saline (PBS). The total cell count in the collected bronchoalveolar lavage fluid (BALF) was determined using a brightfield microscope. For differential cell counts, BALF cells smears were prepared in a glass slide and were stained with Diff-Quik solution. The different cell types were counted (n = 200/slide). BALF was immediately centrifuged at 2000 rpm for 5 min, and the collected supernatant was stored at − 70 °C until measurement of cytokine levels by enzyme-linked immunosorbent assay (ELISA).

2.5. Measurement of cytokine levels

Interleukin (IL)-4(Cat no: E-MSEL-M0008), IL-5(Cat no: E-EL-M0722), IL-13(Cat no: E-EL-M0727), IL-25 (Cat no: E-EL-M0187), and IL-33(Cat no: E-EL-M2642) levels in BALF were quantified by ELISA using Elabscience commercial kits according to the manufacturer's protocol. The sensitivity for IL-4, -5, -13, -25, and -33 assays were 9.38, 9.38, 18.75, 9.38 and 9.38 pg/ml, respectively. The intra and inter assay coefficients of variation for IL-4, -5, -13, -25, and -33 were <10%.

2.6. Measurement of total serum IgE level

At 48 h after the last OVA challenge, mice were anesthetized with phenobarbital sodium, and blood samples were obtained from the abdominal aorta and centrifuged at 3000 rpm for 10 min. Total IgE level in serum was determined by Elabscience ELISA kit(Cat no: E-EL-M3034). Briefly,100 µl of sample was added into each well and incubate for 90 minutes for 37°C, After incubation biotinylated antibody was added and incubated for 1 hr at 37°C followed by a wash of the wells. HRP conjugate was added next and incubated at 37°C for 30 minutes and 90 µl of substrate was added after proper wash to each well and incubated again for 15 minutes. Finally after 15 minutes 50 µl of stop solution was added to each well and OD was measured at 450 nm in Spectramax instrument(10).

2.7. Histological analysis

Forty-eight hours after the final OVA challenge, the mice were euthanized for histological analysis. The lung tissue was extracted, fixed in 10% (v/v) neutral-buffered formalin, dehydrated, embedded in paraffin, and sectioned into 4 µm slices. These sections were deparaffinized using xylene, then stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) stains (both from Sigma-Aldrich). The stained sections were examined under a light microscope (Evos, Thermo Fisher).

The degree of lung inflammation and goblet cell hyperplasia was scored on a scale from 0 to 4. For inflammation, cell counts were conducted blindly across intraluminal, alveolar, peribronchial, and perivascular regions using a five-point grading system: 0 (normal), 1 (few cells), 2 (a ring of inflammatory cells 1 cell layer deep), 3 (a ring of inflammatory cells 2-4 cells deep), and 4 (a ring of inflammatory cells >4 cells deep) (11).

For quantifying goblet cells in the bronchi and bronchioles, a five-point scale was used: 0 (< 0.5% PAS-positive cells), 1 (< 25%), 2 (25-50%), 3 (50-75%), and 4 (> 75%). Five fields per slide were analyzed using a Neubauer's chamber, and the mean score was calculated from six animals. To account for airway size, the number of PAS-positive goblet cells was quantified as the number of PAS-positive cells per mm of basement membrane (12).

2.8. Statistical analysis

Data are expressed as mean ± SD. Statistical multiple comparisons were performed by one-way analysis of variance followed by Sidak test. $p < 0.05$ was considered statistically significant. All the statistical analysis were done in graph pad prism softwere.

3. Results:

3.1. Changes in body and organ weights

The body weight of mice remained at a constant level during the experimental period, and no significant differences were observed between groups. Relative spleen and lung weights were increased in the OVA group as compared with the HK-treated groups and standard group, respectively, but there was no significant difference between both HK treated groups whereas it was significant in the standard group ($p<0.5$), fig 1.(a) and fig 1.(b).

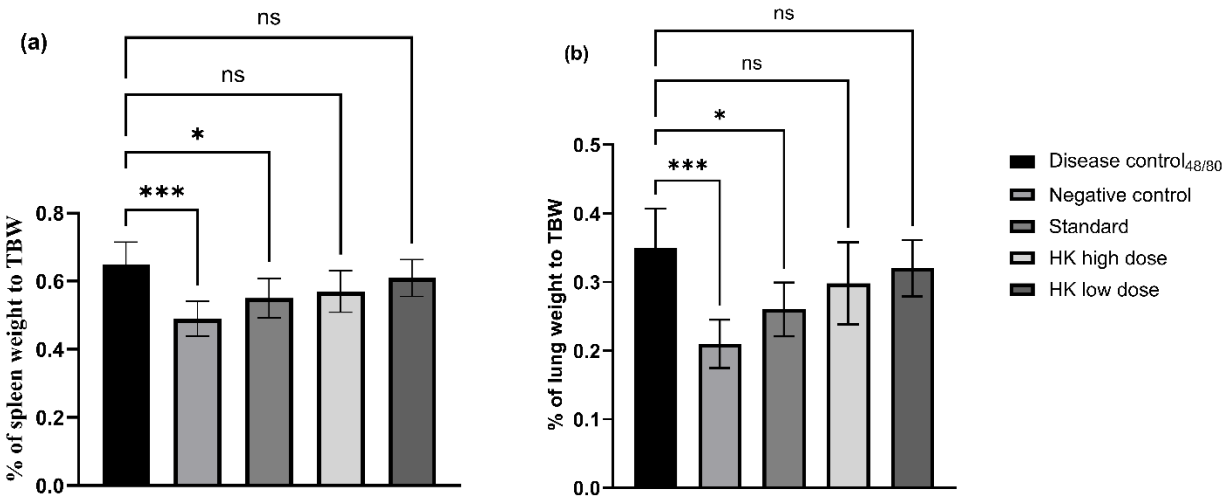


Fig.1: Changes in relative spleen and lung weights of control and OVA-sensitized and challenged mice. Relative spleen (a) and lung (b) weights were calculated using the following formula: relative organ weight = organ weight (g)/terminal body weight (g) × 100%. Bars represent the mean ± SD from five mice per group.

3.2. Cellular changes in BALF

To investigate whether the route of OVA challenge affects the lung inflammatory response, we analyzed the inflammatory cells in the BALF of OVA-induced mice. Following OVA sensitization, both inhaled and intranasal OVA challenges led to a significant increase in the eosinophil population in the BALF compared to the negative control group (Fig. 2). The changes in the percentage of macrophages and eosinophils showed a significant shift in the disease control group ($p<0.001$) compared to the negative control, indicating successful ovalbumin induction. When the disease control group was compared to the standard and HK-treated groups, the results were also significant (macrophages $p<0.5$, eosinophils $p<0.001$ and $p<0.05$, respectively), suggesting that HK may offer protective anti-allergic effects against ovalbumin-induced allergies.

The total cell count and eosinophil numbers in the BALF were significantly higher in the OVA-challenged mice compared to the negative control group (Fig. 3). Notably, the intranasal OVA challenge notably increased the number of neutrophils and lymphocytes compared to the other treated groups. These findings indicate that OVA sensitization and challenge induce eosinophil-dominant allergic inflammation in the lungs, and the route of allergen exposure can influence the pattern of asthma development.

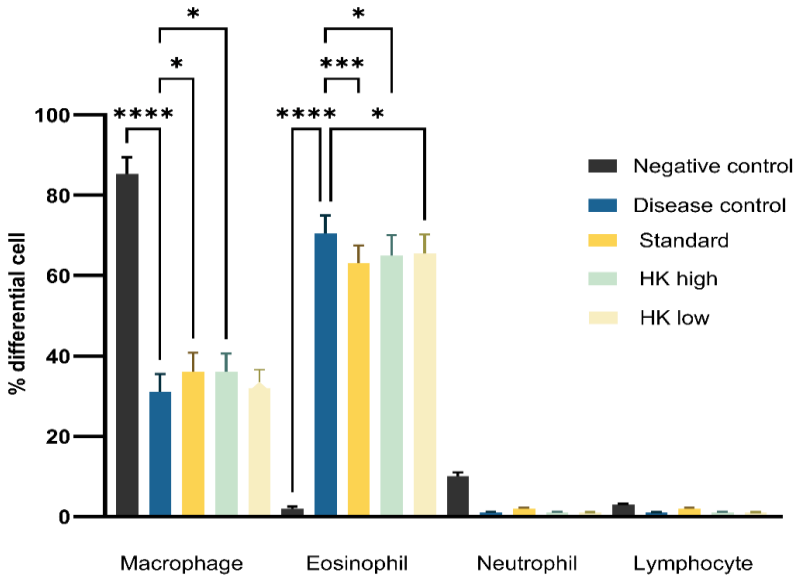


Fig.2: % of different cells in negative control and different treatment groups. Represented comparisons are fit into $p<0.05$.

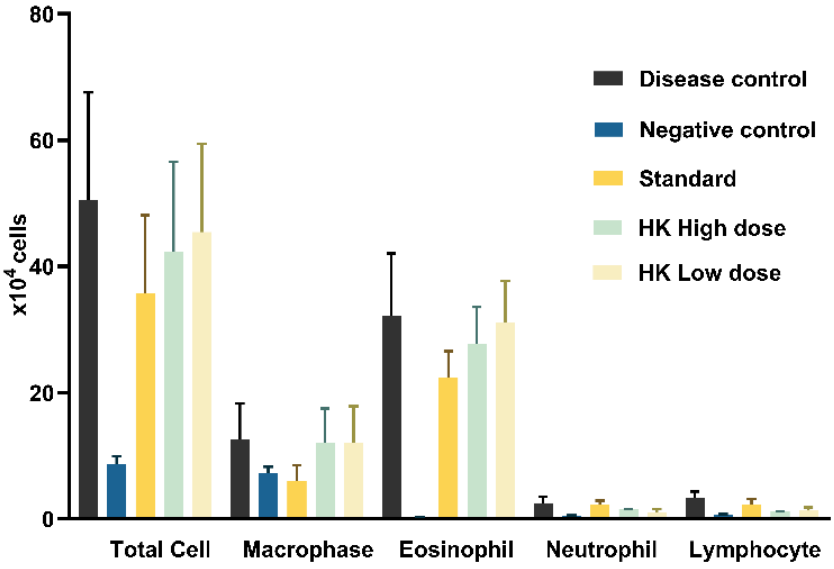


Fig.3: Bars represent mean±SD for % of total cells in BALF (a; disease control, b; negative control, c; standard group, d; HK high dose group, e; HK low dose group).

The number of total cells and eosinophils in BALF was significantly elevated as compared to mice in the negative control group (Fig. 3). Especially, intranasal OVA challenge greatly induced the number of neutrophils and lymphocytes as compared to mice in the other treated groups. These results indicate that OVA sensitization and challenge induce eosinophil-dominant allergic lung inflammation in mice and allergen challenge routes can affect different asthmatic pattern.

3.3. Total serum IgE level

We next investigated whether OVA sensitization and challenge induces IgE-mediated allergic asthma and whether serum IgE is influenced by the OVA challenge route. Total IgE in serum was decreased in the standard group, HK high dose and HK low dose groups relative to the disease control group ($p < 0.001$, $p < 0.01$ and $p < 0.5$ respectively; Fig. 4).

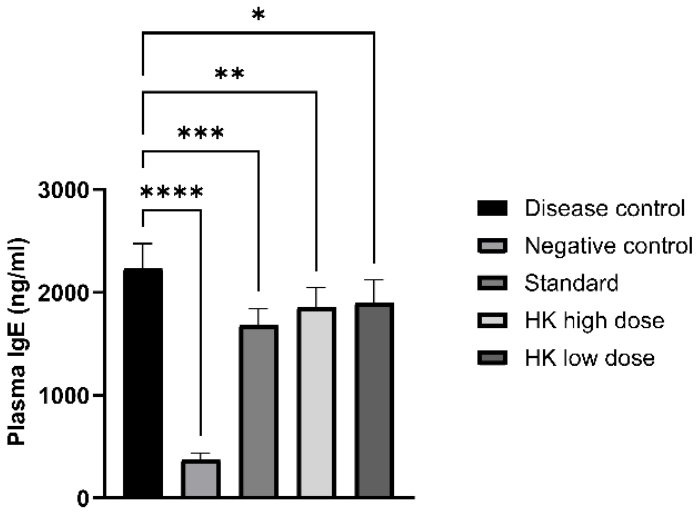


Fig 4: Serum IgE levels in negative control and challenged OVA-sensitized mice groups. Serum samples from all groups were collected 48 hr after the last OVA challenge. Bars represent the mean ± SD from five mice per group.

3.4. Histological changes in lung

We performed a histological analysis to examine the pathological features of OVA-induced allergic lung inflammation and mucus production (Fig. 5i). The typical characteristics of allergic asthma were observed in the OVA-induced disease control group compared to the other groups through H&E staining, with noticeable eosinophilic infiltration in the pulmonary vessels, alveolar ducts, and throughout the lung alveoli (Fig. 5ii). These findings were further

confirmed by histological scoring of inflammatory cell infiltration based on the OVA challenge route (Fig. 6a).

PAS staining revealed a significant increase in the number of PAS-positive cells per mm of basement membrane in OVA-induced mice compared to the other treated groups (Fig. 5.iii, Fig. 6b and 6c). Notably, mucus production in the intranasal OVA group was predominantly observed in the bronchial epithelium. These results suggest that the histopathology of asthma in the lungs is differentially induced depending on the specific treatments administered to the animals.

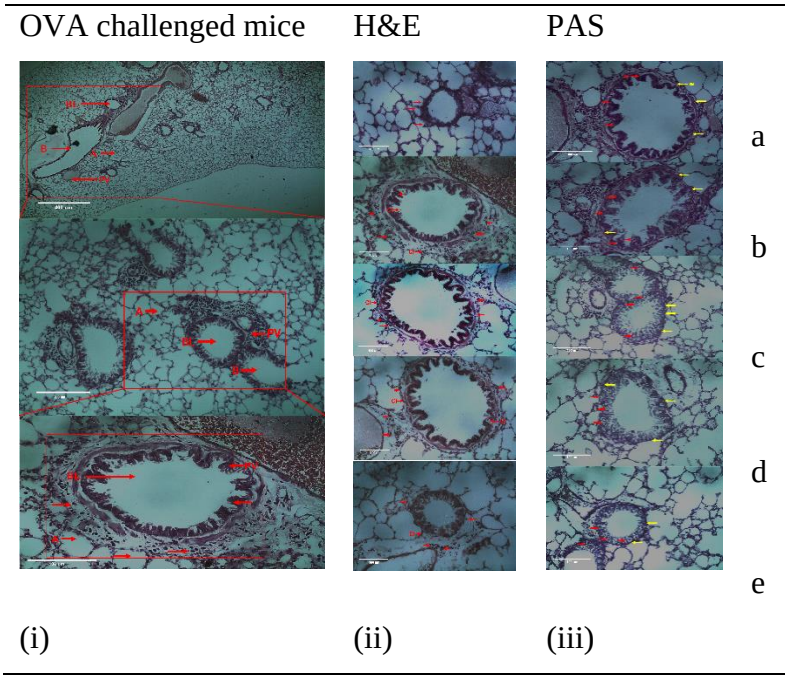
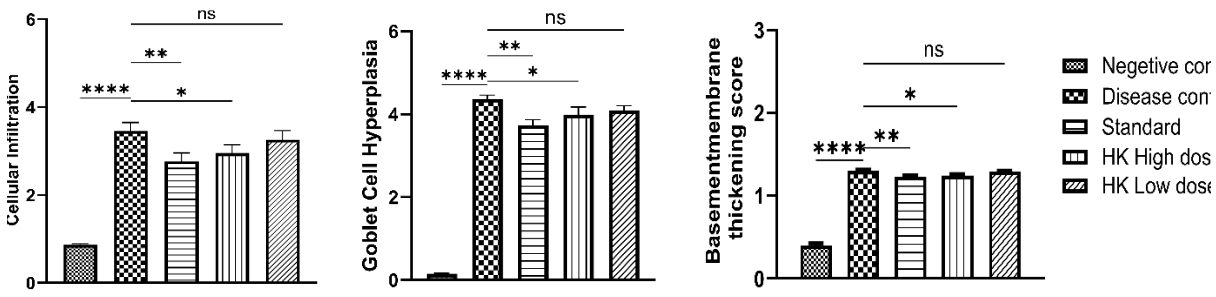


Fig 5: Infiltration of inflammatory cells and mucus secretion in lung tissue from control and OVA-sensitized and challenged mice. Representative H&E and PAS-stained sections of lung and higher magnifications (i) Represent the change in the cellular environment in lung after OVA sensitization and challenge [B; bronchi, BL; bronchiole, A; alveolar, PV; perivascular], (ii) represent H&E stained sections with cellular infiltration(CI) of eosinophil, (iii) represents PAS stained sections of lung for observation of goblet cell hyperplasia(GH) denoted in red arrow and basement membrane thickening(BM) with yellow arrow of a; disease control, b; negative control, c; standard group, d; HK high dose group, e; HK low dose group.



(a) (b) (c)

Fig 6: (a) analysis of cellular infiltration of H&E stained lung sections (b) goblet cell Hyperplasia score in PAS stain and (c) basement membrane thickening score in PAS stained lung sections.

3.5. T helper (Th)2 cytokine levels in BALF

Th2 cytokines play important roles in allergic asthma during airway remodeling and the development of airway resistance (13,14). We assessed Th2 cytokine levels in BALF in the lungs following OVA challenge and found that Th2 cytokine IL-4, -5, and -13 levels in BALF were higher in OVA-induced groups than in other treated groups (Fig. 7a, Fig. 7b and Fig. 7d). In case of IL-4 and IL-5 significant changes were observed in all the groups (standard group was $p< 0.001$ and <0.001, for HK high dose group it was $p<0.001$ and <0.05 respectively, and for HK low dose group it was $p<0.05$ for both the occasion).

However, in the case of IL-13, changes in HK low dose group did not show a significant decrease($p>0.05$), although HK high dose group($p<0.05$) and standard group ($p<0.01$) showed a significant decrease.

3.6. Epithelial cytokine levels in BALF and lung tissues

Epithelial cytokines released in response to various allergens play a key role in promoting allergic asthma (15). To determine whether the method of OVA challenge affects epithelial damage, we measured the levels of epithelial cytokines IL-25 and IL-33 in the BALF (Fig. 7c, Fig. 7e). Our findings revealed that the protein levels of IL-25 and IL-33 were significantly elevated in the OVA-treated group compared to the other treatment groups. We found very highly significant changes in standard treated group ($p<0.0001$) for both epithelial cell-derived cytokine lavel when compared to disease compared group. However, in cases of HK high dose group($p<0.001$) and for HK low dose group ($p<0.05$) it was not as significant as standard group.

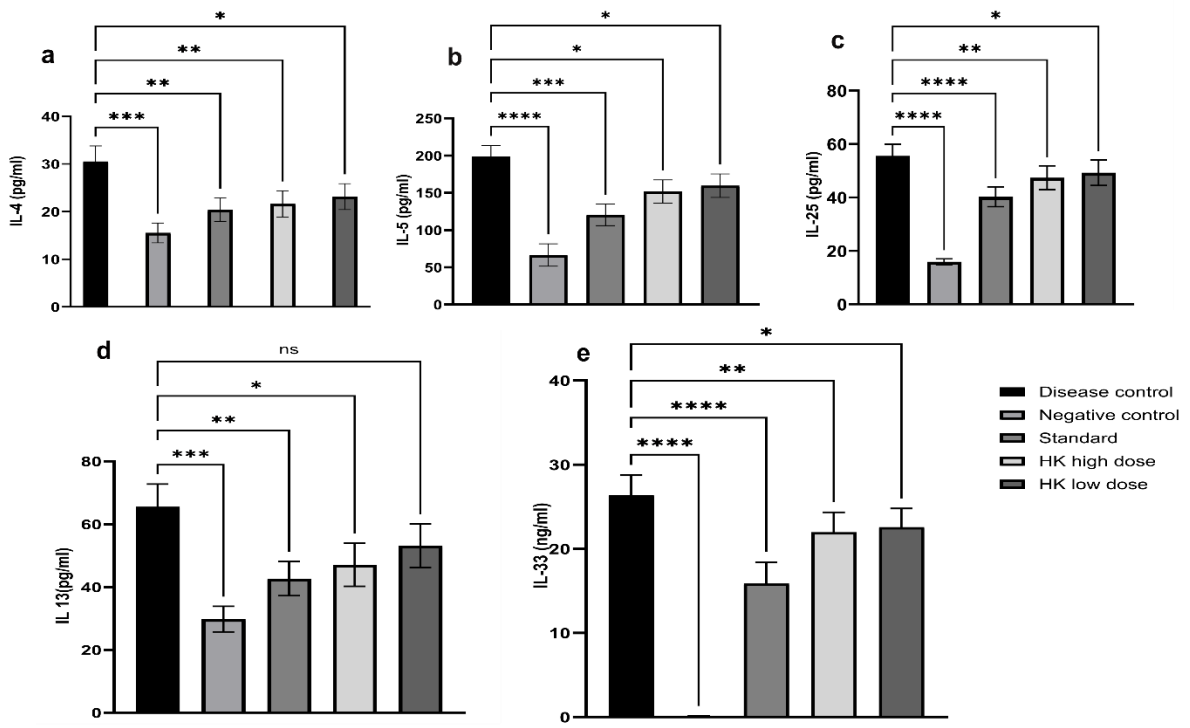


Fig 7: statistical analysis of cytokine levels in BALF (a) IL-4, (b) IL-5, (c) IL-25, (d) IL- 13 and (e) IL-33.

4. Discussion

Ovalbumin is a well-known allergen for generally th2 cytokine and eosinophil upregulation. Activated Th_2 cells produce cytokines IL 4, IL 5, IL 13 which in turn are responsible for IgE production in B cells thereby resulting in eosinophil activation and mucus generation. On the other hand, Th1 cytokines (IFN γ) differentiate from CD4+ naive cells against microbial pathogenic immune induction pathway. Further, IFN γ and IL 4 are known to regulate each other during immune inflammatory reactions. The ratio of Th_1/Th_2 cytokines has been accepted to represent the ratio or balance between IL 4 and IFN γ. In laboratory tests, the dominance of IL 4 in biological fluid indicates Th2 activation while IFN-γ indicates Th1. According to previous ovalbumin lead immunization leads to eosinophil activation and mast cell degeneration.

IL4 regulates allergic inflammation by promoting the th2 cells differentiation, Ig E synthesis and mucus hypersecretion. IL 5 promotes eosinophilic inflammation and infiltration in air ways and IL 13 induces airway hyperresponsiveness.

Epithelial cells in airway are primary line of defence for the respiratory system against foreign stimuli. In case of asthma presents the barrier function of epithelium is undermined by

disruption of tight junction of the central branched airways and long parenchyma. In presence of allergic stimuli lung parenchyma and branched small airways produces huge amount of the Th_2 cytokines responsible for development of Inflammatory response. Moreover these inflammation in digital sites are more severe than lung airways. Damaged epithelial cells secrets IL-25 and 33 which contribute to asthmatic features such as eosinophil infiltration, elevation of Ig E level increase mucus secretion In allergy induced mice. TH2 cytokines are released in type two inner innate lymphoid cells 2 (ILC 2) which are recruited by epithelial cell derived IL25 and 33.

In this study measurement was done for the epithelial cytokines and epithelial damage markers in lung tissue against ovalbumin challenged mice were investigated. Amount of Th2 cytokines, epithelial cytokines in BALF and lung tissue were observed to be increased while it was significantly decreased in different treated groups and in HK treated group in a dose dependent mannar. Due to ovalbumin-induced oversecretion of epithelial cells and dysfunction of epithelial layer while blood cells were recruited in airway pathway and ultimately increased organ weight. Therefore this increased % of organ weight to TBW (disease control) was found to be significantly decreased in other treated groups. This findings maybe correlated with % differential cell count and number of cell count. Eosinophil count was observed to be less that of the disease control group, which otherwise indicates better epithelial layer integrity and less tissue infiltration. Our histopathological findings are also in affirmation for those evedance.

5. Conclusion

From the above discussion, it appears that in different doses of HK, shows antiallergic activity against ovalbumin-induced allergy in a dose-dependent manner. This age-old ayurvedic preparation may be a possible low-cost alternative to the conventional medications that are already used. OVA-sensitised mice in the HK-treated group showed significantly reduced endothelial cytokine, which may promise anti-asthmatic activity.

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CHAPTER VII

Conclusion

Conclusion

The present study explores the antihistaminic potential of two classical Ayurvedic formulations, Haridra Khanda and Maha Manjisthadi Kwatham, both widely utilised in traditional medicine for their purported therapeutic properties. Through a series of pharmacological assays, Haridra Khanda demonstrated superior efficacy in mitigating histamine-induced responses, warranting further investigation into its bioactive constituents and their molecular mechanisms of action.

To elucidate the molecular basis of the observed antihistaminic activity, we undertook a comprehensive phytochemical analysis of Haridra Khanda to identify potential bioactive compounds. Molecular docking studies were conducted to assess the binding affinity of these metabolites to the Fcε receptor, a critical mediator in allergic reactions. Our findings suggest that specific phytoconstituents within Haridra Khanda exhibit a strong binding affinity to the Fcε receptor, potentially inhibiting IgE-mediated mast cell activation and subsequent histamine release.

Histamine, a key mediator of allergic reactions, primarily exerts its effects through the activation of H1 receptors, leading to the classical triple response: flush, flare, and wheal. Beyond its role in cutaneous manifestations, histamine is implicated in a spectrum of allergic conditions by facilitating the release of various cytokines and chemokines. This cascade of immune responses, initiated either by histamine itself or by immunoglobulin E (IgE), plays a pivotal role in the pathophysiology of allergic disorders.

Mast cell degranulation, triggered by allergen exposure, results in the release of histamine and other pro-inflammatory mediators, culminating in immunoinflammatory responses. While these responses are essential for host defence, aberrant immune activation in genetically susceptible individuals leads to exaggerated allergic reactions, significantly impairing quality of life. Such individuals, owing to their genetic predisposition, often require lifelong pharmacological intervention, imposing substantial psychological and financial burdens.

Given the holistic and multi-targeted nature of Ayurvedic formulations, Haridra Khanda emerges as a promising candidate for allergy management. Unlike conventional antihistamines, which primarily target H1 receptors, the bioactive constituents of Haridra Khanda may exert broader immunomodulatory effects, potentially modulating both Fcε receptor activity and mast cell stabilization. These multifaceted mechanisms could offer long-term benefits for individuals suffering from chronic allergic conditions.

From a public health perspective, the integration of Haridra Khanda into clinical practice could provide a cost-effective alternative to conventional antihistamines, particularly in resource-limited settings. In a developing nation such as India, where a significant proportion of the population suffers from allergic disorders, an evidence-based approach to Ayurvedic medicine could bridge the gap between traditional knowledge and modern pharmacology. Furthermore, fostering rigorous scientific validation of classical Ayurvedic formulations could enhance their credibility within the global scientific community, paving the way for standardized, regulatory-approved herbal therapeutics.

This paradigm shift towards an integrative approach may reduce dependence on synthetic pharmaceuticals, thereby minimizing potential adverse effects associated with long-term antihistaminic therapy. The findings from our study underscore the potential of Haridra Khanda as a viable alternative for managing allergic disorders. Through its putative interactions with histaminergic pathways and immune modulators, this classical formulation holds promise for enhancing patient outcomes while reducing the socioeconomic burden associated with chronic allergy management.

Future research should focus on clinical validation, pharmacokinetic profiling, and mechanistic elucidation to establish its role within the broader spectrum of integrative medicine. By bridging Ayurvedic wisdom with contemporary molecular science, we may unlock novel therapeutic avenues for millions of allergy sufferers worldwide.

CERTIFICATES
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Certificate

This is to certify that the project proposal no. **JU/IAEC-22/21** entitled **"Pharmacological evaluation of 2 Ayurvedic formulations in the treatment of Allergy"**, submitted by **Rudranil Bhowmik** has been approved/recommended by the IAEC of Department of Pharmaceutical Technology, Jadavpur University, in its meeting held on 01.06.2022 and 30: Swiss albino mice, 18: Hartley guinea pig (Number and Species of animals) have been sanctioned under this proposal for a duration of next 12 months.

Authorized by	Name	Signature	Date
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Member Secretary	Prof. Pallab Kanti Haldar	P. Haldar	01/6/2022
Main nominee of CPCSEA	Dr. Sankar Maiti	Sankar Maiti	01-06-2022

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Inflammation Research

REVIEW

Immunopathogenesis of urticaria: a clinical perspective on histamine and cytokine involvement

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Abstract

Background Urticaria is a clinical condition characterized by the appearance of wheals (hives), angioedema, or both. Over the last several decades, a better understanding of the mechanisms at play in the immunopathogenesis of urticaria has underscored the existence of numerous urticaria subtypes. Separating the different kinds of urticaria explicitly helps find the best detection method for the management of this skin disorder. Subtypes of urticaria also include both spontaneous and physical types. The conventional ones include spontaneous urticaria, constituting both acute and chronic urticaria. Therefore, a broad and effective therapy is essential for the diagnosis and treatment of urticaria.

Methods To understand the immunopathogenesis of urticaria, various databases, including PubMed, Scopus, and Web of Science, were used to retrieve original articles and reviews related to urticaria. While information on several clinical trials were obtained from clinicaltrials.gov database.

Results This article highlights the immunopathogenesis involved in the intricate interaction between cellular infiltration, immune reactions, coagulation cascades, and autoantibodies that underlie urticaria's pathophysiology.

Conclusion The recent progress in understanding urticaria can help to understand the intricate characteristics in the immunopathogenesis of urticaria and could play a beneficial role in the management of urticaria.

Keywords Urticaria · Diagnosis · Subtypes · Cytokines · Mast cell · Histamine

Abbreviations

AAS	Angioedema Activity Score
ADGRE2	Adhesion g-protein-coupled receptor e2
AECT	Angioedema control test
AE-QoL	Angioedema- quality of life questionnaire
ASST	Autologous serum skin test
AU	Acute urticaria
BCR	B cell receptor
BTK	Bruton's tyrosine kinase
C5aR	Complement component 5 receptor
CholU-QoL	Cholinergic urticaria-quality of life questionnaire
CholU	Cholinergic urticaria

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Research article

Ayurvedic herbal formulations Haridra Khanda and Manjisthadi Kwath (brihat) in the management of allergic rhinitis: A pharmacological study

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ABSTRACT

This study aims to pharmacologically validate Haridra Khanda (HK) and Manjishthadi Kwatham (brihat) (MMK) in allergy management using *invivo* and *invitro* studies to rationalize the prescription of these two ayurvedic polyherbal drug formulations, which are currently used in Indian government hospitals. Experimental animals received HK and MMK orally from day 0 to day 14 and histamine (1 mg/kg b.w/i.v) and 1 % evans blue (EB) (0.1 mL) via tail vein on day 14. The compound 48/80 (intracutaneous) challenged mice model followed the same technique. The former mimicked acute anaphylaxis and the latter mast cell degranulation. For both models, EB dye leakage was quantified spectrophotometrically to determine vascular permeability. Plasma histamine was measured in Compound 48/80-induced animals using LC-ESI-MS/MS. The guineapig received HK and MMK p.o. and 0.6 % histamine sprayed in a histamine chamber to simulate allergic rhinitis. Blood eosinophil count and sneeze rate were measured in histamine-challenged guineapigs. Goat R.B.C. membrane stability assay (mammalian cell membrane toxicity) and intracellular histamine-induced cytosolic Ca^{2+} release assay in Chinese hamster ovary (CHO) cells were performed *in vitro*. For both histamine and Compound 48/80 challenged animals, HK (22.81 % and 14.58 %) and MMK (19.71 % and 22.40 %) significantly reduced EB dye leakage ($p < 0.05$). Both formulations, HK and MMK considerably ($p < 0.05$) decreased plasma histamine (29.62 % and 25.37 % respectively) in mice and eosinophilic count (11.56 % and 9.94 % respectively) and sneeze rate (42.58 % and 29.03 % respectively) in guinea pigs. In membrane stability experiment, HK and MMK reduced RBC lysis. Both HK and MMK raw/dialysate blocked

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