Identification of *Anti-Allergic Natural compounds* through in-situ methods and Docking

A thesis submitted in partial fulfilment of the degree of

Master of Science

In

Biotechnology

Under the guidance of

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Declaration

I hereby declare that the work presented in the thesis entitled "**Identification of *Anti-allergic Natural Compounds* through In-Situ Methods and Docking**" is a bonafide work under the supervision and guidance of **Dr. Atul Kumar Upadhyay,** Professor, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala.

I also declare that this thesis or any other part of this thesis has never been submitted for any degree in this or any other university.

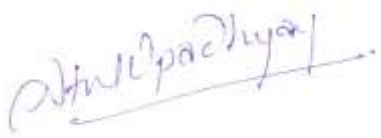
AYUSHI GUPTA

Place: Patiala

Certificate

This is to certify that the dissertation entitled "**Identification of *Anti-Allergic Natural compounds* through in-situ methods and Docking**" submitted by Ms. Ayushi Gupta (Roll no. 302201003) in the partial fulfillment of the requirements for the award of the degree of Master of Science in Biotechnology, Thapar Institute of Engineering and Technology, Patiala is a record of student's work carried out under my guidance and supervision.

It is also certified that the matter embodied in this thesis has not been submitted in part or full to any other institute or university for the award of any degree or diploma.



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In the accomplishment of this project, many people have best owned upon me their blessings and their heart pledged support, this time I am utilizing to thank all the people who have been concerned with this project.

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Abbreviations

S.No.	Abbreviation	Full form
1	AHR	Aryl Hydrocarbon Receptor
2	PDB	Protein Data Bank
3	ADMET	Absorption, Distribution, Metabolism, Excretion, Toxicity
4	IgE	Immunoglobin E
5	NMR	Nuclear Magnetic Resonance
6	mol	Indole-3-butyric acid
7	Kcal	Protocorm-like bodies
8	NIH	National Institute of Health
9	MDCK	Madin-Darby canine kidney
10	DS	Discovery Studio

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ABSTRACT

Abstract

Over the past two decades, food allergies have become an increasing public health concern and need for efficacious anti-allergic agents. We searched for natural compounds that could antagonize the Aryl hydrocarbon receptor (AHR), a transcription factor involved in immune and allergic responses as potential anti-allergic agents. The vast literature review and data mining made possible the selection of 30 natural compounds in a thorough screening process. The AHR structure was obtained from the Protein Data Bank (PDB) and missing residues were incorporated using Swiss-Model to optimize its docking capacity. Sampling with molecular docking (AutoDock Vina) was performed and those having a binding affinity (>–6.0 kcal/mol), were selected for further theoretical/predictive analysis of selectivity as these may be considered suitable candidates to evaluate in cell cultures or animal KING etc.

The molecular docking of high-affinity compounds with the AHR receptor, including detailed analysis on interaction types (hydrogen bond and hydrophobic contact) were validated using Discovery Studio. ADMET 2.0 Lab (online software) was then used to carry out ADMET analysis in order to examine drug-likeness of the shortlist compounds [16]. The Top Candidates: Beta Glucan, Alpha Tocopherol and Gama Tocopherol revealed ideal pharmacokinetic profiles with minimal toxicity.

CHAPTER 1

INTRODUCTION

Introduction

Pollen and dust mites, which are natural allergens, set off allergic reactions, but some plant-based compounds have antiallergic qualities (Gupta et al. 2023). Plant-based treatments such as extracts from turmeric stinging nettle, and butterbur, have shown good results in fighting allergies by changing immune responses and stopping histamine release (Gupta et al., 2023). Brown algae contain phlorotannins like eckol and dieckol, which have shown anti-allergic effects (Sugiura et al. 2021). Herbs such as *Piper longum*, *Ocimum tenuiflorum*, and *Azadirachta indica* are known to have antiallergic and anti-histaminic properties (Saini & Dhiman 2022). These natural substances could be possible replacements for regular antiallergic drugs, which can cause side effects like sleepiness and mental fog (Saini & Dhiman 2022). Plant chemicals, including phenolics, alkaloids, and terpenoids, have shown promise to change inflammatory responses and prevent allergies (Bellik et al. 2012). Many natural product-based formulas are now in clinical trials to test their anti-inflammatory and anti-allergic uses (Bellik et al. 2012).

1. Overview of Food Allergies and the Importance of Identifying Anti- Allergic Compounds

Food allergies are now a major health issue around the world, as more and more people react to everyday foods. About 8% of kids and 10% of grown-ups have food allergies, and these numbers keep going up (Sampson 2014). These reactions happen when the body's defences mistake some food proteins for threats causing symptoms that range from small skin rashes to severe, life-threatening anaphylaxis.

When the body reacts to allergens, it makes Immunoglobulin E (IgE) antibodies. These antibodies stick to the allergen and cause mast cells and basophils to release histamines and other substances that cause inflammation. This reaction leads to the signs of food allergies such as hives, swelling, stomach problems, and trouble breathing (Allen et al. 2020). Because allergic reactions anaphylaxis, can be so unpredictable and serious, they often need quick medical help with epinephrine.

Right now, doctors tell people with food allergies to stay away from foods that cause reactions and to have emergency treatments ready. But these methods don't fix the real problem: the body's mixed-up immune system that causes allergic reactions. We need new ways to stop or lessen allergic responses, so scientists are looking at natural substances that might help fight allergies (Ganesan et al. 2019).

People have used natural substances from plants, fungi, and sea creatures in old-fashioned medicine to treat many health problems, including allergies. These substances seem to work against allergies in different ways. They can stop the body from making IgE, keep mast cells stable, and change how the immune system works (Chen et al. 2021). Some examples are flavonoids, polyphenols, and terpenoids. These substances can reduce inflammation and change how the immune system works, which makes them good options to explore for treating allergies (Ganesan et al. 2019). Finding natural anti-allergic compounds is important for two main reasons: improving patient outcomes and reducing the economic burden of food allergies. Managing food allergies comes with significant costs, including medical care, medications, lost productivity, and decreased quality of life (Sampson, 2014). By developing effective natural therapies, we could alleviate these financial burdens and provide patients with more accessible and safer treatment options.

Additionally, discovering natural anti-allergic compounds aligns with the growing trend towards personalized medicine. Personalized medicine aims to tailor treatments to each patient's individual characteristics. Food allergies can vary greatly from person to person, including the specific allergens involved, the severity of reactions, and the patient's genetic background. This variability requires a personalized approach to treatment. Natural compounds, with their diverse chemical structures and mechanisms of action, have the potential to meet the unique needs of each patient (Allen et al., 2020).

Identifying effective natural anti-allergic compounds poses several challenges:

* The complex nature of allergic reactions, which involve multiple immune pathways and cells, requires a thorough understanding of the molecular mechanisms underlying these responses.
* The safety and efficacy of natural compounds must be rigorously evaluated through preclinical and clinical studies, which can be resource-intensive and time-consuming (Chen et al., 2021).

Despite these challenges, the potential benefits of natural anti-allergic compounds make this an area of significant research interest. The development of therapies that can modulate the immune system and prevent allergic reactions could lead to long-term protection against food allergies, providing a more sustainable solution than current treatment options (Zhou et al., 2021). Natural compounds are also often perceived as safer and more acceptable by patients, especially those seeking alternatives to synthetic drugs.

Food allergies are a growing public health concern that requires new and innovative approaches to treatment and prevention. The identification of natural anti-allergic compounds offers a promising avenue for developing safer, more effective therapies that can improve the quality of life for individuals with food allergies. By exploring the potential of these compounds, researchers aim to address the limitations of current treatments and provide new solutions for managing this global health challenge.

2. Significance of Computational Methods in Allergen Identification and Drug Discovery

The introduction of computer-based methods has changed the field of drug discovery, providing effective tools for finding and improving therapeutic substances. When it comes to identifying allergens and discovering new drugs, computational techniques offer several benefits such as:

* The capability to quickly examine extensive collections of substances
* The ability to forecast how molecules will interact with each other
* The capacity to enhance the chemical characteristics of potential drugs

These techniques are becoming more and more crucial in the quest for new anti-allergic substances. They allow scientists to investigate the healing possibilities of natural compounds in a way that is both more efficient and less expensive.

Molecular docking is one of the most widely used computational techniques in allergen identification. This method allows researchers to model the interactions between a target protein, such as the Aryl Hydrocarbon Receptor (AhR), and potential ligands, including natural compounds. Docking simulations can predict the binding affinity and specificity of a compound to a target protein, providing valuable insights into its potential therapeutic effects (Ganesan et al., 2019). For example, studies have shown that certain natural compounds can bind to AhR and modulate its activity, potentially offering new treatment options for allergic diseases (Zhou et al., 2021).

The AhR is a ligand-activated transcription factor involved in regulating immune responses and xenobiotic metabolism. Dysregulation of AhR signaling has been linked to various allergic and inflammatory diseases, making it an attractive target for therapeutic intervention (Ganesan et al., 2019).

Computational docking studies can help identify natural compounds that bind to AhR and modulate its activity, potentially leading to the development of new treatments for food allergies (Zhou et al., 2021).

In addition to molecular docking, virtual screening is another important computational approach in allergen identification. Virtual screening involves using computer algorithms to search large databases of chemical compounds for those that are most likely to bind to a specific target protein (Chen et al., 2021).

This technique can be particularly useful in identifying natural compounds with potential anti-allergic properties, as it allows researchers to efficiently screen thousands of compounds and prioritize those with the highest likelihood of success (Chowdhury et al., 2021).

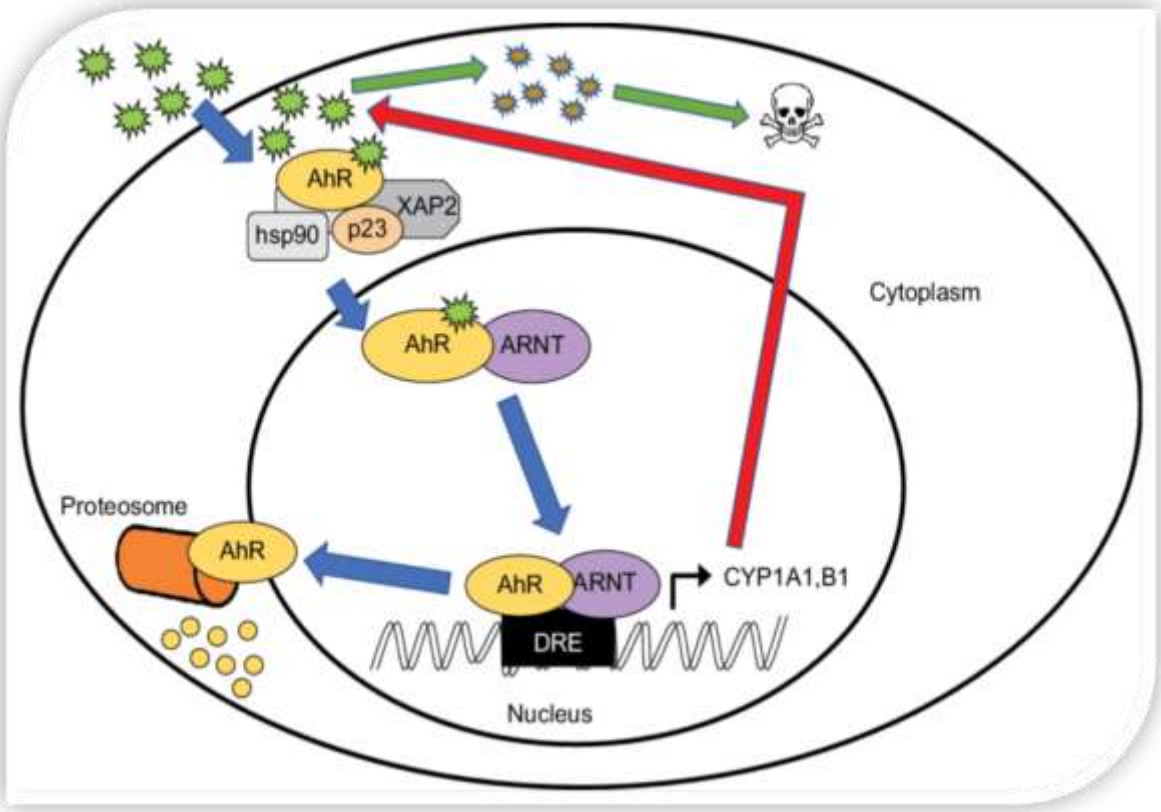


Fig.1 AhR mechanism

Structure-based virtual screening relies on the three-dimensional structure of the target protein, which can be obtained from experimental methods such as X-ray crystallography or nuclear magnetic resonance (NMR) spectroscopy, or from homology modelling techniques (Ganesan et al., 2019). In the context of allergen identification, virtual screening can identify natural compounds that inhibit or modulate key proteins involved in allergic responses. For example, virtual screening has been used to identify inhibitors of enzymes such as histamine release, which plays a central role in developing allergic symptoms (Zhou et al., 2021).

Beyond molecular docking and virtual screening, computational methods such as quantitative structure-activity relationship (QSAR) modelling and molecular dynamics simulations also play critical roles in allergen identification and drug discovery.

QSAR modelling involves developing mathematical models that relate a compound's chemical structure to its biological activity, allowing researchers to predict the activity of new compounds based on their chemical structure (Chowdhury et al., 2021). This approach can optimize the structure of natural compounds to enhance their anti-allergic properties and identify novel compounds with similar chemical features that may also exhibit anti-allergic activity (Jahangir et al., 2021).

Molecular dynamics simulations provide insights into the dynamic behavior of proteins and their interactions with ligands at the atomic level. These simulations can be used to study the conformational changes of a protein upon ligand binding and the stability and flexibility of the protein-ligand complex. Understanding these dynamic interactions allows researchers to gain a deeper understanding of the molecular mechanisms underlying the therapeutic effects of natural compounds and identify ways to optimize their binding affinity and specificity (Ganesan et al., 2019).

Integrating computational methods with experimental approaches is essential for the successful identification and development of anti-allergic compounds. While computational methods provide powerful tools for predicting protein-ligand interactions, experimental validation is necessary to confirm the biological activity and therapeutic potential of the identified compounds (Jahangir et al., 2021). This iterative process of prediction and validation allows researchers to refine their computational models and improve the accuracy of their predictions, leading to the identification of more effective and safer anti-allergic therapies (Zhou et al., 2021).

Moreover, computational methods in allergen identification are not limited to the discovery of new compounds. These methods can also optimize existing therapies by identifying potential off-target effects, predicting drug-drug interactions, and designing more effective delivery systems (Chowdhury et al., 2021). For example, computational modeling can be used to design nanoparticles or other drug delivery vehicles that can enhance the bioavailability and targeted delivery of anti-allergic compounds, reducing the risk of side effects and improving therapeutic outcomes (Allen et al., 2020).

In conclusion, computational methods play a crucial role in the identification and development of anti-allergic compounds. These methods offer numerous advantages over traditional experimental approaches, including the ability to rapidly screen large libraries of compounds, predict molecular interactions, and optimize the chemical properties of candidate drugs. By integrating computational techniques with experimental validation, researchers can identify natural compounds with significant therapeutic potential and develop new treatments for food allergies that are more effective, safer, and tailored to the individual needs of patients. As the prevalence of food allergies continues to rise, the use of computational methods in allergen identification and drug discovery is likely to become increasingly important in the search for novel and innovative therapies

3. Anti-Allergic Compounds and Their Modes of Action

The in silico analysis identified several natural compounds with promising binding affinities to the AhR. Here's a closer look at few of these compounds and their potential anti-allergic mechanisms:

- **Dioxinodehydroeckol (Glycine max)**: This compound, derived from soybeans, exhibited the strongest predicted binding affinity to the AhR (-7.8 kcal/mol). While the exact mechanism of its anti-allergic effect remains to be elucidated, studies suggest it may possess anti-inflammatory and immunomodulatory properties
- **Ergosterol (fungi)**: This sterol found in fungi like mushrooms showed a high binding affinity (-8.6 kcal/mol). Although its role in allergy is not fully understood, ergosterol has been shown to modulate immune responses, suggesting potential benefits in allergic conditions
- **Cordycepin (Cordyceps militaris)**: This fungal metabolite possessed a binding affinity of -6.4 kcal/mol. Cordycepins have been reported to exhibit immunomodulatory effects, potentially suppressing allergic airway inflammation
- **Beta-glucan (Saccharomyces cerevisiae)**: This polysaccharide found in yeast displayed a binding affinity of -6.4 kcal/mol. Beta-glucans are known for their immunomodulatory and anti-inflammatory properties, which may be beneficial in allergies

- **Alpha-tocopherol (Vitamin E)**: This natural antioxidant exhibited a binding affinity of -6.2 kcal/mol. Alpha-tocopherol may offer protection against allergic inflammation by combating free radical activity
- **Gamma-tocopherol (Vitamin E)**: Similar to its counterpart, alpha-tocopherol, gamma-tocopherol (binding affinity = 6.6 kcal/mol) may also provide anti-allergic benefits through its antioxidant properties

A) CORDYCEPS

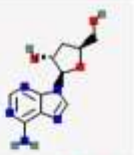
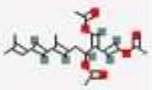
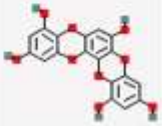
B) PALM OIL

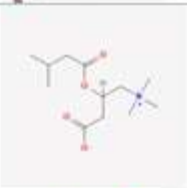
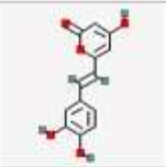
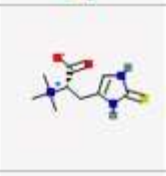
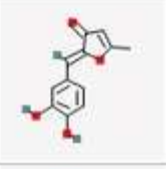
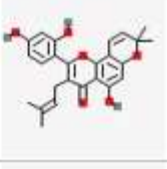
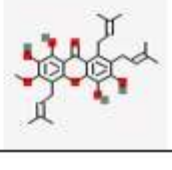
C) OYSTER MUSHROOM

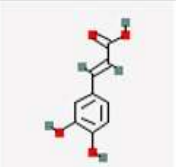
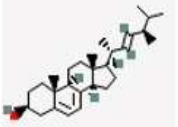
D) HONEY

Fig 2. Sources of anti-allergic compounds

4. List of Anti-allergic compounds

NATURAL SOURCE	COMPOUND	Pubchem ID	2D STRUCTURE
Cordyceps (Ophiocordyceps sinensis)	Cordycepins	6303	
Maitake Mushroom (Grifola frondosa)	Beta-Glucan	439262	
Rhipocephalus phoenix	Rhipocephalin	5281528	
Ecklonia stolonifera	Dioxinodehydroeckol (DHE)	10429214	
Cordia verbenacea	Alpha-Humulene	5281520	
Royal Jelly/Honey	10-hydroxy-2-decenoic acid (10-HDA)	5312738	
Vegetable oil	α-tocopherol	14985	
Palm oil	γ- tocopherol	45356270	
Humicola grisea	3-(2-(4-hydroxyphenyl)-2-oxoethyl)-5,6-dihydropyridin-2(1H)-one	23424618	

Beauveria bassiana	pyridovericin	54697562	
Penicillium griseofulvum	Isovalerylcarnitine	6426851	
Phellinus linteus	HISPIDIN	54685921	
Pleurotus ostreatus (Oyster mushroom)	ergothioneine	5351619	
Phellinus linteus	Inotilone	11644214	
Mori Cortex Radicis	morusin	5281671	
Comfrey plant/urine	Allantoin	204	
fruiting body of Antrodia cinnamomea	antcin A	10004121	
Garcinia nujiangensis	nujiangexanthone A	66553581	

NATURAL SOURCE	COMPOUND	Pubchem ID	2D STRUCTURE
Coffee	Caffeic acid	689043	
Cell membrane of Fungi and Protozoa	Ergosterol	444679	
Feverfew Plant	Parthenolide	7251185	
Yeast, Trout fish, Shrimp, Crayfish	Astaxanthin	5281224	

CHAPTER 2

REVIEW OF LITERATURE

Review of Literature

Allergic diseases like allergic rhinitis, asthma, and food allergies have become more common globally over the years. The World Health Organization (2024) states that these conditions are now major public health issues affecting millions of people around the world. While traditional treatments such as antihistamines and corticosteroids can help relieve symptoms, they often do not address the underlying causes of these diseases.

As a result, there is increasing interest in finding new anti-allergic treatments from natural sources due to their wide range of chemical structures and biological effects. This review aims to provide a comprehensive overview of existing research on anti-allergic compounds, with a specific focus on natural products and how they work in the body. Additionally, it will discuss the role of the Aryl Hydrocarbon Receptor (AhR) in allergic reactions and its potential as a target for future

Prevalence and Impact of Allergic Diseases

Allergic diseases have significantly increased in prevalence over the past few decades. According to a study by Pawankar, Canonica, Holgate, and Rosenwasser (2013), the rates of allergic conditions like allergic rhinitis and asthma have risen sharply, which is linked to changes in environmental factors, lifestyle choices, and genetic predispositions.

Environmental factors such as:

- increased exposure to allergens (substances that trigger allergic reactions)
- higher levels of pollution (harmful substances in the environment)

have been identified as potential contributors to this trend.

Lifestyle changes, including:

1. reduced microbial exposure due to modern hygiene practices
2. shifts in dietary patterns (what people eat)

also play a role in the increasing prevalence of allergies (Bousquet et al., 2020).

The economic impact of allergic diseases is significant. It includes both direct costs like medical expenses and indirect costs such as lost productivity due to illness (Bousquet et al., 2020). Allergies can also have a profound effect on quality of life, with individuals experiencing chronic symptoms that interfere with their daily activities and overall well-being.

This growing burden highlights the urgent need for new and effective treatments for allergic diseases.

Limitations of Conventional Anti-Allergic Medications

Although conventional anti-allergic drugs such as antihistamines and corticosteroids are widely used, these drugs have certain disadvantages. Antihistamines block histamine receptors, blocking its action and facilitating symptoms such as itching, sneezing, and nasal congestion. Nevertheless, antihistamines do not affect the underlying mechanism of the allergic process and, moreover, are also undesirable in terms of side effects such as sedation and xerostomia. Corticosteroids have a pronounced anti-inflammatory effect and are the most effective drugs in this group for reducing inflammation and controlling allergic symptoms. At the same time, the use of corticosteroids is associated with a significant number of undesirable effects: immunosuppression, metabolism disorders, and the risk of severe side effects when taken systematically. These drugs, therefore, do not meet the criteria for safe, effective, and impact on the pathological process.

Natural Products as Anti-Allergic Agents

Natural products are plant-, animal-, or microorganism-derived products with a rich history of use in traditional medicine and are being increasingly sought after for their potential uses in allergic disease treatment. As natural products continue to be identified, preclinical, clinical, safety, and manufacturing applications have become more complex. With their complex structures and the diversity of biological activities, natural products and the purity and activity of their development as drugs are not only attractive but nearly limitless candidates for drug

discovery. Many natural products are found with proven anti-allergic activities against different allergic sources under varied mechanisms:

- **Polyphenols:** Quercetin, a flavonoid present in onions, apples, citrus fruits, among other vegetables and fruits. Inhibits histamine release and has an anti-inflammatory effect. Quercetin can stabilize mast cells and inhibit oxidative stress, highlighting its anti-allergy effect, Curcumin is a polyphenol found in the spice turmeric. It also has a strong anti-inflammatory and antioxidant action, helping to relieve the signs of allergic reaction and inflammation. Resveratrol, also part of the polyphenols, appears to exhibit benefits in the treatment of immune response and allergic inflammation.
- **Alkaloids** – Berberine is an alkaloid obtained from plants such as Berberis species that have been reported to have an effect in moderation of immune response, in addition to anti-allergic effects. The anti-inflammatory and immunomodulatory impacts of berberine are thought to be due to its suppressant effect on the production of pro-inflammatory cytokine. For instance, ephedrine, an alkaloid used in traditional Chinese medicine for respiratory problems, has anti-inflammatory characteristics. Terpenoids – limonene and camphor can inhibit inflammatory reactions and have an immunomodulatory effect. Limonene, a monoterpenic compound found in citrus, is thought to be an anti-inflammatory. Limonene have been verified to promote anti-inflammatory cytokine factories and deactivate the in vitro inflammatory process due to its ability to reduce pro-inflammatory cytokines production.issent on per cent
- **Steroids:** Moreover, naturally occurring corticosteroids called corticosteroids, which are currently found in some plants, have anti-inflammatory properties similar to those of synthetic corticosteroids. Such substances are potentially less likely to cause side effects. For example, forskolin is a compound derived from Coleus forskohlii that has anti-inflammatory properties and is thought to be beneficial for managing allergic conditions.
- **Peptides:** Another group is mast cell-stabilizing peptides that suppress histamine release. It can inhibit the degranulation of mast cells, which reduces the release of histamine and other substances contributing to inflammation. Sakaguchi et al. indicated the peptide isolated from some insects' venom as a known mast cell-stabilizing peptide.

Screening and Isolation of Anti-Allergic Compounds

The discovery and development of new anti-allergic agents from natural sources involve sophisticated methodologies:

High-Throughput Screening (HTS): The discovery and development of new anti-allergic agents from natural sources require complex methodologies. For example, High-Throughput Screening that allows researchers to screen enormous libraries of compounds and identify those that have anti-allergic activity. Modern HTS technologies have significantly evolved over the past few years, facilitating the rapid discovery of novel anti-allergic agents, that can be further developed for clinical use.

Bioassay-Guided Fractionation: Bioassay-Guided Fractionation has been used to identify new natural agents with anti-allergic potential. Bioassay-guided fractionation is based on the ability of specific assays to detect compounds of interest within a complex mixture. This method has been used with a broad range of compounds and extracts, including plant extracts and marine life.

Molecular docking and virtual screening: Molecular docking and virtual screening are another vital approach used to predict how natural compounds may interact with different proteins vital in responding allergic reactions. This would similarly provide information on their energy and the propensity to create solid hydrogen bonds. For instance, molecular docking gives essential information on the possibility that the compounds and ligands may bind to receptors, whereas virtual screening abolishes the number of molecules that may be analyzed. This allows this method to screen a number of particles based on prediction. Thus, Kumar et al. stated the most promising concentration between the best-compounded dock with mucin 1 protein is 6XU5.

Diversity of Anti-Allergic Compounds

Most natural products with anti-allergic properties have a large diversity of chemical structures and biological activities. The broad spectrum of these compounds signifies the potential to target various dimensions of allergic diseases.

Polyphenols: First, polyphenols such as quercetin, curcumin, and resveratrol are remarkable polyphenols with a high anti-allergic capacity. For example, quercetin has an antihistamine effect as it stabilizes mast cells and reduces histamine release, all of which are significant compounds in treating allergic symptoms. Curcumin, which has anti-inflammatory and anti-oxidative effects, also plays a role in the treatment of allergic responses. On the other hand, resveratrol's anti-inflammatory properties through immune regulation are significant indicators of polyphenols in important allergic compounds.

Alkaloids: Berberine and ephedrine are important alkaloids known for their anti-allergic effects. Berberine has immunomodulatory properties and can inhibit the production of pro-inflammatory cytokines, which underscores its potential as an anti-allergic treatment (Zhao et al., 2019). Similarly, ephedrine has been traditionally used for respiratory issues and exhibits anti-inflammatory effects, indicating its possible role in treating allergic diseases (Zhao et al., 2019).

• **Terpenoids:** Limonene and camphor are terpenoids that also show anti-allergic properties. Limonene is effective in reducing inflammation and boosting anti-inflammatory cytokine activity, making it a strong candidate for managing allergic conditions (El-Sheikh et al., 2021). Additionally, camphor's anti-inflammatory and pain-relieving qualities further enhance its potential to relieve allergic symptoms (El-Sheikh et al., 2021).

• **Steroids:** Naturally occurring corticosteroids, like those from Coleus forskohlii, can offer anti-inflammatory benefits and may serve as a safer alternative to synthetic corticosteroids (Newman & Cragg, 2020). These compounds show similar therapeutic effects but may come with fewer side effects.

• **Peptides:** Mast cell-stabilizing peptides present a distinctive method for managing allergies by inhibiting histamine release and lessening allergic reactions. Research has explored their

potential in stabilizing mast cells and reducing allergic responses (Sakaguchi et al., 2020).

Mechanisms of Action

The mechanisms through which anti-allergic compounds work are varied and intricate, showcasing their capacity to influence different facets of allergic responses:

• **Inhibition of Histamine Release:** Numerous natural compounds, including quercetin and mast cell-stabilizing peptides, prevent the release of histamine from mast cells. Since histamine plays a crucial role in allergic reactions, compounds that inhibit its release can help alleviate allergic symptoms and reduce inflammation (Baur & Sinclair, 2006; Sakaguchi et al., 2020).

• **Modulation of Immune Cell Function:** Natural compounds can also affect the activity of immune cells that participate in allergic responses, such as T cells, B cells, and mast cells. By influencing immune cell function, these compounds can change the progression of allergic reactions and enhance clinical outcomes (Jin et al., 2018).

Reduction of Inflammation: Anti-inflammatory compounds, such as curcumin and resveratrol, help alleviate allergic symptoms by reducing inflammation in affected tissues. These compounds inhibit the production of pro-inflammatory cytokines and enhance the activity of anti-inflammatory mediators (Hewlings & Kalman, 2017; Ryu et al., 2014).

•**Targeting Specific Allergens:** Some natural compounds interact directly with allergens, preventing their binding to IgE antibodies and triggering allergic reactions. This mechanism involves the direct interaction of compounds with allergen molecules, reducing their ability to induce allergic responses (Zhao et al., 2018).

The Role of the Aryl Hydrocarbon Receptor (AhR)

The Aryl Hydrocarbon Receptor (AhR) is a transcription factor that is activated by ligands and plays a role in various biological functions, such as immune responses and inflammation. Recent studies indicate that activating AhR could influence allergic reactions, positioning it as a potential target for new therapies (Kiss et al., 2011). When AhR is activated, it affects the differentiation of immune cells, the production of cytokines, and the expression of genes related to allergic inflammation. Ligands that bind to AhR can shift the balance between pro-inflammatory and anti-inflammatory signals, which in turn can impact the intensity of allergic reactions (Tian et

al., 2020). By focusing on AhR, there is a possibility to restore a more balanced immune response and reduce allergic symptoms.

Computational Approaches for Identifying AhR Ligands

Computational methods such as molecular docking and virtual screening are essential for identifying natural compounds that can bind to and activate the AhR. Molecular docking simulations help predict how strongly compounds will bind to the AhR receptor, offering valuable insights into their potential interactions (Morris et al., 2009). Meanwhile, virtual screening techniques enable researchers to quickly assess large libraries of natural compounds for their ability to influence AhR activity (Xu et al., 2020). These computational strategies aid in discovering new AhR ligands that could have therapeutic uses in treating allergic diseases. By combining computational predictions with experimental validation, researchers can pinpoint and develop new anti-allergic agents that have specific mechanisms of action.

Natural products provide a rich and varied source of potential anti-allergic agents, each with its own unique mechanisms and therapeutic benefits. Research into natural compounds like polyphenols, alkaloids, terpenoids, steroids, and peptides has shown their potential in managing allergic diseases through different pathways. The Aryl Hydrocarbon Receptor (AhR) stands out as a promising target for therapeutic intervention, with computational methods helping to identify potential AhR ligands. The integration of traditional knowledge with modern scientific techniques and innovative strategies offers great potential for developing new anti-allergic therapies. More research is necessary to fully understand how these compounds work, confirm their effectiveness through clinical trials, and investigate their possible applications in clinical settings.

CHAPTER 3

MATERIAL AND METHODS

Material and Methods

This chapter describes the materials and methods used for searching of phytochemical whose usage might be helpful for *anti-allergic* treatment in the computer.

Experimental Design:-

Fig 3. Flowchart of different methods used for searching phytochemical

1. Literature Search and Data Mining

Data mining of required material involving anti-allergic natural compounds, Ahr proteins and their role in anti-allergy, pathway of allergic reactions were retrived.

2. Selection of Candidate Compounds

After data mining, the list of potentially anti-allergic natural compounds had been developed. To make the successive selection from this list, the following criteria where used: Selection criteria included:

- **Documented Anti-allergic Activity:** Compounds were ranked higher if scientific literature data testified to their ability to display anti-allergic effect. The present strategy sustained an emphasis on the bad compounds by identifying those that have been known to have therapeutic effects in allergy.
- **Chemical Structure and Target Interaction:** The chemical structures of the compounds were analyzed ad potential of compounds to bind to the AhR protein, the selected target receptor because of its efficacy and a known link to allergic reactions. Based on the structural features that appeared to be related to favourable interactions with AhR, the following compounds were considered for further analysis.
- **Availability and Safety Profile:** The chemical substances used were evaluated in terms of their being available and their risk on the patients. Ideally, the chosen compounds were more easily available for additional studies, and the essential compounds had an organoleptically acceptable safety profile in the event of their subsequent development as anti-allergic products.

Thus, using these criteria, about 30 natural compounds were selected for the further study based on the docking simulations. These chosen compounds included:

- Maackiain (Astragalus membranaceus)
- Ergosterol (fungi)
- Inotilone (various plants)
- Smallest of the ninty distinct parasites, Antcin A derived from Parthenium hysterophorus.
- Alpha-tocopherol (Vitamin E)
- Vegetarian sources and alternative sources – Astaxanthin (from several marine organisms)

- Beta-glucan (Saccharomyces cerevisiae) Parthenolide (Tanacetum parthenium)
- Cordycepin (Cordyceps militaris)
- Dioxinodehydroeckol (Glycine max)
- 3-(2-(4-Hydroxyphenyl)-2-oxoethyl)-5,6-dihydropyridin-2(1H)-one (Pyridovericin)
- Gamma-tocopherol (Vitamin E)
- Morusin (Morus alba)
- Nujiiangexanthone A found in Millettia griffoniana
- Hispidin (various plants)
- Rhipocephalin (Rhipocephalus phoenix)
- Alpha-Humulene (Cordia verbenacea)
- 10-hydroxy-2-decenoic acid (10-HDA)
- Pyridovericin (*Beauveria bassiana*)
- Isovalerylcarnitine (Penicillium griseofulvum)
- Ergothioneine (Oyster mushroom)
- Inotilone (Phellinus linteus)
- Allantoin (comfrey plant)
- Antcin A (fruiting body of Antrodia cinnamomea)
- Caffeic acid (coffee)
- Parthenolide (feverfew plant)
- Astaxanthin (yeast)

3. Purchasing and Synthesis of Compound Templates

The 3D conformation of the selected natural compounds was obtained from the PubChem database, a centralized repository of information related to small molecules sponsored by the NIH. PubChem gives structures in a variety of formats ideal for the computational modelling.

4. Recruitment and Characterization of AHR Protein Structure

The AHR protein was selected because it was the intended receptor for docking simulations of the investigated ligands; conversely, it is known to play a critical role in mediating allergic

reactions. AHR protein plays a role in immunomodulation and it was associated with different courses of allergic diseases. Although an AHR structure was initially retrieved from PubChem, the structure had missing residues that could compromise the fidelity of docking simulations.

To counteract this problem, two other structures of AHR were obtained with PubChem IDs 5V01 and 5Y7Y. These structures were subsequently rebuilt to the desired conformations using the available Swiss-Model online homology modelling software. A common technique of homology modelling is compared with known structures with relatively high identity to the target protein (AHR in the present study) to create an improved and more accurate model. The changed protein structures were then downloaded.

Residues in the remodelled protein structures were also confirmed in PyMOL a molecular modelling tool to ensure that there were no missing residues. PyMOL enables a viewer to manipulate and analyze models of protein in the three-dimensional perspective. After this, the proposed structures of the AHR remodelled to exclude missing residues were verified and downloaded for other purposes.

5. Docking Simulations

- **Ligand and Protein Preparation:**

The structures of both the AHR protein and the natural compounds were converted into formats compatible with AutoDock Vina. This may involve converting the structures to PDBQT format, a format specifically designed for docking simulations.

Kollman charges were assigned to the protein and polar hydrogens were added to both the protein and the compounds. These steps help to account for electrostatic interactions that are crucial for binding affinity.

Any unnecessary atoms or charges were removed from the compounds to optimize the docking process. For example, water molecules or solvent molecules might be removed as they are not directly involved in binding to the protein.

- **Defining the Binding Site**:

The binding pocket of the AHR protein, the specific region where ligand binding occurs, was defined for docking simulations. This can be achieved using various methods, such as utilizing knowledge from existing literature on AHR ligand binding or by identifying cavities within the protein structure using software tools.

- **Docking Runs and Analysis:**

AutoDock Vina was used to perform multiple docking runs for each natural compound with each remodelled AHR structure. Each docking run generates a set of possible binding poses for the compound within the protein's binding pocket.

The binding affinity of each docked pose, typically measured in terms of Gibbs free energy (kcal/mol), was calculated by AutoDock Vina. A lower binding energy signifies a more favorable interaction between the compound and the protein.

PyMOL was used to visualize the docked complexes and analyze the interactions between the natural compound and the AHR protein. This may involve examining hydrogen bonds, hydrophobic interactions, and other factors that contribute to binding affinity.

- **Selection of Candidate Compounds:**

Based on the docking results, the top 16 compounds with the most favorable binding affinities (below -6 kcal/mol) were selected for further analysis. The selected compounds included:

    - 3-(2-(4-Hydroxyphenyl)-2-oxoethyl)-5,6-dihydropyridin-2(1H)-one (Pyridovericin)
    - Alpha-tocopherol (Vitamin E)

- A (Parthenium hysterophorus)
- Beta-glucan (Saccharomyces cerevisiae) (-6. 4 kcal/mol).
- Peptides (Phallus deliciarius) (-7.)
- Dioxinodehydroeckol (Glycine max) : 7. 8 kcal/mol
- Ergosterol (fungi) (-8 . 6 kcal/mol)
- Gamma tocopherol vitamin E (6 . 6 Kcal/mol)
- Inotilone (Various Plant) (6. 7 kcal/mol)
- Hispidin (var., plants) – 7. 1 kcal/mol
- The strategy Kenko recommends to strengthen stomach comprised herbs such as Maackiain (Astragalus membranaceus) (-7. 6 kcal/mol).
- Morusin isolated from the fruits of Morus alba (8. 4 kcal/mol)
- Millettia griffoniana – Nujiiangexanthone A (- 8627.2 kJ/mol)
- Extract of Tanacetum parthenium (semiqu quantitative structure activity relationship Parthenolide 6. 9 kcal/mol.

6. 2D Interactions and Visualization Analysis

The theoretical binding modes of the 16 selected compounds with AHR protein were eventually explored by using the Discovery Studio software. Discovery Studio also has a number of tools for molecular interaction visualization as well as interaction analysis.

• Two-dimensional (2D) plots of the selected natural compounds with the amino acid residues of the AHR protein were ‘observed’ using the software. This offered information on the nature of interactions that have an impact on binding energy such as hydrogen bond interactions, pi-pi stacking interactions.

• It also offered the ability to distinguish between one type of bond or another that occurs between the compound and the protein, which were helpful to understand the binding mechanism.

7. In-Silico ADMET Prediction

In order to evaluate the possible toxicity and carcinogenic effects of 16 chosen compounds in-silico ADMET software was used. The use of ADMET prediction is useful to give an early insight of safety challenges that may be associated with a drug in the course of its discovery.

• The PubChem database was used to get the SMILES notations, the canonical SMILES strings of small molecules identifier of the selected compounds.

• These SMILES strings were then entered to online ADMET prediction servers or software programs.

• Computationally, the software forecasted more ADMET properties of the compounds such as the absorption ability, ability to cross blood-brain barrier, and carcinogenicity.

Analysis of ADMET Prediction Results: Analysis of ADMET Prediction Results:

The deduced ADMET properties of the 16 compounds were thus modeled and predicted with considerable care. Absorption aids, low BBB permeability and moderate drug-like were the selected compounds. Furthermore, according to a model of toxic and carcinogenic risk, any compound expected to have high toxicity/carcinogenicity was screened out from the topical system.

Integration with Docking and 2D Interaction Analysis:

Therefore, ADMET prediction of in-silico results was combined with the outcomes from docking simulation and 2D interaction evaluation. Such an approach let for a better analysis of the prospects of the chosen natural compounds, including their effectiveness.

For instance, a compound that has been found to have great binding affinity for the AHR protein and good interaction the AHR protein compared to other proteins may be rejected if ADMET in-silico prediction showed high toxicity. In contrast, a compound which has moderate binding affinity but beneficial toxicity profile may be considered for a further investigation into an enhancement of its binding characteristics.

Thus, based on the synchronous results, the lists of more effective natural compounds with the potential anti-allergic activity and tolerable toxicity were defined for further in vitro and in vivo.

• **Absorption:** This property relates to the capacity of a compound to reach the systemic circulation, from the site of administration. In this aspect, software used for the prediction of intestinal absorption and human oral bioavailability was applied

• **Blood-Brain Barrier (BBB) Penetration:** The BBB is a complex border which controls the exchange of materials between blood and the brain. Software suggested its possibilities for compounds' ability to pass the BBB which is useful while evaluating possible side effects.

• **Drug-likeness:** The physicochemical characteristics that determine the possibility of a compound under consideration to be developed into a drug. Some of the properties forecasted by the software included molecular weight, lipophilicity and number of hydrogen bond donors/acceptors to establish the drug-likeness of the compound.

• **Toxicity:** The toxicity which refers to the ability of a compound to cause harm to the body was compared. Software made predictions of different toxicity parameters including acute toxicity, Ames gene mutation and Carcinogenicity.

CHAPTER 4

RESULTS

Results

1. Selection of Anti-Allergic Compounds

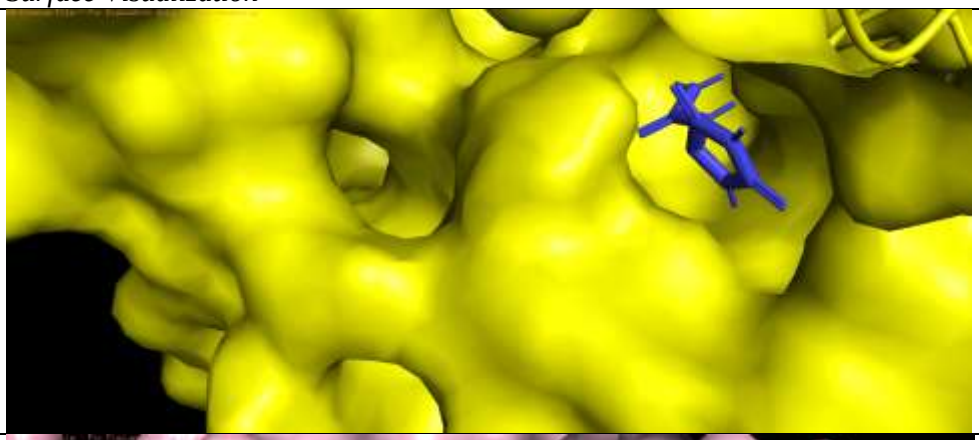
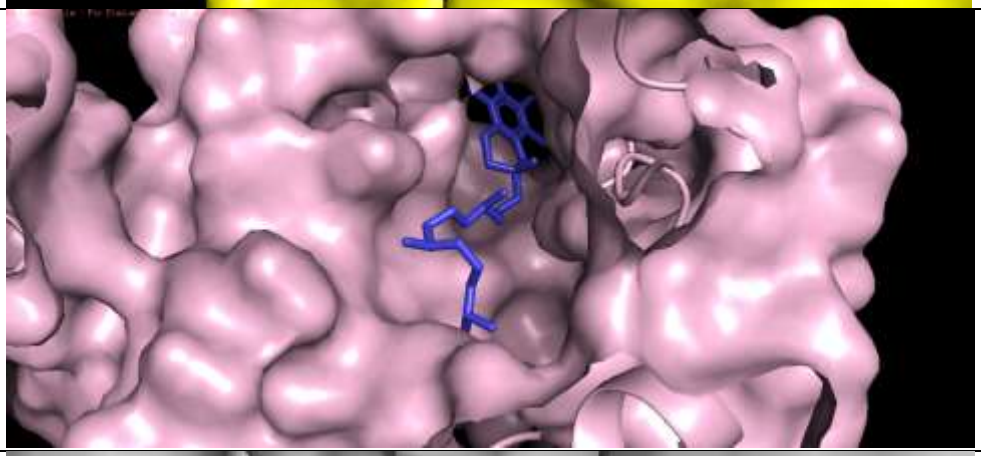
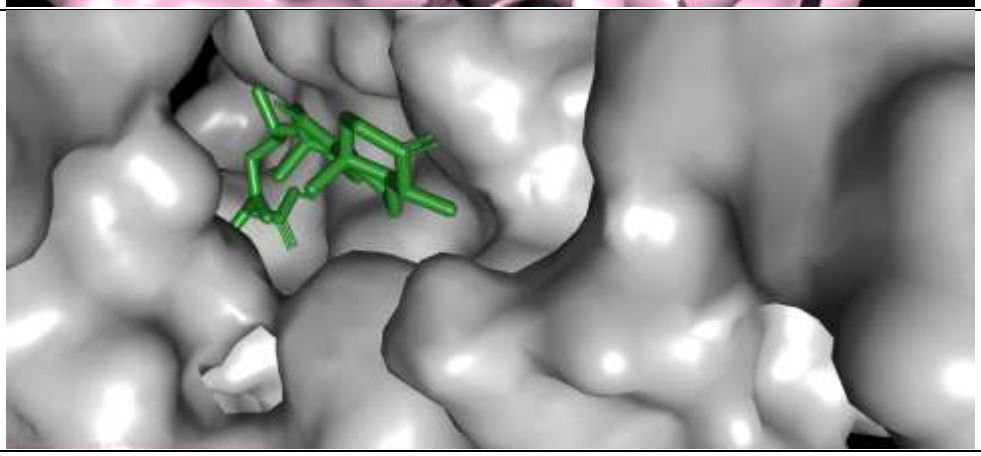
A thorough selection process was carried out to find potential natural anti-allergic compounds. We identified 30 natural compounds through an extensive review of literature and data mining from scientific databases. These compounds were chosen for their known anti-allergic properties and were then assessed for their potential interaction with the Aryl hydrocarbon receptor (AHR). This receptor is crucial in regulating immune and allergic responses, making it an important focus for our study due to its role in controlling the expression of genes associated with inflammatory and immune processes.

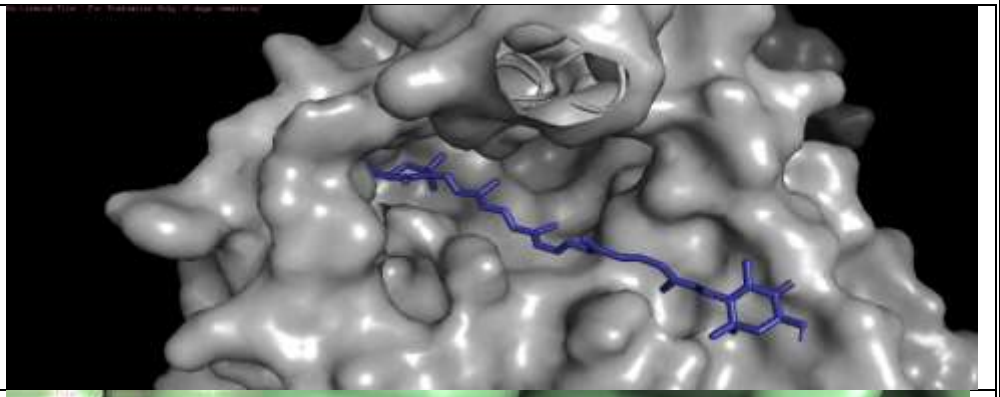
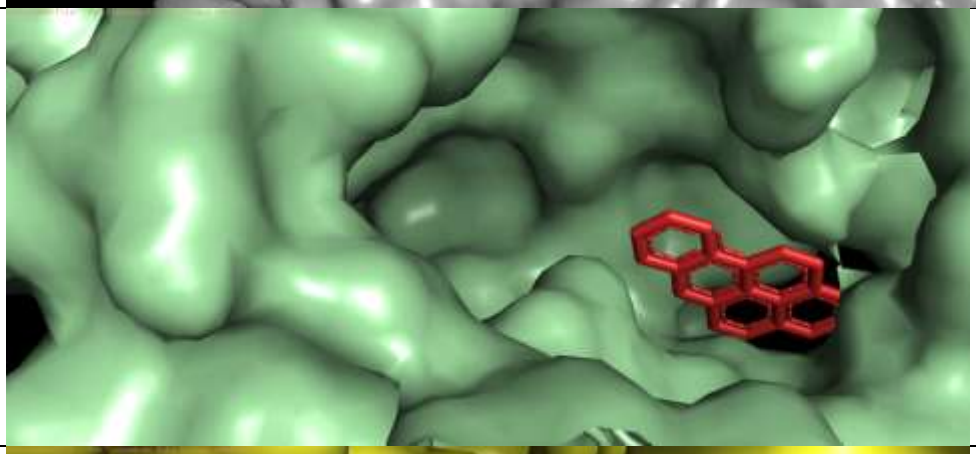
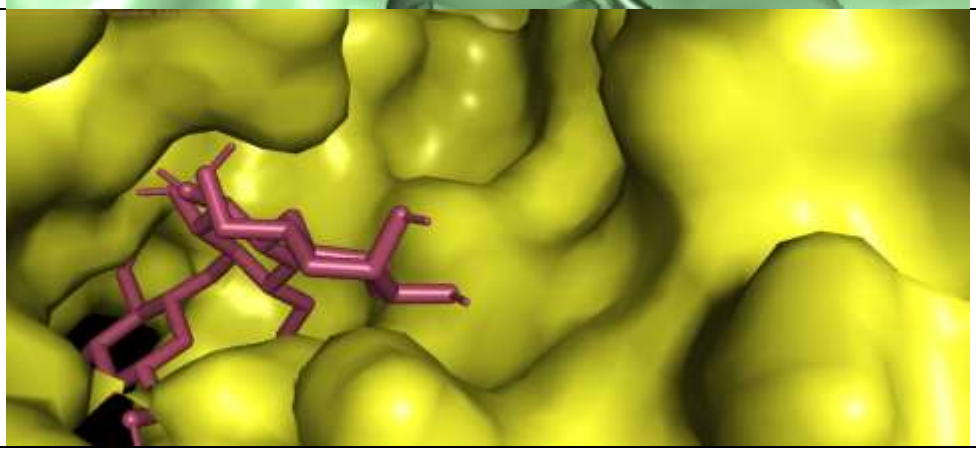
2. Molecular Remodelling of AHR Receptor

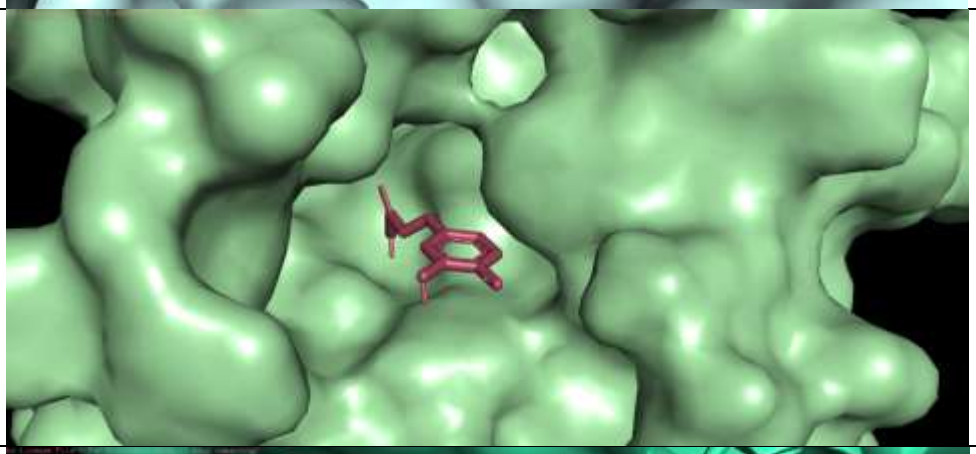
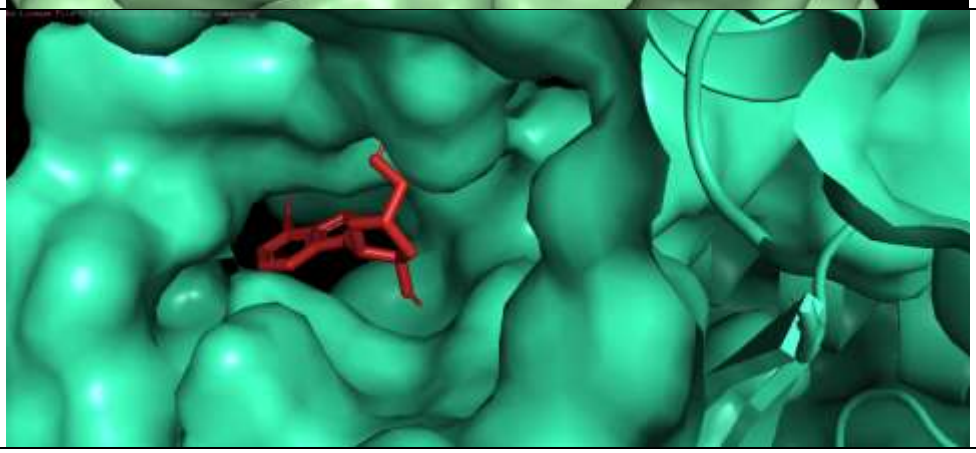
The initial three-dimensional structure of the AHR receptor was obtained from the Protein Data Bank (PDB). However, a closer examination with Pymol software revealed several missing residues in the receptor structure, which could affect the accuracy of future docking simulations. To resolve this issue, two homologous AHR structures from Homo sapiens were sourced and merged using Swiss-Model, a reliable homology modelling tool. This method enabled the reconstruction of a complete and structurally accurate AHR receptor, making it suitable for high-precision docking studies.

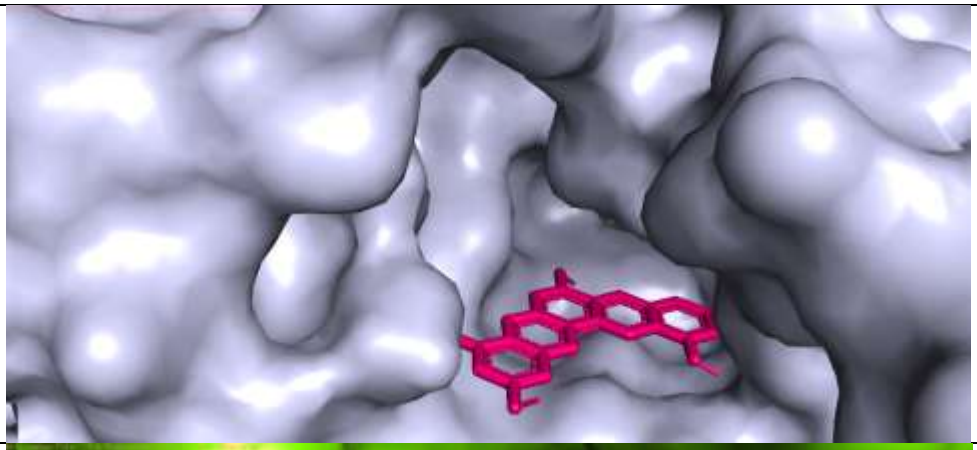
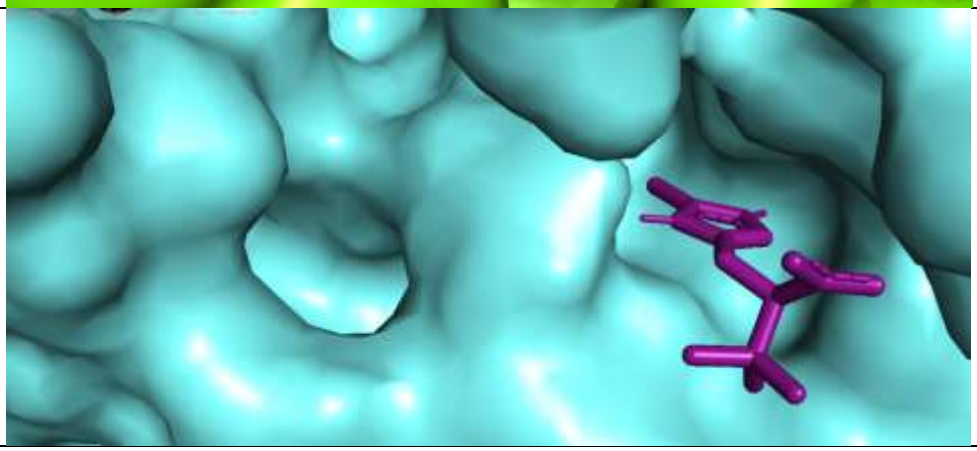
3. Docking Studies

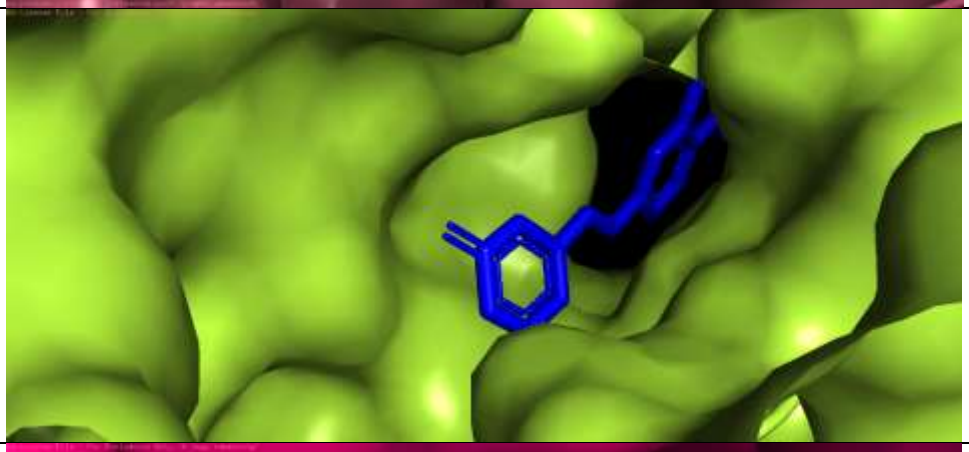
Using the remodelled AHR receptor, we performed molecular docking studies with AutoDock Vina, a well-known tool for virtual screening and docking simulations. We docked each of the 30 selected compounds into the active site of the AHR receptor to predict their binding affinities and interaction modes. The docking results were evaluated based on binding affinity scores, with compounds showing a binding affinity greater than -6.0 kcal/mol being shortlisted for further analysis. This threshold was established based on the receptor's known ligand-binding characteristics to ensure that we selected compounds with significant interaction potential.

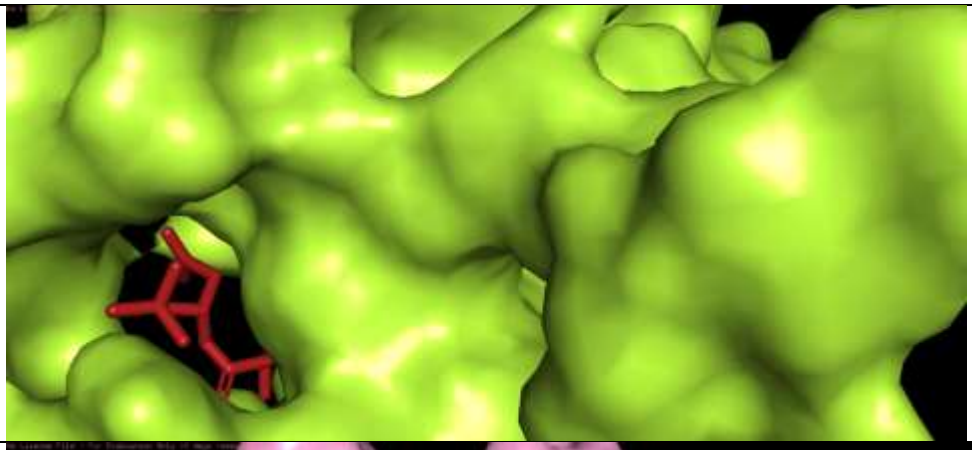
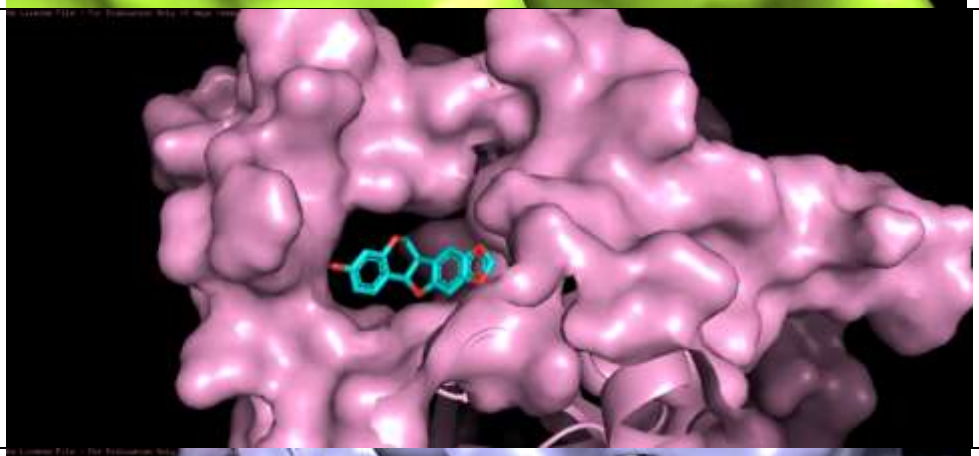
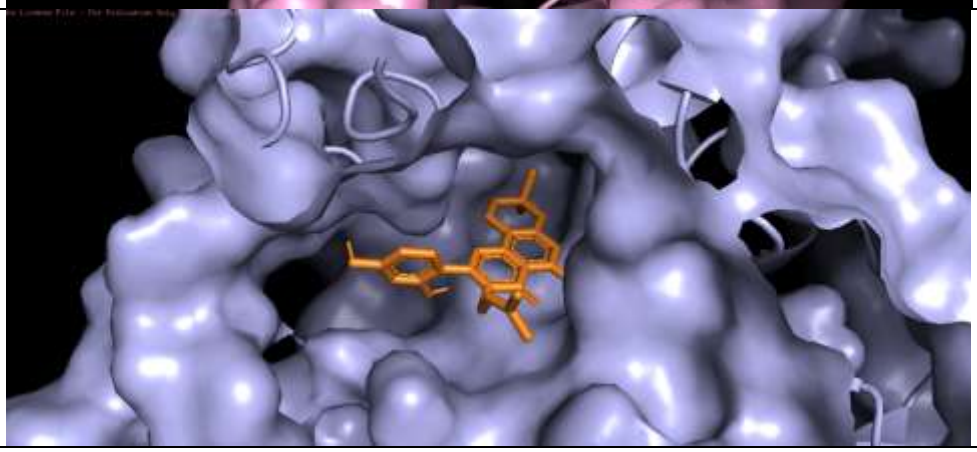
Compound Name	*Affinity Score*	*Surface Visualization*
Allantoin	*-5.4*	
Alpha tocopherol	*-8.5*	
Antcin A	*-8.2*	

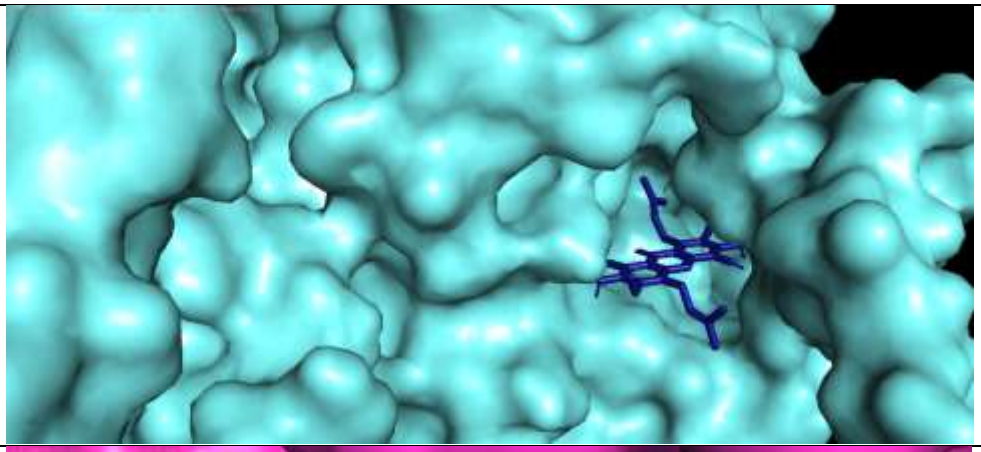
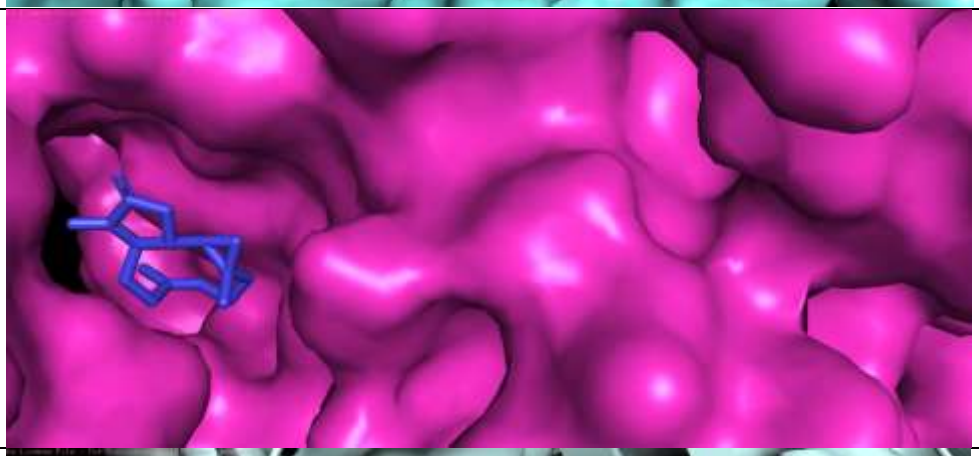
Astaxanthin	*-8.8*	
Benzo Apyrene	*-9.7*	
Beta glucan	*-6.4*	

Bisphenol A	*-6.8*	
Caffeic acid	*-5.7*	
Cordycepin	*-6.4*	

Dioxinodehydroeckol	*-7.8*	
Ergosterol	*-8.6*	
Ergothioneine	*-4.6*	

Gama_tocopherol	*-6.6*	
Hispidin	*-7.1*	
Inotilone	*-6.7*	

Isovalerylcarnitine	*-5.2*	
Maackiain	*-7.6*	
Morusin	*-8.4*	

Nujiangexanthone A	*-8.2*	
Parthenolide	*-8.2*	
Pyridoviricin	*-7.1*	

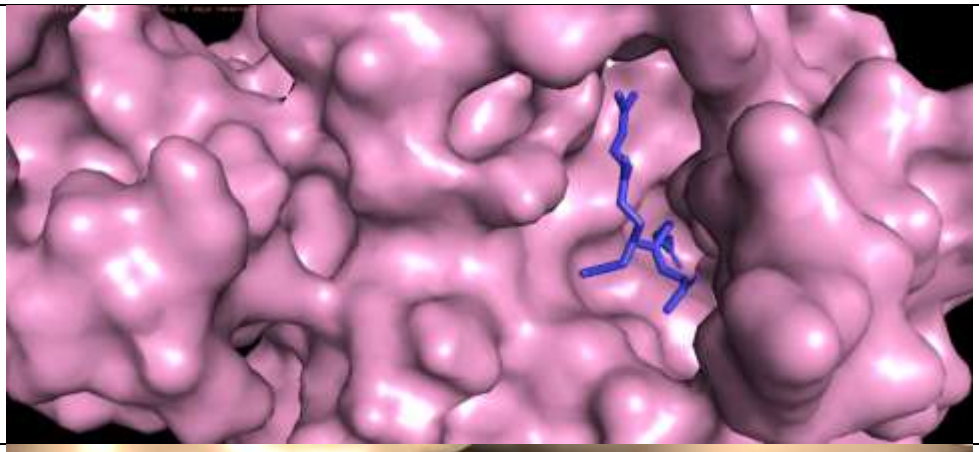
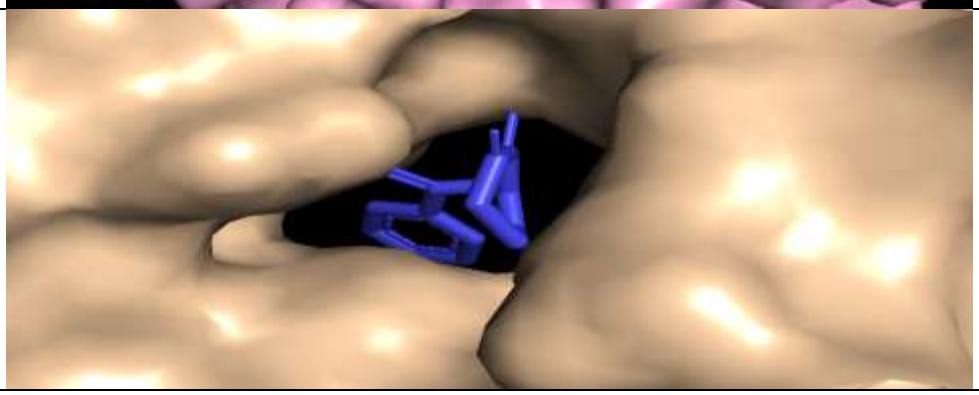
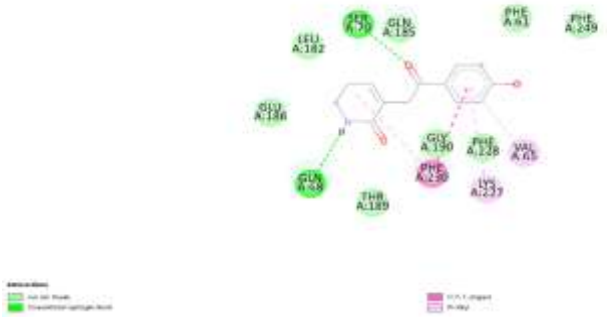
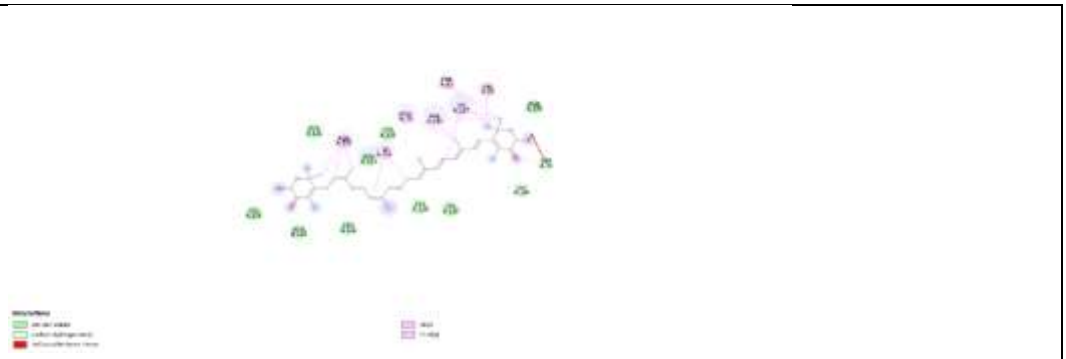
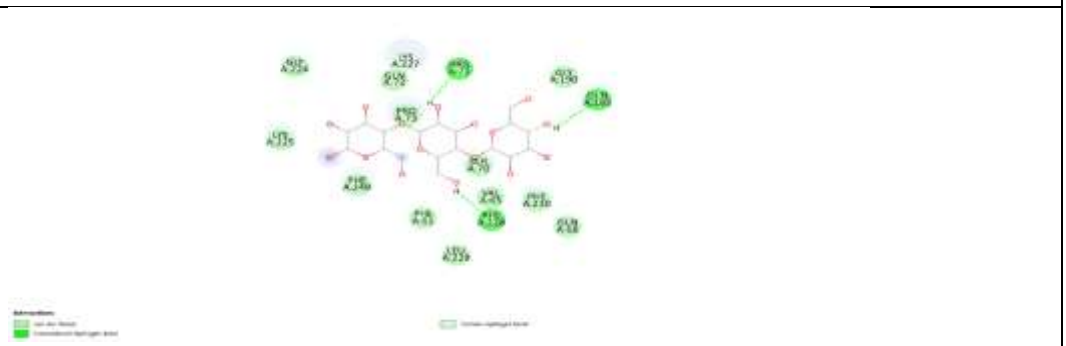
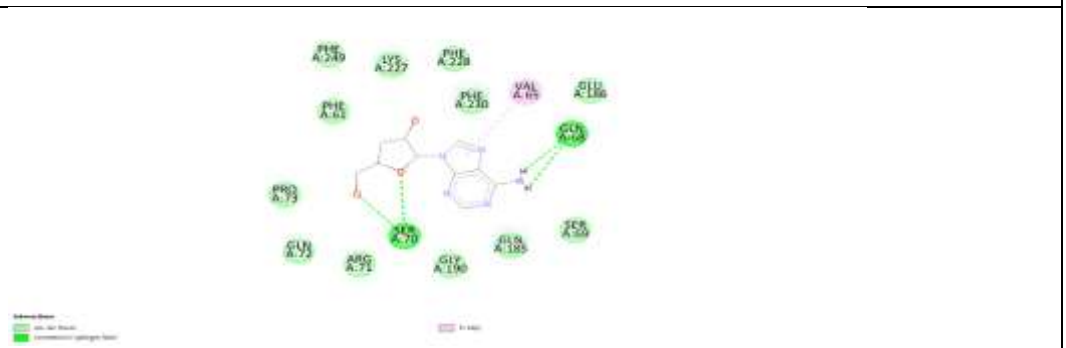
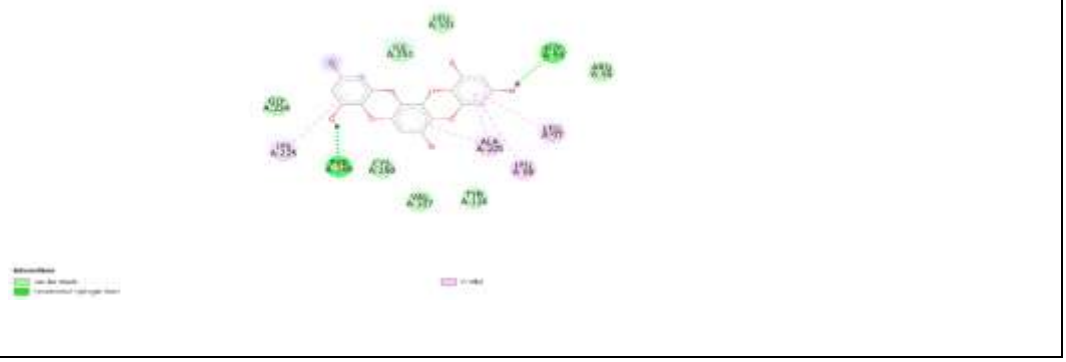
Rhipocephalin	*-6.0*	
3-(2-(4-Hydroxyphenyl)-2-oxoethyl)-5,6-dihydropyridin-2(1h)-one	*-7.0*	

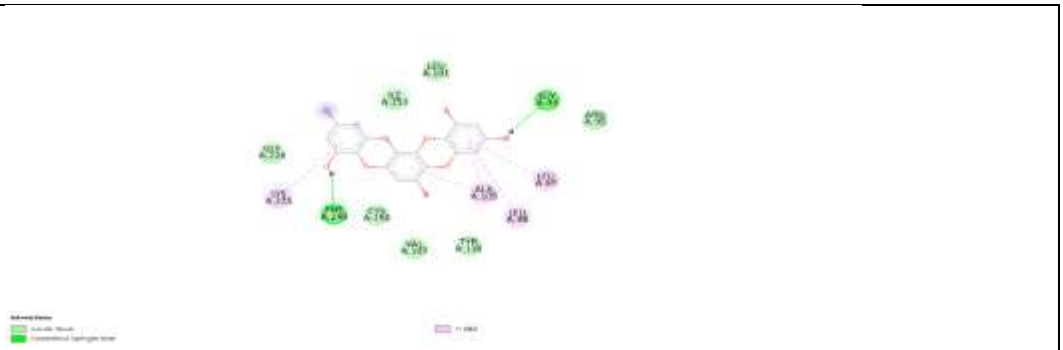
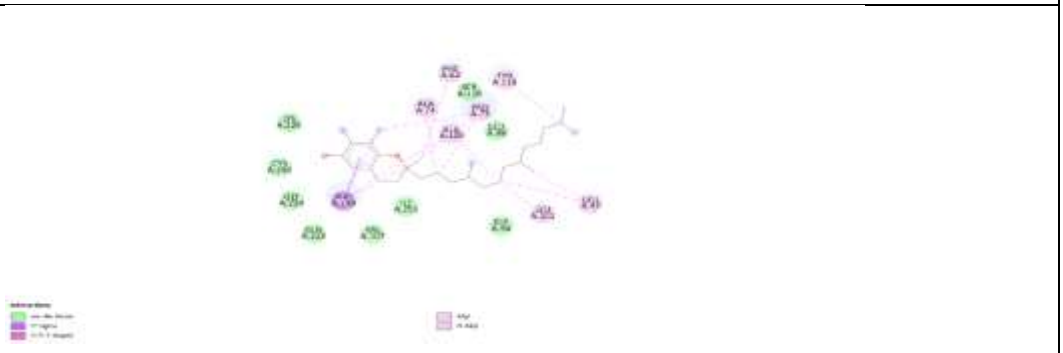
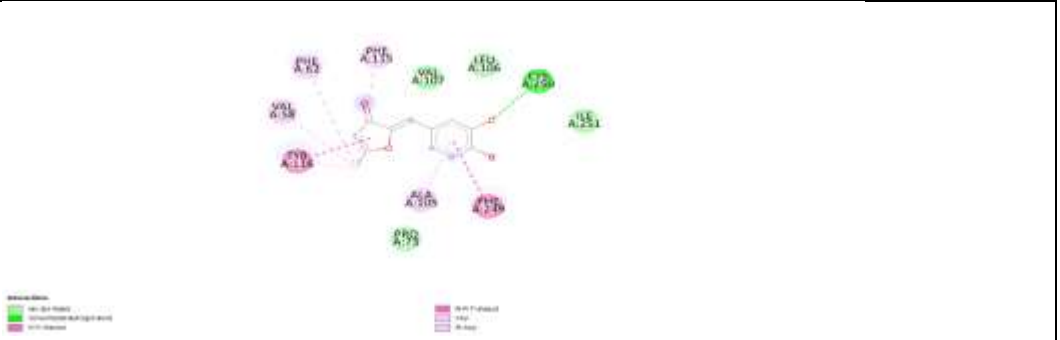
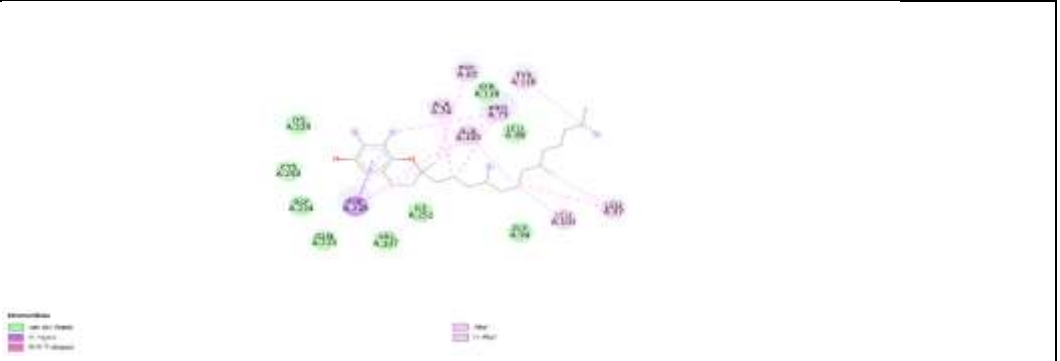
TABLE 2:- Compounds with their affinity score and visual representation

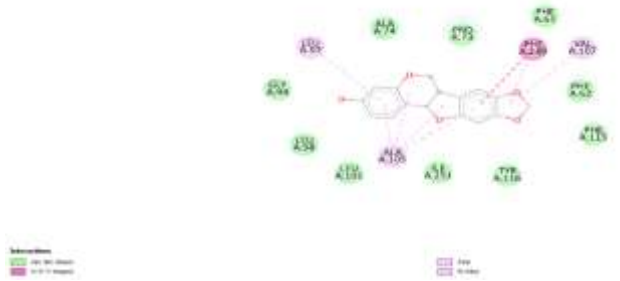
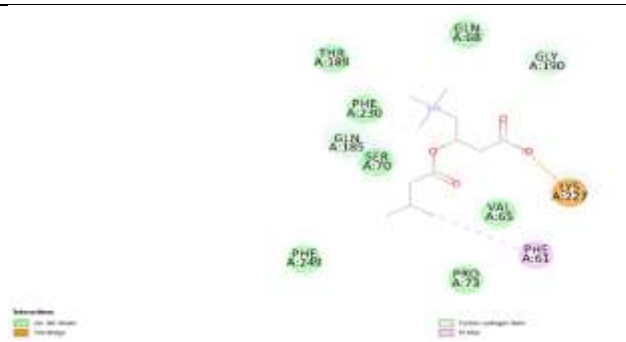
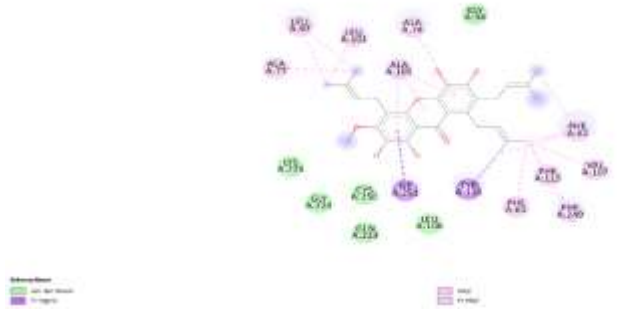
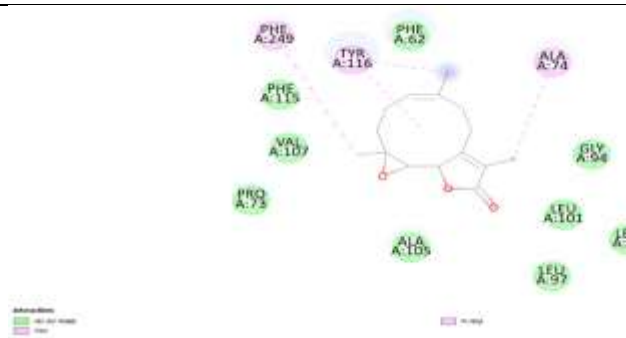
4. Discovery Studio Analysis

After the docking studies, the selected compounds were further validated using Discovery Studio, a robust suite for molecular modeling and simulation. This tool was utilized to closely examine the molecular interactions between the high-affinity compounds and the AHR receptor. The analysis aimed to identify and visualize important interactions, including hydrogen bonds, hydrophobic contacts, van der Waals forces, and π-π stacking interactions. These interactions are vital as they enhance the stability and effectiveness of the compound-receptor binding, which in turn affects the compound's potential to combat allergies.

COMPOUND	*2D INTERACTION*
3-(2-(4-Hydroxyphenyl)-2-oxoethyl)-5,6-dihydropyridin-2(1h)-one	
Alpha tocopherol	
Antcin A	

Astaxanthin	
Beta-Glucan	
Cordycepin	
Dioxinodehydroeckol	

Ergosterol	
Gama-Tocopherol	
Inotilone	
Hispidin	

Maackiain	
Morusin	
Nujiangexanthone A	
Parthenolide	

Pyridovirivin	

TABLE 3:- Compound and their 2D interactions

5. ADMET Analysis

To evaluate the drug-like properties of the selected compounds, we performed an ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) analysis using the ADMET 2.0 Lab online software. This analysis was designed to predict the pharmacokinetic and toxicity profiles of the compounds, ensuring they not only demonstrate strong receptor binding but are also likely to be safe and effective in a biological setting. We assessed parameters such as intestinal absorption, blood-brain barrier penetration, hepatic metabolism, renal clearance, and potential toxic effects. The aim was to identify the most promising candidates with favorable ADMET profiles, making them suitable for further preclinical and clinical development as anti-allergic agents.

S. no.	compound	Absorption				Distribution				Metabolism							Excretion		Toxicity											Toxicophore rule			
		caco-2 permeability	MDCK permeability	Pgp-inhibitor	HIA	PPB	VD	BBB Penetration	Fu	Cyp1a2 inhibitor	Cyp2c19 inhibitor	Cyp2c9 inhibitor	Cyp2D6 inhibitor	Cyp2D6 substrate	CYP3A4 inhibitor	Cyp3a4 substrate	CL	T 1/2	Herg BLOCKERS	H-HT	DILI	AMES toxicity	Rat oral acute toxicity	FDAMDD	Skin sensitization	carcinogenicity	Eye corrosion	Eye irritation	Respiratory toxicity	Acute toxicity rule	Genotoxic Carcinogenicity Rule	Non Genotoxic Carcinogenicity Rule	FAF-Drugs4 Rule
	3-(2-(4-hydroxyphenyl)-2-oxoethyl)-5,6-dihydropyridin-2(1H)-one	-4.674	2.1e-05	—	—	43.720%	0.755	-	66.381%	+	-	-	—	+	—	-	10.756	0.778	—	—	-	-	-	—	+++	—	—	+	+	0 alert(s)	0 alert(s)	0 alert(s)	2 alert(s)
	Alpha tocopherol	-4.776	7.1e-08	—	—	101.230%	6.837	++	1.588%	—	—	—	—	—	—	—	8.380	0.022	—	—	—	—	—	—	+++	—	—	-	—	0 alert(s)	0 alert(s)	0 alert(s)	1 alert(s)
	Astaxanthin	-5.660	2e-05	+++	—	98.010%	5.904	—	—	—	—	—	+	+++	—	++	0.343	0.200	-	--	---	-	--	+++	+++	—	—	—	+	0 alert(s)	1 alert(s)	0 alert(s)	4 alert(s)
	Beta glucan	-6.210	0.00087	—	+++	10.524%	0.229	-	58.959%	—	—	—	—	—	—	—	0.794	0.609	—	—	—	—	—	—	—	—	—	—	—	0 alert(s)	0 alert(s)	0 alert(s)	0 alert(s)
	cordycepin	-3.889	5.4e-06	—	+++	14.943%	2.012	+	83.084%	—	—	—	—	—	—	—	11.802	0.503	—	+	+++	-	—	++	+	+	—	—	—	0 alert(s)	3 alert(s)	0 alert(s)	1 alert(s)
	Dioxonodehydro roseol	-5.400	3.7e-05	—	-	84.840%	0.671	—	25.963%	+++	-	+	—	-	-	—	11.347	0.913	—	+++	+++	+	-	+++	++	-	—	+	—	0 alert(s)	3 alert(s)	1 alert(s)	2 alert(s)
	ergosterol	-4.747	8.8e-08	+++	—	99.844%	1.388	+	1.627%	—	—	-	—	-	-	+++	15.231	0.028	—	—	—	-	-	+	—	—	—	—	+++	0 alert(s)	0 alert(s)	0 alert(s)	0 alert(s)
	Gaina Tocopherol	-4.855	7.8e-08	—	—	100.603%	5.329	++	1.844%	—	-	-	-	-	-	-	8.244	0.029	—	—	—	—	—	—	+++	—	—	-	-	0 alert(s)	0 alert(s)	0 alert(s)	1 alert(s)
	[illegible]	-4.842	2.7e-05	—	—	96.307%	0.387	—	1.984%	+++	-	-	+	-	+	-	10.143	0.902	—	+	-	-	++	+++	+++	-	+	+++	+++	0 alert(s)	2 alert(s)	1 alert(s)	
	Hispidin	-4.895	1.5e-05	—	—	97.289%	0.414	—	4.506%	++	—	-	-	++	+		14.912	0.911	—	—	+	—	-	+	+++	+	—	+++	-	0 alert(s)	0 alert(s)	0 alert(s)	2 alert(s)
	Maackiain	-4.926	2.9e-05	—	—	98.359%	1.180	—	2.181%	+++	+++	++	+++	+++	+++	-	17.311	0.188	—	—	-	++	-	+	++	—	—	-	-	0 alert(s)	0 alert(s)	0 alert(s)	2 alert(s)
	Morusin	-4.874	1.5e-05	—	—	97.679%	[illegible]	—	3.704%	++	+++	—		-			4.881	0.310	—	+++	+++		+	++	-	++	—	-	+	0 alert(s)	0 alert(s)	0 alert(s)	1 alert(s)
	[illegible] A	-4.946	1.1e-05	++	+	74.163%	0.798	—	30.942%	-	-	+	—	-	—	-	6.798	0.385	—	+++	+++	—	-	—	++	—	—	-	-	0 alert(s)	4 alert(s)	1 alert(s)	3 alert(s)
	parthenolide	-4.591	2.6e-05	—	—	92.408%	2.255	—	3.641%	—	-	-	—	—	—	—	17.573	0.089	—	—	-	—	+	+	-	++	—	—	+++	0 alert(s)	0 alert(s)	1 alert(s)	2 alert(s)
		-5.022	4e-08	—	—	98.820%	0.223	—	1.137%	++	-	++	++	-	++	-	9.489	0.934	-	-	+	+	++	+++	+++	-	—	—	+	0 alert(s)	1 alert(s)	1 alert(s)	3 alert(s)

TABLE 4:- ADMET properties of different compounds

Selected Compounds with Favorable ADMET Profiles

1. The selection criteria include: • Low Toxicity Alerts: We prioritize compounds that have minimal or no toxicity alerts. • Favorable ADMET Properties: We look for compounds that exhibit strong absorption, distribution, metabolism, and excretion characteristics, while also showing low interaction with cytochrome P450 enzymes and reduced risks of carcinogenicity or toxicity. Selected Compounds Considering these criteria, the following compounds are noteworthy:

   Beta Glucan

   - **Toxicity**: No toxicity alerts.
   - **ADMET Profile**:
     - Caco-2 permeability: -6.210
     - MDCK permeability: 0.00087
     - High intestinal absorption (HIA): 10.524%
     - Moderate distribution and low plasma protein binding.
     - No cytochrome P450 interactions.
     - Clearance (CL): 0.794, half-life (T1/2): 0.606
   - **Toxicophore Rule**: No alerts.

2. **Alpha Tocopherol**
   - **Toxicity**: Only one toxicity alert.
   - **ADMET Profile**:
     - Caco-2 permeability: -4.776
     - MDCK permeability: 7.1e-06
     - High intestinal absorption (HIA): 101.236%
     - High distribution, moderate plasma protein binding.
     - No cytochrome P450 interactions.
     - Clearance (CL): 8.280, half-life (T1/2): 0.022
   - **Toxicophore Rule**: No alerts.

3. **Gama Tocopherol**

- **Toxicity**: Only one toxicity alert.
- **ADMET Profile**:
  - Caco-2 permeability: -4.855
  - MDCK permeability: 7.6e-06
  - High intestinal absorption (HIA): 100.603%
  - High distribution, moderate plasma protein binding.
  - No cytochrome P450 interactions.
  - Clearance (CL): 8.243, half-life (T1/2): 0.029
- **Toxicophore Rule**: No alerts.

The analysis revealed that the following compounds exhibited the most promising ADMET profiles while posing minimal toxicity risks:

• Beta Glucan

o No toxicity concerns reported.

o Displays moderate permeability, effective distribution, and limited interaction with cytochrome P450 enzymes, suggesting good metabolic stability.

• Alpha Tocopherol

o Reports one toxicity concern.

o Exhibits excellent absorption and distribution, with no notable interactions with cytochrome P450.

• Gamma Tocopherol

o Reports one toxicity concern.

o Like Alpha Tocopherol, it shows strong absorption and distribution with few metabolic complications.

Each of these compounds showed a blend of promising ADMET properties, positioning them as strong contenders for additional simulations and possible clinical trials.

6. Conclusion and Future Directions

The compounds Beta Glucan, Alpha Tocopherol, and Gamma Tocopherol show significant promise for anti-allergic therapies, thanks to their favorable docking affinities, stable molecular interactions, and low toxicity. These results open the door for additional simulations and in vivo studies to assess their effectiveness in clinical applications.

CHAPTER 5

DISCUSSION

Discussion

The study focused on discovering natural compounds that could help alleviate allergies by interacting with the Aryl hydrocarbon receptor (AHR), which plays a significant role in regulating immune responses. The docking studies revealed several compounds, including Alpha Tocopherol, Beta Glucan, and Gamma Tocopherol, that exhibited strong binding affinities, indicating their potential as anti-allergic agents. These findings align with existing research that emphasizes the activation of AHR as a means to reduce allergic reactions by influencing gene expression related to immune functions. The compounds identified in this research not only demonstrated strong binding to AHR but also showed promising ADMET profiles, suggesting they are suitable candidates for further development as therapeutic agents.

Comparison with Other Computational Approaches for Allergen Identification

This study combined molecular docking with ADMET analysis to create a well-rounded method for selecting compounds. Unlike other computational techniques that might only focus on either docking or ADMET, this integrated approach provides a more comprehensive screening process. By including ADMET analysis, it ensures that the chosen compounds not only interact well with the target receptor but also have drug-like characteristics, such as low toxicity and good pharmacokinetics. This method stands in contrast to traditional approaches that might miss important aspects like metabolism and toxicity, which can result in selecting compounds that, although effective in vitro, may not succeed in later phases of drug development.

Potential Mechanisms of Action of Selected Anti-Allergic Compounds

The selected compounds, especially Beta Glucan, Alpha Tocopherol, and Gamma Tocopherol, showed strong binding affinities and established stable interactions with AHR. These interactions are likely to facilitate AHR activation, which in turn modulates gene expression to suppress inflammatory and allergic responses. The types of interactions noted, including hydrogen bonding and hydrophobic contacts, indicate that these compounds help stabilize the active form

of AHR, boosting its capacity to provide anti-allergic effects. This mechanism is backed by existing literature that highlights AHR activation as a key factor in alleviating allergic inflammation.

Molecular Dynamics Simulations to Study the Pharmacological Properties of Selected Compounds

To further validate the findings, it is essential to conduct molecular dynamics (MD) simulations on the chosen compounds. These simulations will shed light on the stability and behavior of the receptor-ligand complexes in physiological conditions. By observing the interactions over time, we can predict the long-term stability, binding modes, and possible side effects of the compounds in a dynamic environment. This approach will enhance our understanding of the pharmacological properties of the compounds and their potential as therapeutic agents.

In Vitro and In Vivo Studies to Validate the Anti-Allergic Effects of Selected Compounds

The predictions made in this study require validation through experimental methods. In vitro studies with cell cultures can evaluate how well the compounds activate AHR and influence gene expression associated with allergic responses. Once in vitro results are promising, in vivo studies in animal models will be essential to determine the effectiveness of the compounds in alleviating allergic symptoms and to examine their safety profiles. These investigations are vital for turning the computational findings into real-world therapeutic applications.

Clinical Trials to Assess the Safety and Efficacy of Lead Compounds

The compounds that show the most promising ADMET profiles, especially Beta Glucan, Alpha Tocopherol, and Gama Tocopherol, should be prioritized for clinical trials. These trials will assess the safety, tolerability, and effectiveness of these compounds in human participants. The initial Phase I trials will aim to establish a safe dosage range, while the following Phase II and III trials will focus on the compounds' effectiveness in treating allergic conditions. If the clinical trials are

successful, these compounds could be validated as new anti-allergic medications, providing additional treatment options for allergy sufferers.

CHAPTER 6

CONCLUSION

Conclusion

Natural products represent a vast resource that can offer therapeutic compounds and serve as self-medicators against allergies and other allergic diseases, each with unique forms and effects. The main classes of bioactive compounds involved include polyphenols, alkaloids, terpenoids, steroids, and peptides, and the potential of these compounds in managing allergic diseases has been explored through various pathways.

Potential therapeutic targets have been identified through computational analysis of molecular data, with the Aryl Hydrocarbon Receptor (AhR) standing out as particularly noteworthy. The combination of extensive knowledge in traditional medicine with experimental and molecular approaches, along with current treatment ideas for allergic disorders, holds significant promise for advancing treatment options.

Overall, more comprehensive studies are needed to understand the mechanisms of action of these compounds, and their effectiveness should be evaluated through well-designed clinical trials to assess their potential clinical applications.

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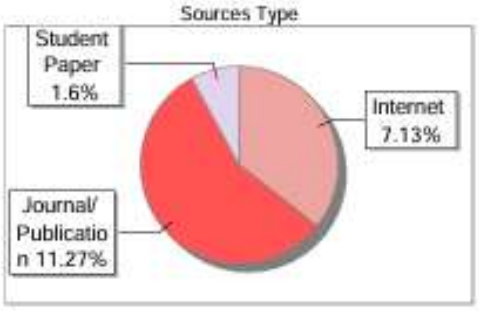

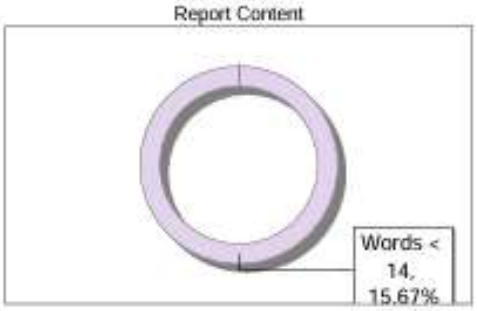

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