DECIPHERING THE THERAPEUTIC POTENTIAL OF *MYRICA ESCULENTA* IN ASTHMA TREATMENT: AN IN-DEPTH SYSTEMIC PHARMACOLOGY INVESTIGATION INTEGRATING MOLECULAR DOCKING, SIMULATION, UNSUPERVISED MACHINE LEARNING, AND GENE EXPRESSION ANALYSIS

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CANDIDATE'S DECLARATION

I, Sakshi shyamala, Roll no. 2k22/MSCBIO/43 student of M.Sc. Biotechnology, hereby declare that the project Dissertation titled Deciphering the Therapeutic Potential of *Myrica esculenta* in Asthma treatment: An In-Depth Systemic Pharmacology Investigation Integrating Molecular Docking, Simulation, Unsupervised Machine Learning, and Gene Expression Analysis which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi, in partial fulfillment of the requirement for the award of the degree of Master of Science, is original and not copied from any source without proper citation. This work has not been previously formed the basis for the award of any Degree, Diploma associateship, Fellowship, or other similar title or recognition.

Sakshi shyamala

Candidate Signature

This is to certify that the student has incorporated all the correction suggested by examiner in the thesis and the statement made by the candidate is correct to the best of our knowledge

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Deciphering the Therapeutic Potential of *Myrica esculenta* in Asthma treatment: An In-Depth Systemic Pharmacology Investigation Integrating Molecular Docking, Simulation, Unsupervised Machine Learning, and Gene Expression Analysis

Sakshi Shyamala

ABSTRACT

Asthma is increasingly prevalent worldwide, especially in developed nations. Traditional medicinal models have utilized plants, prompting many patients to seek alternative therapies due to the side effects of current asthma treatments. Ayurvedic and Indian texts document the medicinal use of plants, including Myrica esculenta (ME), which is rich in phytochemicals with significant medicinal potential. These compounds have shown promise in treating diabetes, ulcers, tumors, asthma, and stress. This study employed a comprehensive network pharmacology approach encompassing drug-likeness evaluation, target identification. By protein-protein interaction key constituents like Myricanol, Arjunolic acid, Gallic acid, Ellagic acid, Caffeic acid, Cyanidanol, and Quercetin were analyzed and therapeutic genes identified included IL6, STAT3, JUN, NFKB1, and RELA.

Enrichment analysis revealed the pivotal roles of genes such as STAT3, IL6, JUN, NFKB1, and RELA in transcriptional machinery and cytokine production pathways, crucial in mediating allergic responses underlying asthma pathogenesis. Particularly, inhibition of STAT3 was identified as a potential strategy to suppress Th17 cell differentiation, thereby reducing allergic effects in asthma. Furthermore, disease pathway analyses unveiled a significant association between asthma symptoms and respiratory syncytial virus (RSV) bronchiolitis during infancy, shedding light on potential early-life origins of asthma. Importantly, ellagic acid emerged as a promising therapeutic agent for asthma, exhibiting high interaction and binding affinity with STAT3. Molecular docking, dynamics simulations, and chemical map spacing validated this finding, corroborating ellagic acid's potential as a targeted treatment for asthma.

Expression analysis further confirmed the relevance of STAT3 in lung-related diseases, particularly asthma, underscoring its potential role as a therapeutic target in respiratory disorders. These collectively provide valuable insights into the molecular mechanisms underlying asthma pathogenesis and offer promising avenues for the development of novel therapeutic interventions targeting STAT3, and related pathways.

Keyword- Myrica esculenta, asthma, phytochemicals, protein-protein interaction (PPI), STAT3

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LIST OF ABBREVIATIONS

GEO - Gene Expression Omnibus

NMR- Nuclear Magnetic Resonance

LC-MS/MS- liquid chromatography-mass spectrometry

FcεRI- Fc epsilon RI

PC- Phytochemical

ME- Myrica esculenta

ADME- Absorption, distribution, metabolism, and excretion

BBB- Blood-brain barrier

TPSA- Topological Polar Surface Area

CTD- Comparative Toxicogenomics Database

OMIM- Online Mendelian Inheritance in Man

PCIDB- PhytoChemical Interactions Database

IMPPAT- Indian Medicinal Plants, Phytochemistry And Therapeutics

Dr Duke- Dr. Duke's Phytochemical and Ethnobotanical Databases

PharmGKB- Pharmacogenomics Knowledge Base

FDA- Food and Drug Administration

PPI- Protein-Protein Interaction

GO- Gene ontology

BP- Biological process

MF- Molecular function

CC- Cellular compartment

KEGG pathway- Kyoto Encyclopedia of Genes and Genomes pathway

RNA Pol II- RNA polymerase II

TF- Transcription factor

PDB- Protein Data Bank

RSV- Respiratory syncytial virus

MD- Molecular dynamics

NMA- Normal Mode Analysis

RMSD- Root Mean Square Deviation

RMSF- Root Mean Square Fluctuation

SASA- Solvent Accessible Surface Area

PCA- Principal Component Analysis

GTEx- Genotype-Tissue Expression

IL- Interleukin

TNF- Tumor necrosis factor

CHAPTER 1

INTRODUCTION

1.1 Research and development of drug using natural products

Natural products, illustrates a broad spectrum of biological reactions and structures of chemicals sourced from various organisms, are extremely valuable in the discipline of researching and developing new drugs. Their medicinal history extends centuries, as natural substances have been the basis for many drugs, ranging from well-known ones like aspirin to innovative antibiotics like penicillin [Atanasov et al (2015)]. Inspiration for the synthesis of analogues and derivatives comes from natural products, which improves pharmacological properties like potency, selectivity, and bioavailability, in addition to their direct therapeutic applications. Natural product research offers vital insights into biological pathways and mechanisms, frequently identifying new therapeutic targets for intervention [Newman and Cragg (2016)]

Natural products are still very important in the pharmaceutical industry today, especially when it comes to treating illnesses. Leveraging high throughput techniques, they constitute an extensive reservoir of powerful resources for assessing chemical effects performance in drug discovery. Novel methods provide insights into therapeutic effects, such as estimating gene networks regulated by compounds found in medicinal plants [Zhang et al (2019)]. The paradigm is changing to multi-target drug development in recognition of the shortcomings of single-target approaches, with network pharmacology establishing itself as a viable framework. By building complex "protein–compound/disease–gene" networks, this integrative methodology elucidates the mechanisms behind the synergistic therapeutic effects as mentioned in the Fig-1.1 [Zhang et al (2013)].

As a new area of drug development and discovery that combines information science and systematic medicine, network pharmacology is rapidly developing. Although it was initially viewed with some scepticism and overhype, it has developed into a widely used strategy that is fueling innovation in contemporary drug discovery procedures. The paradigm has changed from the conventional "single-target, single-drug" models to the more complex" multi-component, network-target therapies" approach as a result of this revolutionary breakthrough [Zhang et al (2019)].

The potential to cure a variety of illnesses using revolutionary bioactive compounds in medicine is encouraging. With ongoing developments in network pharmacology, there is

growing hope for the treatment of numerous illnesses. This field has enormous potential to spur innovation and change the pharmaceutical industry as it develops further [Casas et al (2019)].

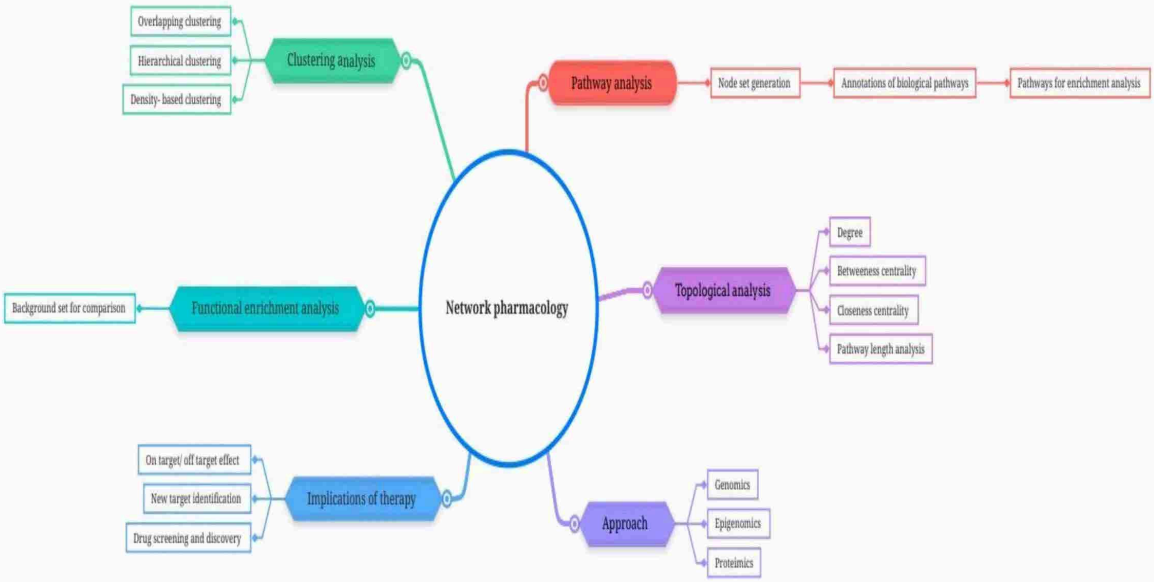


Figure 1.1: System pharmacology approaches- Every nodes of the circle is the method utilized for these studies by researcher.

Comprehending conventional pharmaceuticals and medicinal plant pharmacology requires an understanding of the synergy of traditional drugs. This idea is intrinsically related to pharmacology and conventional medical practices like ayurveda, which have historically depended on the use of herbal remedies. The chemical profiles of these intricate herbal formulations are crucially characterized by metabolomics, which makes translational research in traditional medicine easier [Chandran et al (2017)]. In order to identify and quantify metabolites, methods like LC-MS/MS, GC-MS, and NMR spectroscopy are essential. These techniques provide both targeted and untargeted approaches. With the use of these technologies, multi-molecular 15 herbs and herbal formulations can undergo thorough chemo profiling, revealing information about their therapeutic qualities and modes of action. Through the use of metabolomics, scientists can clarify the complex relationships between the bioactive components of conventional medications, opening the creation of new treatments and the incorporation of traditional knowledge into contemporary healthcare practices [Banerjee (2021)].

Systems biology sheds light on the interactions between individual molecules to create molecular network, which helps understand molecular toxicology and pharmacology in cells. These networks are maps of pathways that make it possible to analyze how

pharmacological reactions occur in intricate biological systems. Through examining the ways in which chemical compounds disrupt these linkages or networks, scientists can acquire a more profound comprehension of drug effects and toxicity processes.

Systems biology uses comprehensive data from omics technologies to identify gene and protein expression profiles in alongside analyzing pathways. Large volumes of data are produced by methods like high-precision mass spectrometry, high-performance sequencing, and DNA microarrays, which allow scientists to map the molecular landscape of cellular responses to different stimuli [Banerjee (2021)]. Proteomics databases offer useful information on protein expression profiles, and publicly accessible databases such as the GEO offer to utilize a wealth of data from DNA microarrays. Researchers can understand the complex molecular mechanisms underlying drug responses and toxicological effects by combining data from various sources. This knowledge can then be used to inform the creation of medicines that are secure and more successful [Barrett et al (2013)].

1.1.1 Challenges and Advancement in Natural Product Research

The isolation, characterization, and optimization of natural products have been facilitated by advancements in computational biology, combined the field of chemistry and analytical techniques despite having difficulties brought on by their complexity of structure and sourcing logistics. Combining traditional knowledge with modern technology also makes it possible to explore natural resources sustainably and strikes a balance of developing drugs and preservation of biodiversity. Natural products have a long history of helping treat serious medical conditions like cancer, infectious diseases, cardiovascular conditions, and multiple sclerosis. This highlights their continued importance in contemporary pharmacotherapy [Wagenaar (2008)].

1.1.2 Natural Products Significance in Modernism Pharmacotherapy

When compared to their synthetic counterparts, natural products have distinct properties such as greater molecular rigidity, higher molecular masses, and remarkable scaffold diversity. Their significance in drug discovery is highlighted by their defiance of Lipinski's rule of five. They have undergone evolutionary optimization to cater to particular biological functions, such as modifying endogenous defense mechanisms and interacting with other organisms. When compared to conventional synthetic libraries, the repertoire of bioactive compounds derived from traditional medicine practices is more expansive in terms of chemical space [Cragg et al (1993)].

1.2 Asthma

Chronic inflammation of the lower airways, especially in those with upper respiratory tract disorders, is the cause of asthma, a chronic inflammatory disease. Although the cause is mostly unknown, bronchial hyper responsiveness and variable airway blockage are its hallmarks. Breathing difficulties, coughing, chest tightness, and wheezing are common symptoms of asthma (Fig-1.2). Reduced airway diameter leads to airway

Figure 1.2: Pathophysiology of asthma- In these smaller circle represent the effect individual suffer from these condition which are all connected to the main circle as asthma.

obstruction, which is the primary characteristic of asthma. Features of asthma such as bronchial hyper responsiveness are caused through persistent airway wall inflammation, which is marked by stimulated immune cells and infiltration. Nevertheless, continuous clots of mucus in the narrower airways and additional pathophysiological mechanisms such as airway remodeling may prevent severe asthma from returning to normal following treatment. The definition and path-physiology of asthma should be understood by medical professionals that treat patients with it [Nakagome and Nagata (2021)].

The relationship among mast cell-resident IgE and FcεRI and basophiles starts the allergic cascade. The pro-inflammatory Th2 milieu is enhanced by this interaction, which also releases neutral proteases, lipid mediators, Th2-associated cytokines, and histamine. During the allergic reactions frequent phase, IgE increases cellular recruitment and lung vascular permeability. Smooth muscle cells in the airways react to IgE during the early phases of an allergic reaction, resulting in effector functions in asthma. Allergens induce inflammation and bronchoconstriction during the early stages of asthma by binding to IgE on mast cells and basophils. Moreover, IgE has the ability

to attach to FcεRI on dendritic cells, improving the way allergens are presented to memory Th2 lymphocytes. As a result, there is a decreased threshold for the initiation of allergen-specific Th2 cell responses, which increases B cells' production of allergen-specific IgE [Banafea et al (2022)].

Corticosteroids, anti-leukotrienes, and antihistamines are examples of anti-frightening agents that work by sensitizing mediators or inhibiting the expression of specific genes. However, they have limited clinical application and do not effectively inhibit allergic reactions due to flaws and unfavorable reactions. Long-term use of monoclonal anti-IgE antibodies can boost efficacy and safety while also increasing the risk of drug resistance. More research is needed to find medication that are both less likely to cause adverse reactions and are also efficient [Ducharme (2002)]

1.3 Objective

Using *Myrica esculenta* (ME) anti-allergic and antioxidant qualities, this study intends to investigate the plant's therapeutic potential in the treatment of asthma. It is chronic inflammatory condition named asthma which is characterised by airway remodelling, inflammation, and hyper-responsiveness. Allergies and oxidative stress frequently make asthma worse. The quest for new therapeutic agents is necessary due to the drawbacks and adverse effects of existing treatments. Traditional medicinal plant ME has demonstrated strong antioxidant and anti-allergic properties in early research, indicating that it may be a useful natural asthma treatment. The multi-target effects of ME and its underlying molecular mechanisms in the context of asthma can be better understood with the aid of network pharmacology, an emerging field that integrates systems biology and bioinformatics. Developing ME for a novel, multi-targeted drug for the dealing with the condition as finding its target for the therapy.

1.4 Research hypothesis and pipeline

Using a systemic pharmacology approach, this study seeks to explore the therapeutic potential of ME in treating asthma by utilizing its well-known anti-allergic and antioxidant properties. According to the theory, the bioactive components of M. esculenta can successfully alter pathways linked to asthma. An extensive methodology has been developed to test this hypothesis. Phytochemicals (PC) from ME will be identified and assessed using the Swiss ADME tool, with a focus on compounds with promising drug-likeness and bioavailability profiles. Target genes linked to these PC will be predicted by computational tools, and a list of genes linked to asthma will be compiled through extensive data mining. To find therapeutic genes impacted by the metabolite from M. esculenta, protein interaction networks will be built. Gene set enrichment analysis will then show which biological pathways these therapeutic genes significantly affect. To perform toxicity profiling of active PC for therapeutic use, the study will make use of in silico tools. Molecular docking and molecular dynamics

simulations are two structure-based validation techniques that will be used to forecast and confirm the binding affinity and stability of compound-protein interactions. Using unsupervised machine learning and bioinformatics techniques, chemical space mapping will validate ligands by examining gene expression in diverse tissues. Ensuring these PC are safe for use as medicine is the aim.

1.5 Thesis outline

Chapter 2: A brief description of the role of theoretical details of the plant and its various functions. This section includes metabolite profiling and the activity for anti-allergy that would be used in the development of the asthma in this study.

Chapter 3: This chapter includes the methodology and the platform used for the identification of metabolite and validation of the protein. Each subsection gives a brief description of processes used by each online server. Online tool used perform various prediction and validation functions.

Chapter 4: This chapter provides the results of each prediction and evaluation that we have performed

Chapter 5: This chapter provides the significance and the inference of the method result we have obtained.

Chapter 6: This section provides a conclusion of the results that we have obtained from the study and mentioned the perspective in treating disease condition.

CHAPTER 2

LITERATURE REVIEW

According to Coombs and Gell's original definition, type I allergy (T1A) mechanism are characterized as IgE-induced release of mediators of inflammatory processes produced by basophils and mast cells such as histamine. As much as 30% of individuals in countries of the West suffer from allergic rhinitis, allergic anaphylaxis, food allergies, atopic dermatitis, and allergic asthma; these reactions are critical in the development of these allergies. They arise from an improper reaction to specific antigens, or allergens, mediated by Th2 immunity, which results in the synthesis of allergen-specific immunoglobulin E antibodies. These antibodies, known as IgE, attach to the FcεRI on mast cells along with basophiles, sensitizing them to activation upon further exposure to the allergen. A key element of classical type I allergy by this basophil and mast cell stimulation mechanism driven by adaptive IgE [Vitte et al (2022)]. There were no effective medications. It is now understood that asthma is a complicated, persistent inflammatory illness whose course is influenced by a wide range of genes linked to various aspects of the illness, such as changed lung growth and reaction to the environment stimuli, fixed blockage of the airway and reaction to treatment. Over time, our understanding of asthma has completely changed. The most interesting thing is that one of the contributing factors is now thought to be being susceptible to allergies [Church (2017)].

2.1 Myrica esculenta

Native to India, M. esculenta (ME) is a dioecious smaller everlasting tree, thrives particularly well in mountainous regions. Its leaves are distinctive, with a light green underside and a darker green upper surface, lanceolate and obovate in shape. Anatomically, the plant features a multilayered cork, a single-layered polygonal epidermis, and a secondary cortex made up of rectangular-polygonal parenchymatous cells (Fig-2.1). M. esculenta typically grows in mixed forests, agricultural areas, and nitrogen-depleted soils, demonstrating significant adaptability to diverse environmental conditions. The plant is renowned for its edible fruits and byproducts, which are highly valued in local communities. These products provide a vital source of income for tribes in Meghalaya and the sub-Himalayan regions, underscoring the economic importance of M. esculenta (Fig-2.2). The cultivation and harvesting of M. esculenta not only support the livelihoods of local populations but also contribute to sustainable agricultural practices in these areas, highlighting the plant's potential for socio-economic development and environmental conservation. [Kabra et al (2019)]

Figure 2.1: Myrica esculenta - Illustrates it's the leaves and fruit of the ME plant.

Recent studies have brought to light the anti-allergic, antihistaminic, and anti-asthmatic properties of various medicinal plants, many of which have long-standing traditional uses in treating respiratory ailments. One such plant is M. esculenta (ME), also known as kaiphal, recognized in Ayurvedic medicine for its efficacy in managing conditions like bronchitis and asthma. ME's fruits and bark are esteemed for their expectorant and anti-inflammatory attributes, offering relief to patients and facilitating easier breathing.

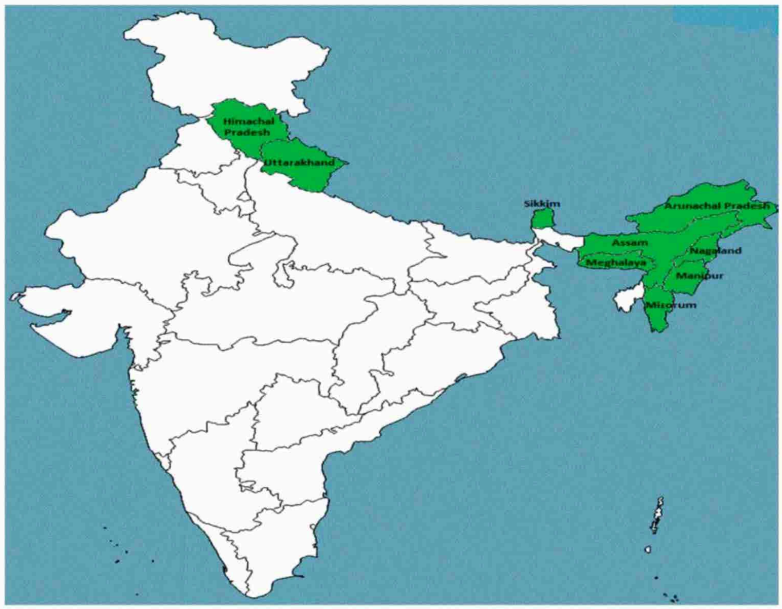


Figure 2.2: Distribution M. esculenta - The shaded region illustrates the natural distribution of ME in its native habitat across India

These findings validate the traditional knowledge surrounding ME's medicinal properties and underscore its potential as a natural remedy for respiratory disorders. Incorporating ME into modern therapeutic approaches could provide alternative and complementary strategies for managing asthma and related conditions, contributing to improved healthcare outcomes and improving the impacted people's standard living [Taur and Patil (2011)].

2.1.1 Profiling of phytochemicals

The study discovered that the high concentration of PCs like phenolics and flavonoids in M. esculenta extracts of leaves give them with properties protect cells from free radical damage, lowering the risk of chronic diseases, boosting immune health, and slowing aging showing antioxidant function . It also prevent or kill bacterial growth, help avoid infections, treat bacterial illnesses, and mitigate antibiotic resistance, promoting overall health and well-being act as antibacterial.

In order to identify and characterize these compounds for possible health benefits, more research is required [Kabra et al (2019)]. M. esculenta showcases a diverse array of Medicinal properties entail a variety of advantageous outcomes, such as antitumor growth-inhibiting anti-cancer actions, beneficial effects against ulcers, alleviation of allergic reactions, anxiolytic qualities that lessen anxiety, and neuroprotective mechanisms that guard against damage to the nervous system, all of which contribute to overall health and well-being. Its bioactive compounds, such as diaryl heptanoids like myricanol, contribute to these benefits. Additionally, it exhibits muscle relaxant properties. These findings underscore the potential of ME in treating various health conditions, from cancer to allergies, and its role in promoting overall well-being. Its multifaceted therapeutic actions make it a valuable candidate for further research and potential utilization in medical treatments and pharmaceutical formulations [Ahmad et al (2022)]

2.1.2 Pharmacology of ME Plant

Myrica esculenta (ME), commonly known as the kaiphal plant, has demonstrated significant therapeutic properties, particularly anti-inflammatory and anti-allergic effects. Research has shown that administering specific dosages of an ethanol extract from the plant's aerial parts to mice reduces vascular permeability and allergic pleurisy. This indicates its potential in managing allergic responses and inflammation. Furthermore, M. esculenta's bark from the stem has significant airway relaxants relax and widen airways, easing breathing in conditions like asthma. They're crucial for managing symptoms and improving airflow in respiratory disorders and anti-anaphylactic properties (Fig-2.3). It has been shown to effectively prevent egg albumin-induced anaphylaxis and acetylcholine- caused in experimental animals a bronchoconstriction when administered at the recommended dosage. These findings highlight its potential in treating respiratory conditions and allergic reactions.

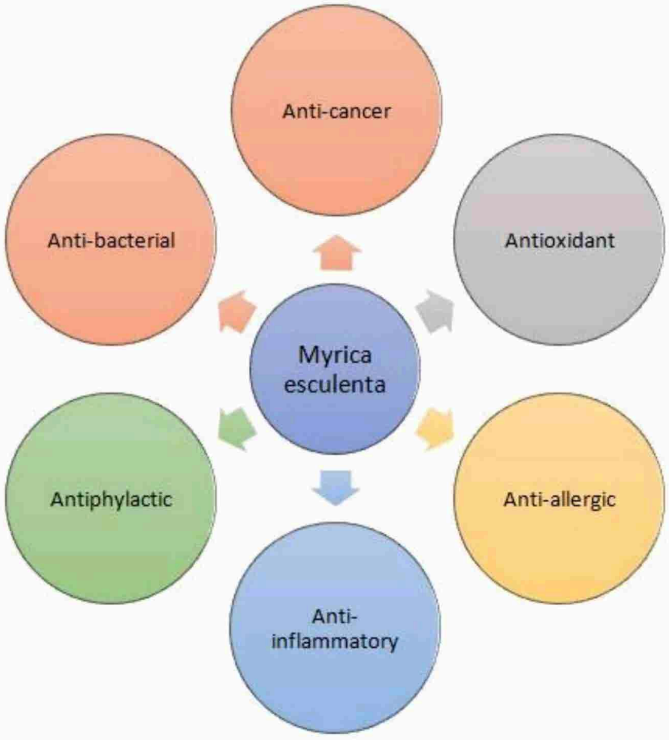


Figure 2.3: Biological property of M. esculenta

M. esculenta demonstrates a capacity to relax the ileum and trachea in guinea pigs exposed to histamine and acetylcholine, further supporting its use in respiratory therapy. The plant's ability to counteract bronchospasm and anaphylaxis suggests it could be a valuable natural remedy for conditions such as asthma and other allergic disorders. Overall, the therapeutic properties of ME make it a promising candidate for developing treatments for inflammatory and allergic conditions. Its effectiveness in reducing allergic symptoms and improving respiratory function underscores its potential benefits in clinical applications. [Ahmad et al (2022)].

CHAPTER 3

METHODOLOGY

3.1 Screening for the active ingredient

3.1.1 Acquiring of Phytochemicals for ME

A comprehensive list of phytochemicals (PC) found in ME was compiled using three open-source databases that provide information on natural products, PCIDB, Dr Duke's, and IMPPAT. Data on medicinal uses, PC, and aromatic Indian plants are gathered in the IMPPAT 2.0 database. Complying in Silico library that provides PCs with helpful property annotations and drug-like attributes were filtered. By applying cheminformatics to the database, the molecular complexity and structural diversity of medicinal plants found in India are characterized [Vivek-Ananth et al (2023)]. One of the best sources of ethnobotanical information is Dr. Duke's metabolite and ethnobotanical databases, which enable searches for plants, chemicals, bioactivity, and ethnobotany. It was made with extensive compilations put together by Duke, the former Chief of the Economic Botany Laboratory at the USDA. The database contains years' worth of quantitative data and PCs information that users can explore and search in a number of user-focused ways [Duke (1994)]. A specialized resource that lists interactions between metabolite and biological targets is the PhytoChemical Interactions Database (PCIDB). It supports research in drug discovery and the creation of plant-based therapies by offering comprehensive information on the bioactive compounds present in plants, their therapeutic effects, and possible interactions. These PC was located, given an individual identification number, and its chemical properties were taken out of the PubChem database using canonicalized SMILES and PubChem Id.

Based on structural features, ClassyFire is an automated chemical classification system that classifies compounds into a hierarchical structure. Through the methodical classification of compounds into classes, superclasses, and subclasses, it facilitates the comprehension of chemical characteristics and biological effects documented across diverse chemical databases. SMILES were converted to InChI after being acquired from PubChem. After that, these were uploaded to ClassyFire for categorization. The Alluvial plot was used to display the results [Djoumbou Feunang et al (2016)]

3.1.2 Swiss ADME

Among the selection criteria were Different aspects of pharmacokinetics and the degree to which a compound will resemble a drug are predicted using a web tool called Swiss ADME [Daina et al (2017)]. Drug development and discovery professionals use a set of

standards known as Lipinski's Rule of Five to evaluate a chemical molecule's potential for use as a medication. It is ideal to have the molecular weight of less than 500 daltons. It is desirable for the octanol-water partition coefficient (Log P) to be less than 5. It is preferred to have up to five hydrogen bond donors. There is a need for fewer than ten hydrogen bond acceptors. Several of these guidelines may work against a compound's potential to become an oral active medication. Compounds with no blood-brain barrier permeability, strong gastrointestinal absorption and a 0.55 bioavailability score with rotable hydrogen bond must be less than ten. These standards also enhance the potential of drug candidates to achieve therapeutic targets and improve overall bioavailability by optimising them for efficient oral delivery.

3.2 Target prediction

3.2.1 M. esculenta associated PCs genes

Currently, determining whether a molecule can interact with target with relevant pharmacology is a crucial step in the advancement of drug development. For this purpose, target predictions of ME compounds were conducted using STITCH [Kuhn et al (2008)], SWISS Target Prediction [Daina et al (2019)], and CTD [Davis et al (2023)]. The use of three tools having different methodologies was used to diversify the amount of target predictions which could be then screened systematically through network analysis using canonical smiles. Swiss target prediction is a web server which accepts the smiles of the molecules and predicts the targets determined by the concept of similarity which is conducted via a Swiss Target Prediction compares compound structures to known ligands in opposite screening to predict protein targets, which helps with drug discovery in pharmaceutical research and development. The smiles of the active compounds were submitted to Swiss Target Prediction server using following /, “Homo sapiens” species was selected and targets were predicted, the predictions were saved in csv format and the entries with predictions score equivalent to zero were discarded as they serve no meaning to the study. STITCH is a search tool that combines information from drug-target relationships crystal structures, metabolic processes, and binding studies to comprehend how small molecules and proteins interact. Chemical structure similarity, text mining, and phenotypic effects are used to predict chemical relations. The database has information on how various compounds interact with one another. Second tool used is CTD is a special a source for researchers’ community because it combines diverse cross-species data on chemical ingestion and its biological effects. It gathers and connects data on chemicals, genes, phenotypes, anatomy, diseases, species, and exposure through literature. The database now contains 20% more data about chemicals, genes, phenotypes, diseases, and exposure statements. In order to construct molecular mechanistic pathways, information blocks made up of four units are generated by the CTD Tetramers tool.

3.2.2 Data mining for asthma related genes

Data mining for asthma related genes was performed on following electronic databases: OMIM database [Amberger et al (2019)], GeneCards [Stelzeret et al (2016)],

PharmGKB [Gong et al (2021)] along with Drug bank [Wishart et al (2008)]. The keywords used for the queries were “Asthma”. OMIM is an online knowledge repository which includes catalog of human genomics and genetic characteristics that is openly accessible; it includes over 16,000 the genes and details on mendelian illnesses. Scientists can rapidly examine and establish connections between a vast array of individual genes, conditions variants proteins, cells, and processes in biology with the aid of GeneCards, an extensive human gene compendium.. Thanks to a redesigned infrastructure, the latest version of offers a better user experience, faster data updates, and more accurate data queries. For GeneCard relevance score ≥ 1 is taken in account. PharmGKB is an online resource devoted to comprehending how drug responses are influenced by genetic variations. It contains details about prescription drugs, clinical recommendations, genetics, illnesses, and drug labels. Numerous published papers and a computer-based natural language processing tool are used in the compilation of the database. Explanations of how to use the website and analyze results for metabolize phenotypes or genotypes associated with pharmacogenomic variants are given in the article. Drug Bank is a comprehensive resource for drug discovery, design, docking, metabolism prediction, and interaction prediction. It has expanded significantly since its launch in 2006, bringing in more FDA-approved biotech and small molecule drugs as well as new drug entries. The most recent version includes additional protein target information, experimental ADME data, and food-drug interactions as new data fields.

3.3 Building PPI Networks and Discovering Therapeutic Genes

Prior to constructing the system for PPI , the genes associated with asthma that were common to the predicted targets of ME active ingredients was done using a web tool Venny 2.0 [Oliveros (2007)] which was accessed to make Venn diagrams were also made. To understand the mechanism of targets involved and their visualization, PPI networks aids in this task. STRING web tool was used to for the constructing of protein network of genes that were common.
[Szklarczyk et al (2021)], integrating PPI associations is the STRING tool, both functional and physical, through experiments, predictions, text mining, and systematic transfers. It covers multiple organisms and offers enhanced text-mining, scoring, and user interface features, enabling comprehensive genome-wide queries using experimental data. The common drug-disease genes were submitted to the multiple proteins input section, and Homo sapiens were selected as the organism of choice. Nodes having interaction score of confidence score of 0.9 or above was shortlisted only for further network analysis as this node have the meaningful interactions within the network of the PPI. Then by means TSV as the format was used for export it was exported in TSV format which is compatible with Cytoscape.

Network Analyzer in Cytoscape [Doncheva et al (2012)] is a powerful tool used to calculate topological parameters of biological networks, such as degree, closeness, and betweenness centrality. These parameters help in understanding the importance and role of individual nodes (genes or proteins) within the network. Degree this parameter

calculates the quantity of direct connections between a node along with various nodes. In biological terms, a high-degree gene or protein is often referred to as a hub, indicating its significant role in cellular functions and interactions. Closeness centrality shows the distance between a node and all the others in the system. A gene with high closeness centrality can quickly influence the entire network, reflecting its potential impact on biological processes. The number of times a node serves as a connecting point along the shortest route between the two other nodes is determined by betweenness centrality. Genes with high betweenness centrality are crucial for controlling information flow and can be key regulators in biological pathways. In a typical analysis, Network Analyzer calculates these parameters for each gene in the network. The median values for degree, closeness, and betweenness centrality are then determined. Genes with parameter values equal to or exceeding these median values are selected for further analysis represent as the following equation 3.1;

$$\boldsymbol{Median} = \left(\frac{\mathbf{n+1}}{\mathbf{2}}\right)^{\mathbf{th}}$$

In these 'n' shows the number of terms and 'th' is given for the n(th) number. This subset of genes is used to form a sub network, which is more focused and manageable compared to the entire network.

3.4 Gene enrichment

The enrichment of identified hub genes was conducted through DAVID [Sherman et al (2022)], its well-known computational biology system provides web services and a web server for analyses of enrichment and annotation of gene lists. It features a variety of functional analysis tools and an extensive knowledge base. The identified therapeutics genes were submitted to this server in "Official Gene Symbols", "Homo sapiens" was selected as organism of choice and type of list was selected as "Gene List". The results pathways the for KEGG [Kanehisa and Goto (2000)] and Gene ontology [Ashburner et al (2000)] and GAD disease pathway were imported having p-value and count of genes, enrichment bubble plots and enrichment bar with color were constructed using SR Plots [Tang et al (2023)] web tool.

3.5 Construction active ingredient-Gene Target-Pathway and Disease system

In our methodology, we utilized Cytoscape 3.10.1 to construct networks illustrating the poly-pharmacological activity of M. esculenta PCs and their targeted genes. These networks were instrumental in visualizing any observed synergism, shedding light on the complex interactions between multiple active ingredient and their respective target genes. Specifically, we created networks depicting the relationships between active ingredients and target genes, as well as the enrichment of target genes within specific pathways. This approach allowed for a comprehensive understanding of how ME

phytochemicals exert their effects on biological systems, providing valuable insights for further investigation and potential therapeutic applications.

3.6 Toxicity profiling

Toxicology analysis is performed using the Protox (Banerjee et al (2018)] web tool with input of canonical SMILES strings. Users choose toxicity parameters such as cytotoxicity, immunogenicity, mutagenicity, and carcinogenicity by entering the SMILES representations of chemical compounds. Based on these parameters, Protox then analyses the chemical structures and produces comprehensive toxicity profiles. This tool is very helpful for drug development and safety evaluation because it helps researchers predict adverse effects. It's automated, effective, and dependable procedure guarantees the security of novel therapeutic candidates while assisting in the identification of potentially hazardous compounds.

3.7 Structure based method for validation

3.7.1 Molecular docking

Molecular docking, enabled by PyRx software [Dallakyan and Olson (2015)], predicts the binding interactions between specific target proteins and selected ligands. Understanding these interactions is crucial for effective drug discovery, as it helps identify precise drug candidates. Key proteins are then shortlisted based on their degree within the target PPI sub network, with their crystal structures taken from the PDB [Burley et al (2017)]. Ligands chosen for docking are also selected based on their degree within the compound-gene-pathway network, with their structures sourced from the PubChem repository [Kim (2016)].

Molecular docking involves several key steps including protein preparation, identification of the active site, ligand preparation, and docking. This process of docking begins by loading the target protein into the program and transforming it into a macromolecule. Selected ligands are loaded into the software using Open Babel and changed to the pdbqt format for energy minimization. Docking is performed using Vina Wizard by selecting all the pdbqt files of the ligands. Before running the docking process, a grid box is set around the protein binding site. After docking, the binding affinities for each ligand against the macromolecule are stored. The output files of each docked ligand are saved for subsequent 2D analysis, which shows the interactions and hydrogen bonds between the ligand and macromolecule using Discovery Studio [Pawar and Rohane (2021)].

3.7.2 MD simulation

3.7.2.1 iMODS

Internal coordinates for NMA is a technique that facilitates the exploration and generation of transitions, even for substantial molecules, between structures that are similar. A flexible server, iMODS helps with movement illustrations, morphing trajectories, and evaluation of vibrations, making it accessible for both non-specialists and advanced users. This server allows users to quickly characterize potential conformational changes and customize model resolution as needed. iMODS offers advanced visualization tools to show collectively movements, such as an improved arrow representation of realm patterns based on the affine model. Upon submission of an atomic structure or retrieval of a Protein Data Bank ID entry, iMODS performs standard NMA in internal coordinates. To help users investigate potential collective motions, the lowest frequency normal modes are determined by the web server using internal the coordinates. The server provides effective motion representations, such as modal animations, affine-model arrows, and vector fields. Mobility profiles, deformability properties, eigen value computations, covariance maps, and connectivity matrices are all included in the evaluation [López-Blanco et al (2014)].

3.7.2.2 GROMACS

To further understand the binding stability and conformational changes of top identified MPLE phytochemicals in their respective targets, full atom-based MD simulation using the GROMACS2020 version [Van Der Spoel et al (2005)]. The docked conformation was used as starting point for the dynamics. The CHARMM GUI server was used for producing the MD simulation's input files [Lee et al (2016)]. The TIP3P water model was utilized to accommodate the water model in a rectangular box and the distance between the protein complex and box's edges were set to 10 Å. The system was neutralized to physiological pH 7.4 by addition of the proper number of counter ions (Na^+ and Cl^- ions) at 0.15M concentration. To optimize energy consumption, the steepest descent algorithm was applied. By iteratively adjusting parameters in the direction of the steepest energy decline, it minimized potential energy, stabilizing the system. This refined the initial setup, ensuring a low-energy, stable configuration for accurate further analysis or simulations for prepared system. equilibration at NVT (Noose Hoover thermostat) and NPT (Berendsen thermostat) ensemble for duration of 100ps each. The final production step was carried out at 300.15K and at 1 atm pressure for duration of 10ns with time step of 2fs. Trajectory analysis involved the examination of generated trajectories through metrics such as RMSD, RMSF, Hydrogen Bond count, as well as the generation of plots SASA and Principal component analysis of eigen vectors. These plots were created using the XMgrace software package (GRACE v5.1.19) [Turner (2005)]

3.8 Chemical map spacing for ligand validation

The similarity of PCs with STAT3 protein inhibitors was visualized using an unsupervised machine learning-based dimensionality reduction technique (PCA). The SMILES strings for STAT3 inhibitors were retrieved via the database known as ChEMBL [Zdrazil et al (2024)] (CHEMBL ID: CHEMBL4026) PubChem fingerprints

were generated from these SMILES strings, which were then used to construct a PCA plot. This plot facilitated a subsequent chemical space analysis. All processes were executed within the use of KNIME Analytics [Berthold et al (2009)] by system utilizing RDKit along with KNIME nodes [Landrum (2016)].

3.9 Validation of target gene

Researchers in the field of medicine use the GTEx database to examine the relationships between gene expression and genetic genotypes in humans. Several types of human tissue and donor samples are included in the most recent release of the dataset. With RNA-seq samples, the musculoskeletal system is the most abundant tissue. Transcripts corresponding to human genes, including those that code for proteins and those that do not, were examined in the study. Target identification by gene ID was required by the GTEx database, which then analyzed the data based on exon and bulk tissue gene expression.

The study looked at transcript isoforms in genes that code for proteins using the Top-ranked transcript isoform display web-interface (TREGT), which uses GTEx transcript TPM expression data files and gene symbols for the search, user-friendly website that allows visitors to explore the most highly identified transcript isoforms across a variety of healthy human tissues. The protein-coding genes were ordered by highest-ranking transcript isoform and expression level. Inferential studies were performed to analyze variations in the pattern of expression of transcript isoforms across genes that code for proteins. Potentially important Rank1 switch event genes were identified by determining the variance in transcript ranked across tissues [Tung et al (2020)].

CHAPTER 4

RESULT

4.1 M. esculenta derived metabolite

To screen the active ingredient from metabolite, the study collected data from three open-source databases. A total of 67 PC were acquired from the websites PCIDB, Dr. Duke, and IMPPAT (Table IV.I: Comprehensive list of PC from M. esculenta). After removing the duplicates from this set of PCs, 49 unique PCs were left, which are subsequently used for further investigation. We were able to obtain 45 of their canonical smiles from the PubChem server, so those were taken. Subsequently, Swiss ADME performs metabolite filtering, selecting only molecules that exhibit no violations of the Lipinski rule, are impermeable to the BBB, have good GI absorption along with a rotable H-bond and bioavailability score. The screening criteria were only met by Myricanol, Arjunolic acid, Gallic acid, Caffeic acid, Cianidanol, Ellagic acid along with Quercetin (Table-4.1).

To analyze the chemical properties of various phytochemicals, their structures were first taken in SMILES format from the database of chemical structure called PubChem. These SMILES strings were then converted to InChI format using cheminformatics tools for compatibility with the ClassyFire classification system. The InChI representations were uploaded to ClassyFire, which categorized the compounds into hierarchical levels including kingdom, superclass, and subclass based on their structural features.

Table 4.1: Attributes of active ingredient

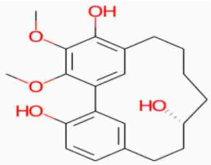
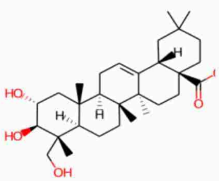
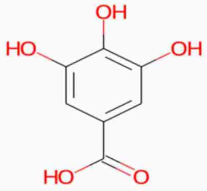
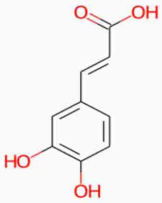
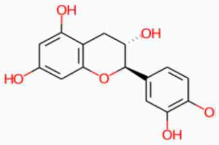
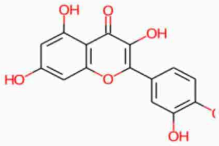
S.No	IMPPAT ID	Compound	Structure	Molecular weight	Formula	Bioavailability
1.	IMPHY005413	Myricanol		358.43	C21H26O5	0.55
2.	IMPHY011627	Arjunolic acid		488.7	C30H48O5	0.56

Table 4.1 (continued)

3.	IMPHY012021	Gallic acid		170.12	C7H6O5	0.56
4.	IMPHY005537	Ellagic acid		302.19	C14H6O8	0.55
5.	IMPHY011933	Caffeic acid		180.16	C9H8O4	0.56
6.	IMPHY014854	Cianidanol		290.27	C15H14O6	0.55
7.	IMPHY004619	Quercetin		302.24	C15H10O7	0.55

The classification results revealed that all the active ingredients fall under the organic compound kingdom. They were further divided into superclasses, with five active ingredients categorized under Phenylpropanoids and polyketides. Specifically, myricanol was classified as a diarylheptanoid, ellagic acid as a tannin, caffeic acid as a cinnamic acid, and both cianidnol and quercetin as flavonoids. Conversely, arjunolic acid was classified under lipid-like molecules. The results of this classification were visualized using an Alluvial plot (Fig-4.1), which highlighted the distribution and relationships between the different classes and subclasses. [Djoumbou Feunang et al (2016)].

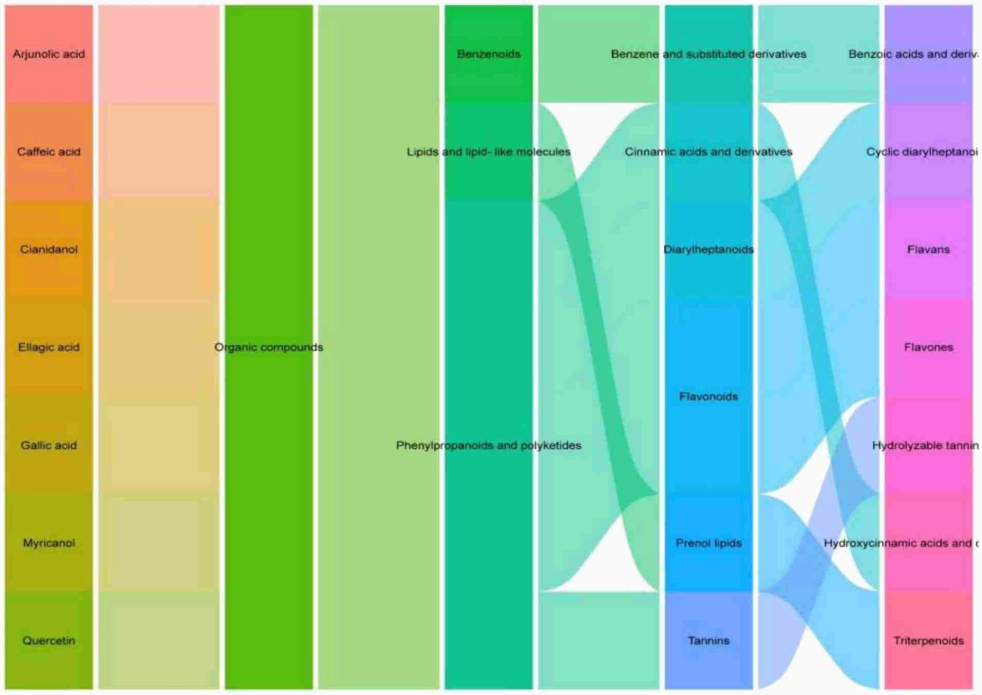


Figure 4.1: Classification of active ingredient – With the help of hits value Classyfire tool classify the data into kingdom then into subclasses.

4.2 Prediction of active ingredients and asthma target

For the active ingredient, a comprehensive list of 895 ME-related genes was identified by three web servers, Swiss Target Prediction, STITCH, and Comparative Taxicogenomics Database (CTD) and the interaction between PC with their target genes in Fig-4.2 is shown. Of these, only 483 genes were found to be unique. Similarly, for asthma, a list of 3560 genes was identified from four different web tools, including OMIM, GeneCard, PharmGKB, and Drug Bank, with 2945 genes remaining unique after redundant data was removed.

Total 226 common genes (Fig-4.3) between asthma and ME were found using a Venny 2.0 server these genes will be utilized for further investigation and network construction. For the sake of the research was to find compounds that could be targets for asthma. The goal of the study was to offer insightful information about PC's potential for treating this type of disease.

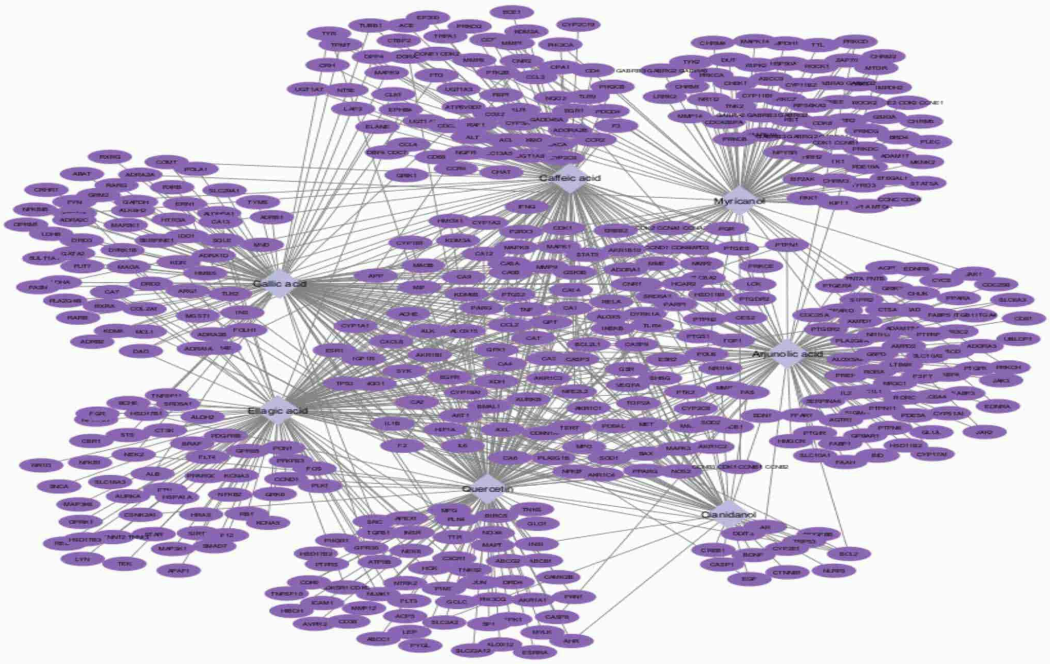


Figure 4.2: Compound- target gene network- showing interaction between target genes (purple) and active ingredient (Mauve) containing the value of 496 nodes and 854 of edge

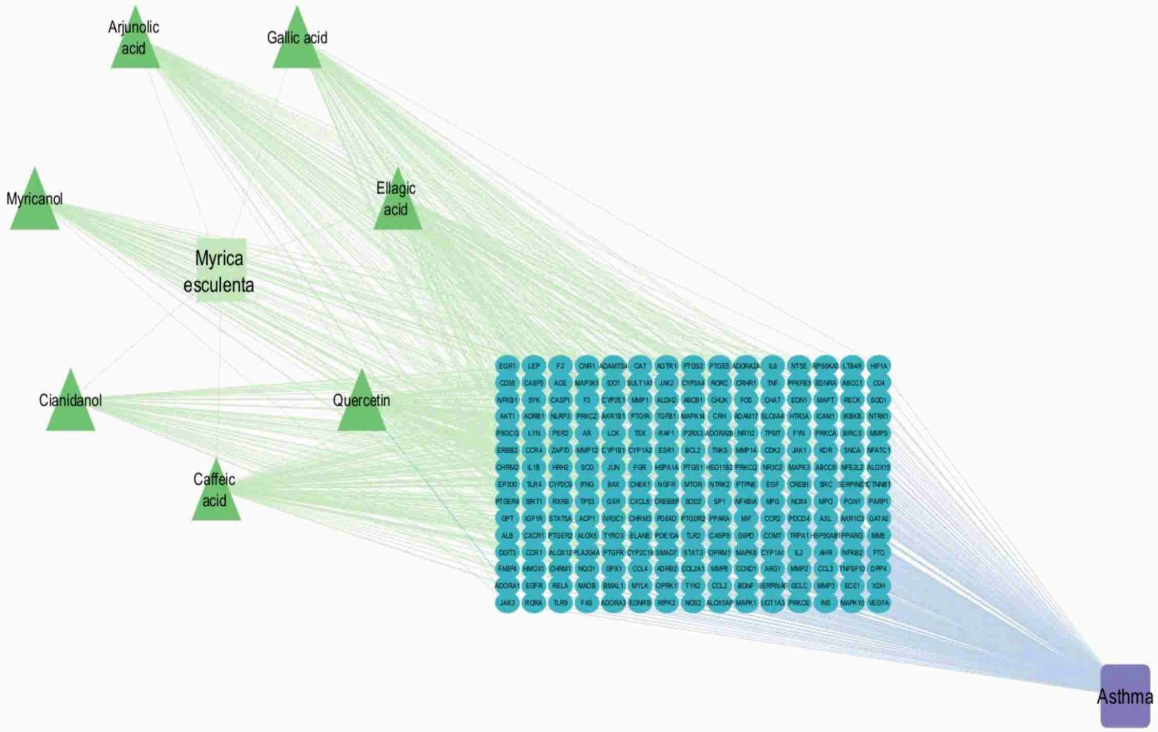


Figure 4.3: Identification of common genes – Illustrating the interaction between active ingredient and asthma (light green- M. esculenta, green- active ingredient, blue- common genes, and the purple- asthma)

4.3 PPI network

To visualize the protein-protein interactions among common genes, we utilized Homo sapiens data with the highest confidence level, resulting in a network comprising 190 nodes and 784 edges (Fig-4.4). This network was then transferred to Cytoscape for further analysis. Utilizing network analyzer, we assessed parameters for the topological analysis by means degree, betweenness centrality, and closeness centrality by calculating their mean values for the gene network. Employing this method, we filtered the topological parameters based on a median value greater than or equal to 5. Subsequently, sub-network-1 was formed, with values of 0.0744879 and 0.41256068. Through this process, we identified highly interactive genes within the network, leading to the formation of consecutive sub-networks, namely sub-networking-2 and sub-networking-3 as shown in diagrammatic illustration in Fig-4.5 (see Table IV.II: Gene list for topological analysis). Notably these are IL6, STAT3, JUN, NFKB1 and RELA having 5 nodes and 10 edges emerged as potential therapeutic genes (refer to Table-4.2).

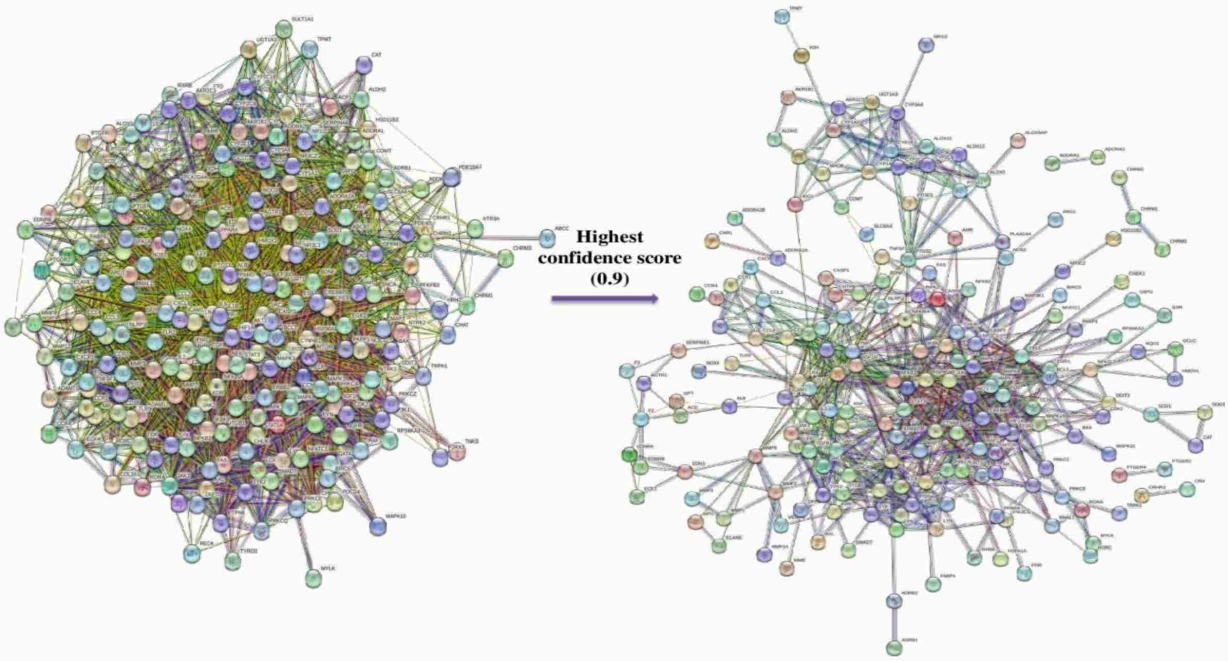


Figure 4.4: Protein- protein interactions (PPI) of common genes by STRING – For these PPI network is filtered by applying the confidence level of 0.9.

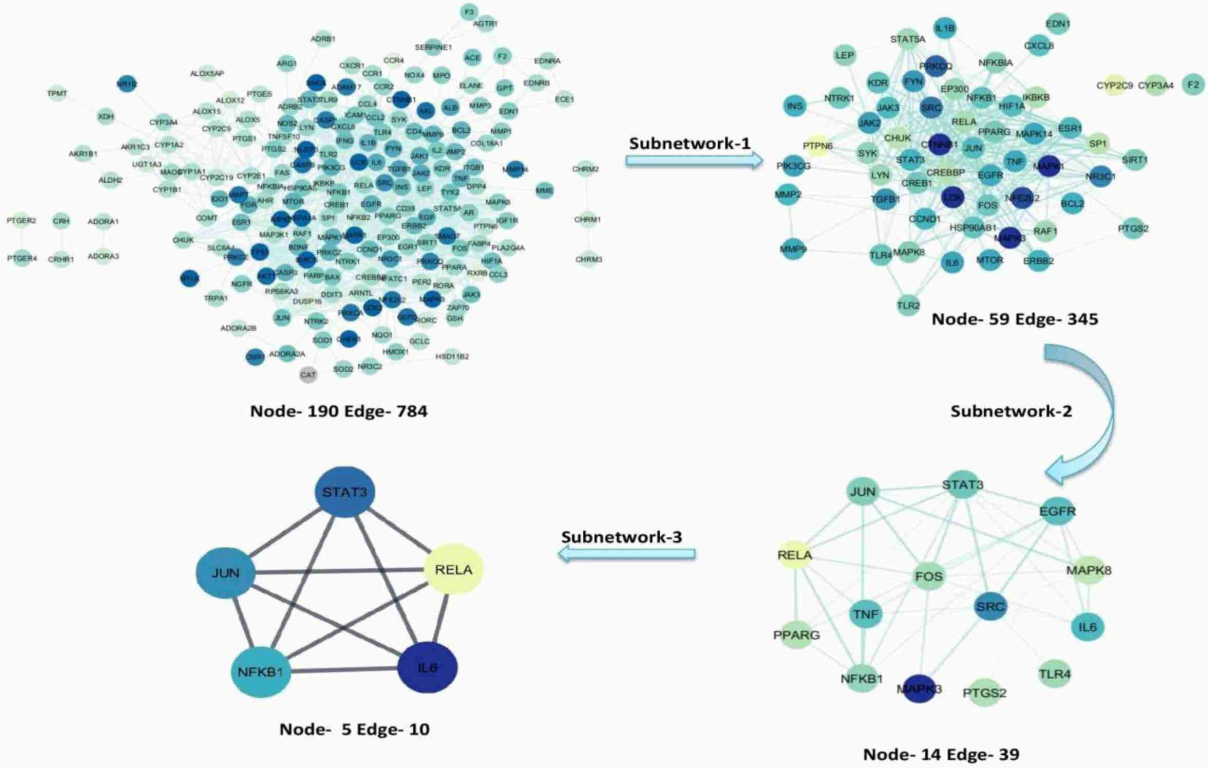


Figure 2: Streamed line filtration of complicated PPI network- Utilized for the identification to therapeutic genes based on parameter of topological (color pattern is based on the same parameter).

This sub network highlights genes that are potentially significant in maintaining the integrity and function of the biological system under study. By identifying these critical genes, researchers can pinpoint potential therapeutic targets. These targets are often involved in key regulatory mechanisms, resulting in them attractive options for the discovery of novel medications along with therapeutic intervention. This approach ensures that the selected genes are not just randomly chosen but are statistically significant within the network, enhancing the reliability of the findings.

In inflammatory processes, including asthma, transcription factors like STAT and NF-kB are essential. Research indicates that STAT6 may be a target for IL-4 effect blocking, providing an avenue for treating allergic asthma. STAT3 is required for the stimulation of cells that contribute to the development of asthma. Lung inflammation severity can be decreased by inhibiting STAT3. Prolonged inflammation such as pneumonia Th cells along with macrophages, leading to symptoms such as respiratory tract remodeling, hyper-reactivity, and neutrophil infiltration [Nikolskii et al (2021)]. Studies have demonstrated the importance of mitochondrial STAT3 for histamine secretion and mast cell degranulation, and the reduction of MC degranulation by its

inhibitor. This demonstrated that since STAT3 controls the activities of mast cells, It might be a fresh focus for the treatment of allergic illnesses [Erlich et al (2014)]. With its highest degree value of 39, STAT3 is the main protein to target when treating diseases (Table-4.2).

Table 4.2: Topological parameter for therapeutic genes

S.No	Gene symbol	Name	Degree	Betweenness Centrality	Closeness Centrality
1.	RELA	RELA proto-oncogene, NF-kB subunit	29	0.561225	0.636363636
2.	NFKB1	nuclear factor kappa B subunit 1	28	0.751435	0.685714286
3.	JUN	Jun proto-oncogene, AP-1 transcription factor subunit	31	1.085781	0.543478261
4.	STAT3	signal transducer and activator of transcription 3	39	0.728363	0.642857143
5.	IL6	interleukin 6	32	1.81196	0.520661157

4.4 Analysis for enrichment

Gene enrichment analysis plays a pivotal role in network pharmacology, providing insights into drug mechanisms, target identification, adverse effect prediction, personalized medicine, and drug repurposing. By identifying sets of enriched genes related to specific pathways or diseases, researchers can understand how drugs or natural compounds act at the molecular level. This understanding helps prioritize drug targets, predict adverse events, customize treatments based on individual genetic profiles, and discover new therapeutic uses for existing drugs. For this study, the DAVID tool from NIH bioinformatics was utilized. DAVID allows researchers to uncover and interpret the biological significance hidden within a set of genes. Three biological themes were investigated for the hub genes: KEGG Pathways, GAD disease ontology, and Gene Ontology such as Molecular Functions, Cellular Components, and Biological Processes. After that, the enriched ontology was imported, and significance was checked to make sure the results were statistically significant.

The gene ontology (GO) analysis identified various processes related to the transcription mechanism, with the top-scoring processes selected for graph plotting. In the biological process (BP) category of gene ontology (Fig-4.6b), the highest value was assigned to the

positive regulation of transcription from a DNA template. Among these processes, those are involved in the positive control of IL-6 and IL-8 secretion. Asthma, inflammatory bowel disease, and arthritis share similar inflammatory responses, but they also differ due to factors unrelated to NF-κB. In asthma, IL-5 secretion promotes eosinophilic inflammation, and a chemokine regulated by NF-κB amplifies the disease-specific inflammatory process [Barnes and Adcock (1998)]. As IL-6 activates STAT3 produces the anti-allergic response. The pathway for the signaling mediated by cytokines, the response to discomfort and increased production of vascular endothelial growth factor. Th1 T-helper cells produce cytokines, which are involved in allergic reactions, asthma, atopic dermatitis, and macrophage activation, among other inflammatory responses [Sprague and Khalil. (2009)]. Growth factors and cytokines contribute the significant function in angiogenesis, were process of forming new vessels of blood. In active angiogenesis, IL-6 expression is elevated [Cohen et al (1996)].

For cellular compartment (CC), the highest scores were observed for the transcription factor complex, along with chromatin, nucleoplasm, nucleus, and other transcription factors. When transcription factors bind to particular sequences or activate gene promoters, messenger RNA is formed and RNA polymerase II is activated, which starts transcription. These ubiquitous factors coordinate cellular responses by controlling gene expression in cells. For maximum gene expression, interactions between transcription factors, like NF-κB and CAAT/enhancer binding protein, may be required [Barnes and Adcock (1998)]. This indicates that therapeutic genes are involved at the molecular level, particularly in transcription (Fig-4.6b).

For the molecular function (MF) one of them transcription regulatory region-sequence-specific DNA binding comes under the top pathway in MF (Fig-4.6b) along with additional sequence-specific DNA binding TF that involve RNA Pol II. Transcription is initiated when transcription factors bind to specific recognition sequences or activate target gene promoters. This leads to the formation of messenger RNA and the enhancement of gene transcription through the activation of RNA polymerase II. Binding to specific motifs in the promoter region can alter transcription by interacting with cofactors or other components of the basal transcription machinery [Latchman (1997)].

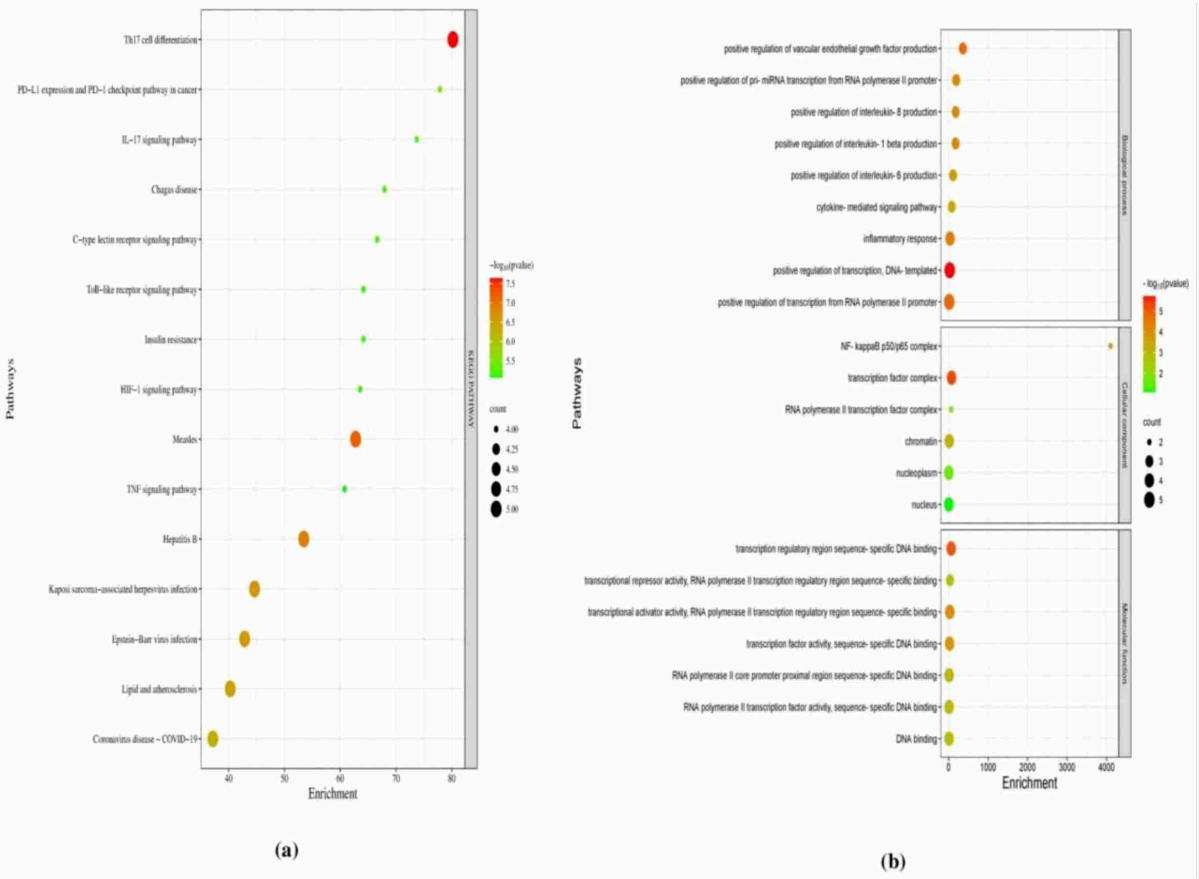


Figure 4.6: Enrichment analysis (a) KEGG pathways and (b) Gene ontology- The red highlighted circle in the KEGG pathway (a) shows the highest value among the other pathway which is Th17 cell differentiation. For GO analysis (b), biological process reveal the pathways that are involved in the cytokine signaling, cellular component shows complex involved in the transcription machinery and molecular function protein required for the trancription including RNA Pol II and transcription factor.

The top 15 KEGG pathways with the highest relevance to Th17 cell differentiation, a critical process in the allergic response, include those involving Toll-like receptor and TNF signaling pathways. These pathways are crucial in the immune response and inflammation. Key cells that produce IL-17, plays the important roles in these pathways are played by immune system components such mast cells, eosinophils, macrophages basophils, T cells, and dendritic cells. When IL-17 binds to its receptor (cytokine-cytokine receptor interaction), it triggers Th17 cell differentiation. This process involves several critical signaling pathways, including MAPK, Apoptosis along with NF-kB pathways in the signaling (Fig-4.6a).

Immune responses require the release of a variety of cytokines and chemokines, which occur when these pathways are active. Neutrophils get drawn in and activated more easily by chemokines and cytokines, which intensifies the inflammatory response. This recruitment is crucial in the context of allergic reactions and autoimmune conditions, where an overactive immune response can lead to tissue damage and chronic inflammation (Fig-4.7). Understanding these pathways and their interactions provides insight into the mechanisms underlying allergic responses and autoimmune diseases. This knowledge can help in developing targeted therapies to modulate Th17 cell differentiation and the associated inflammatory responses, potentially leading to more effective treatments for conditions like asthma and other allergic diseases.

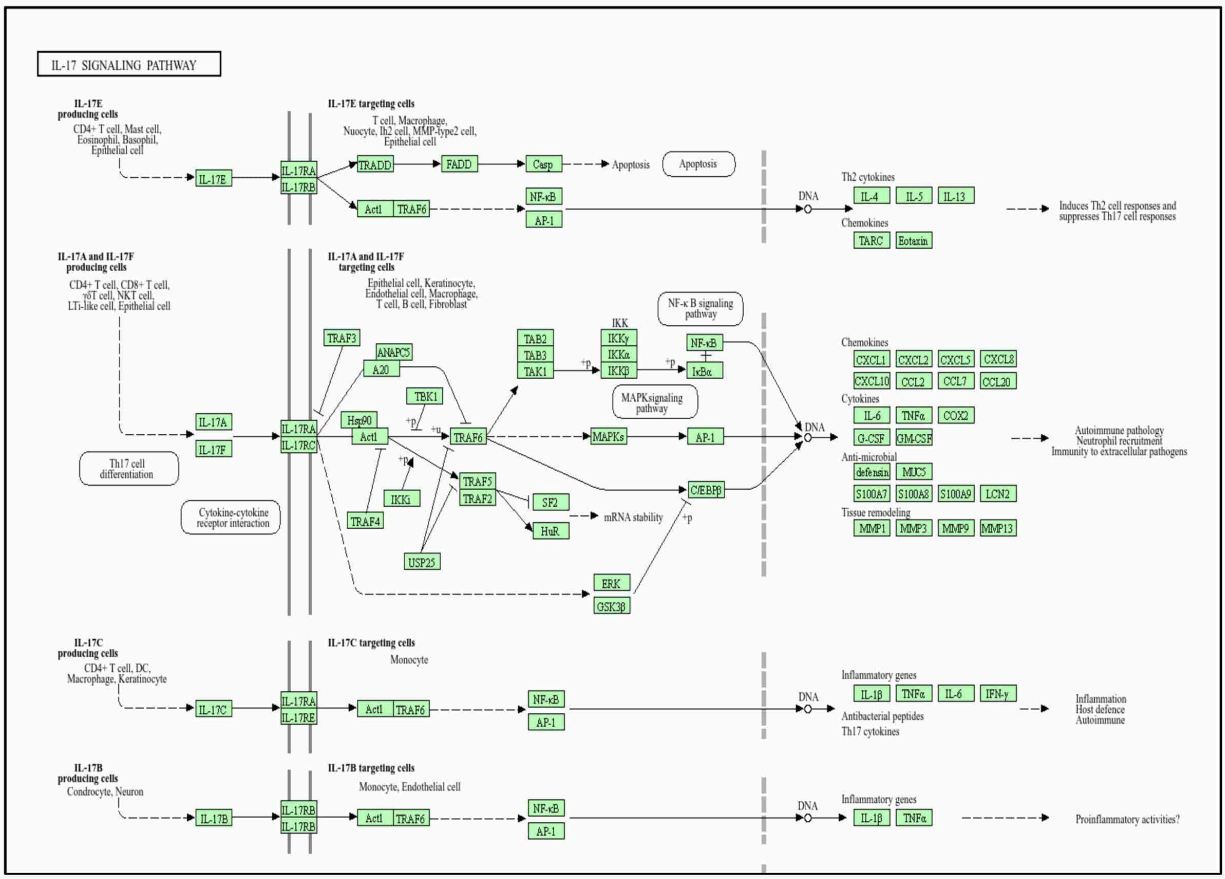


Figure 4.7: KEGG pathway- For Th17 cell differentiation which is the target pathway were the interleukin binds to its receptor to induce the signaling the pathways involving NF-κB at the molecular level leading to production of the cytokine thus causing recruitment of the immune cells to site producing allergic response.

For the disease ontology from GAD diseases for the top 10 diseases (Fig-4.8) which revealed respiratory syncytical virus bronchiolitis with highest value among other illness condition. Research increasingly suggests a strong correlation between respiratory

syncytial virus (RSV) bronchiolitis in infancy as well as the subsequent development of the asthma-related symptoms in childhood. Evidence indicates that children experiencing lower respiratory symptoms during RSV infection in their early years face an elevated risk of exhibiting asthma-like symptoms as they progress through school. RSV-induced bronchiolitis is characterized by inflammation and obstruction of the small airways, which can lead to wheezing and difficulty breathing.

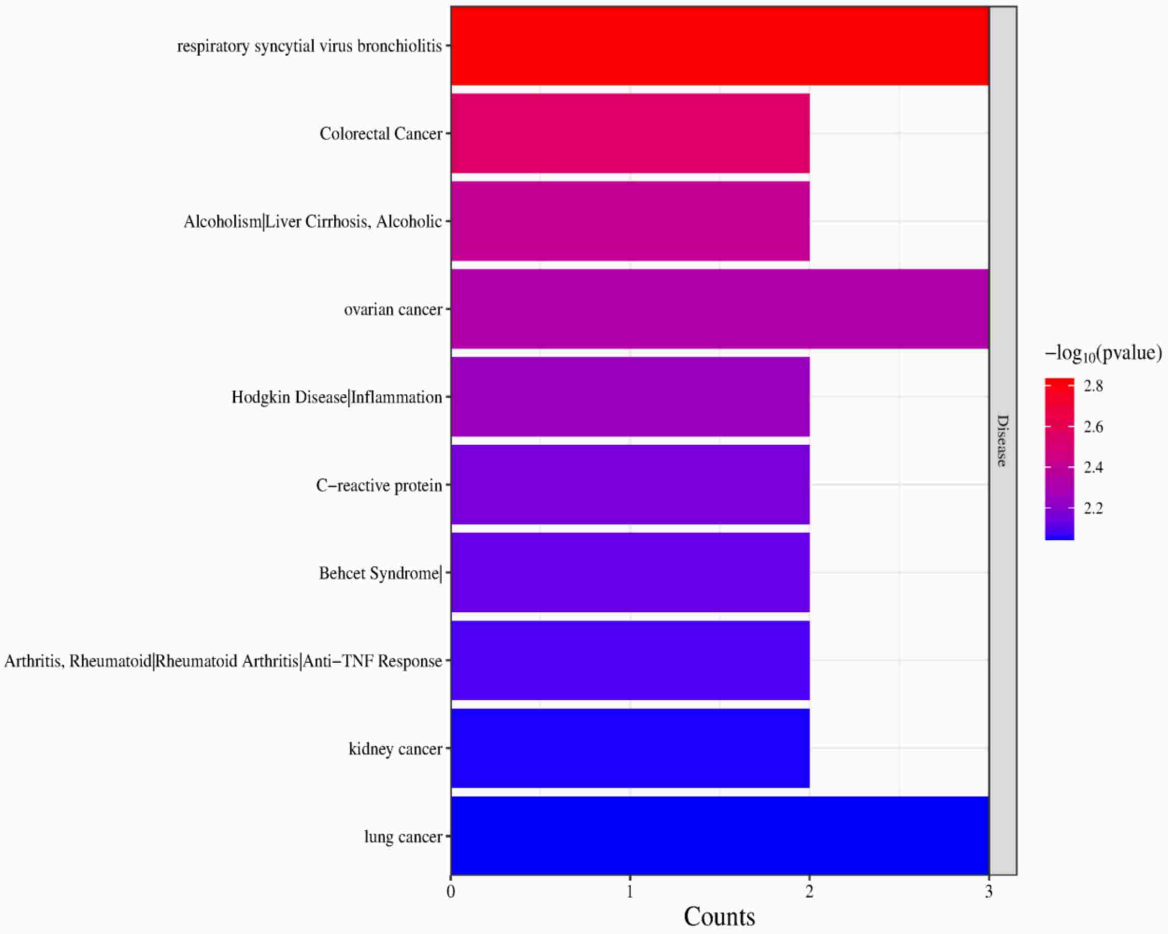


Figure 4.8: Top 10 pathway by GAD DISEASES- The highlighted red color represents the respiratory syncytial virus (RSV) bronchiolitis is a common lung infection in children, marked by inflammation of the small airways. This condition can increase the risk of developing asthma later in life due to the long-term impact on the respiratory system's structure and immune response.

These respiratory symptoms, often severe during RSV infection, may persist or recur in later years, resembling asthma manifestations. The inflammatory response triggered by RSV infection can cause lung damage and alter the airway structure, potentially

contributing to the development of chronic airway inflammation characteristic of asthma. Furthermore, RSV-induced bronchiolitis may disrupt the immune system's development, predisposing individuals to allergic sensitization and asthma. Overall, the association between RSV bronchiolitis in infancy and subsequent asthma-like symptoms underscores the importance of early-life viral respiratory infections in shaping long-term respiratory health outcomes [Martinez (2003)].

4.5 Tripartite network

In the tripartite network constructed using Cytoscape, M. esculenta (ME) extract PCs were linked to their therapeutic targets, which were further connected to the top KEGG pathways identified through gene enrichment analysis. Notably, Ellagic acid showed interactions with all the therapeutic genes and had the highest degree among other PC, with all these genes being enriched in the identified KEGG pathways (Fig-4.9). According to research, ellagic acid, which is well-known for having antioxidant qualities, may be utilised as a medication to lessen inflammation in the allergic airways. By enhancing immune clearance processes and focusing on inflammatory pathways in vivo study on animal model, it may pave the way for novel therapies for allergic respiratory disorders. This implies that ellagic acid may be used to treat allergy-related illnesses [Alves el al (2013)]. This visualization demonstrates the potential multi-target effects of M. esculenta PC on key genes involved in various allergic pathways, indicating their therapeutic relevance for asthma treatment. By targeting key genes within these pathways, ME phytochemicals present promising potential for addressing the underlying mechanisms of asthma.

To delve deeper into the probable molecular mechanisms underlying ME and therapeutic genes in this pathway, a Hierarchical network was constructed. A detailed examination of the tripartite network reveals that the majority of M. esculenta PC interact with genes enriched in the Th17 cell differentiation, IL-17, Toll-like receptor, and TNF signalling pathways in KEGG (Fig-4.10).

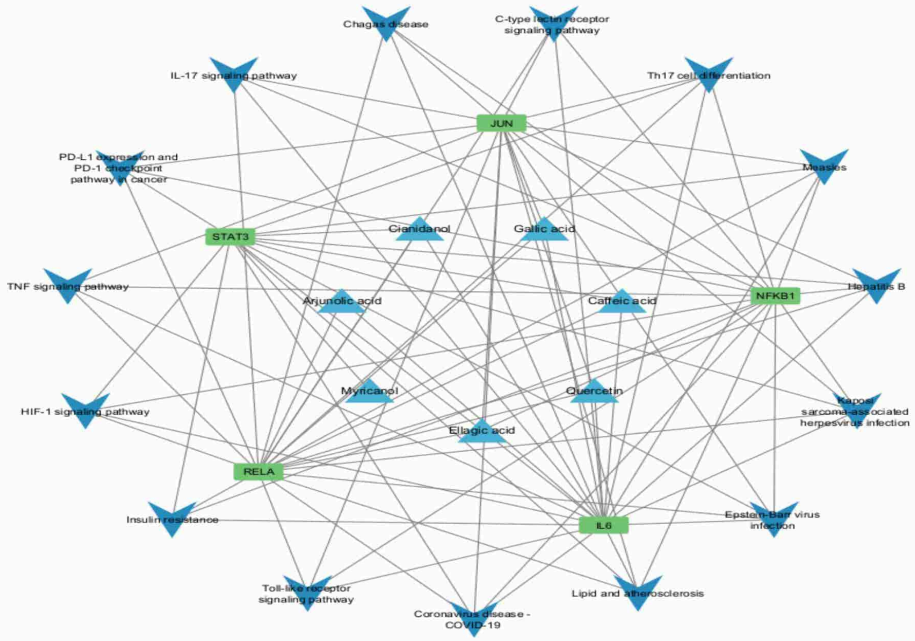


Figure 4.9: Tripartite network- illustrating complex interaction of Compounds-therapeutic genes-KEGG pathway (Blue- compound, Green- therapeutics genes, Dark blue- KEGG pathway)

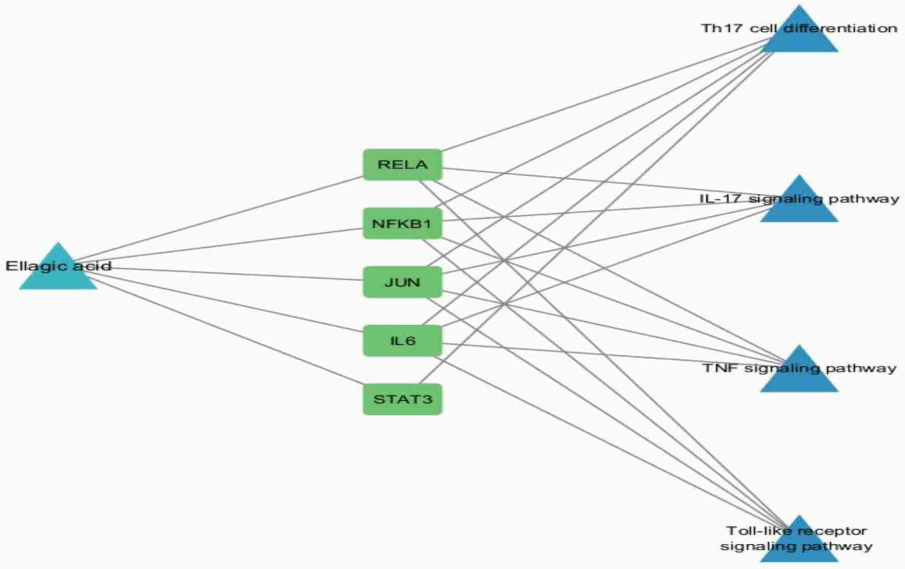


Figure 4.10: Hierarchical network [illustrating interactions of the ME Compounds with the cytokine and T-cell signaling pathway. (Blue- compound, Green- therapeutics genes, Dark blue- KEGG pathway)]

4.6 ADME and toxicity analysis

The assessment of Ellagic acid's pharmacokinetics reveals that it meets all the criteria for a drug-like molecule and its attributes (Table-4.3). It has a bioavailability score of 0.55 without any violations of Lipinski's rule. Its solubility in water is moderate, facilitating absorption in the gastrointestinal tract while maintaining impermeability to the blood-brain barrier. With a synthetic accessibility score of 3.17, Ellagic acid is reasonably easy to synthesize. Toxicity analysis indicates that it lacks immunogenic, mutagenic, carcinogenic, or cytotoxic properties, simplifying predictions of its metabolic behavior and making it advantageous for clinical use and drug development. These characteristics also increase the likelihood of drug candidates achieving therapeutic goals.

Table 4.1: Molecular property of Ellagic acid

Property	**Value**
Molecular weight	302.19
Topological Polar Surface Area (TPSA)	141.34 Å²
Rotable bonds	0
Number of atoms	22
H-bond acceptors	8
octanol/water partition coefficient(logP)	1.31
H-bond donors	4
Bioavailability score	0.55
Molecular refractivity	75.31

4.7 Validation by structure based method

4.7.1 Molecular docking analysis

Docking studies were conducted on a set of active ingredients from seven selected ligands, including the well-known STAT3 inhibitor, Stattic (PubChem ID-2779853) as a reference, targeting the STAT3 protein (6NJS). These studies were crucial in identifying potential new inhibitors of STAT3, a protein implicated in numerous signalling processes, such as those connected to EGF and platelet-derived growth factor, and IL-6 family cytokines. Using the PyRx software for the docking process, the ligands were evaluated for their binding affinities to the STAT3 protein. Our analysis revealed ellagic acid to be the most notable compound, exhibiting remarkable binding affinity. With a binding affinity of -8.2 kcal/mol, it demonstrated the highest level of interaction with the target molecule. Moreover, ellagic acid's RMSD value of 6.178 showed the extent of conformational change upon binding. This strong binding affinity and noteworthy

RMSD value highlight ellagic acid's potential effectiveness as a bioactive substance among other active ingredient. These results underscore its possible therapeutic utility and call for more research into its mechanisms of action and potential advantages for medication development.

With a binding affinity value -5.7kcal/mol, the inhibitor of the STAT3 is Stattic was significantly outperformed by ellagic acid. It demonstrates ellagic acid, higher binding affinity value -8.2 kcal/mol, suggesting that it could be a more useful substance for therapeutic applications involving STAT3 targeting others remaining docking analysis with their binding energy is given in Table-4.3 and the 2D interaction is represented in Fig-4.11.

Table 2.3 Molecular docking score – For the protein STAT3 against active ingredients and reference

S.No.	PDB ID	Pubchem ID	Ligand	Binding energy (kcal/Mol)
1	6NJS	5280343	Quercetin	-8.1
2	6NJS	161779	Myricanol	-7.3
3	6NJS	5281855	Ellagic acid	-8.2
4	6NJS	73641	Arjunolic acid	-8
5	6NJS	689043	Caffeic acid	-6
6	6NJS	370	Gallic acid	-5.8
7	6NJS	9064	Cianidanol	-7.6
Drug				
1	6NJS	2779853	Stattic	-5.7

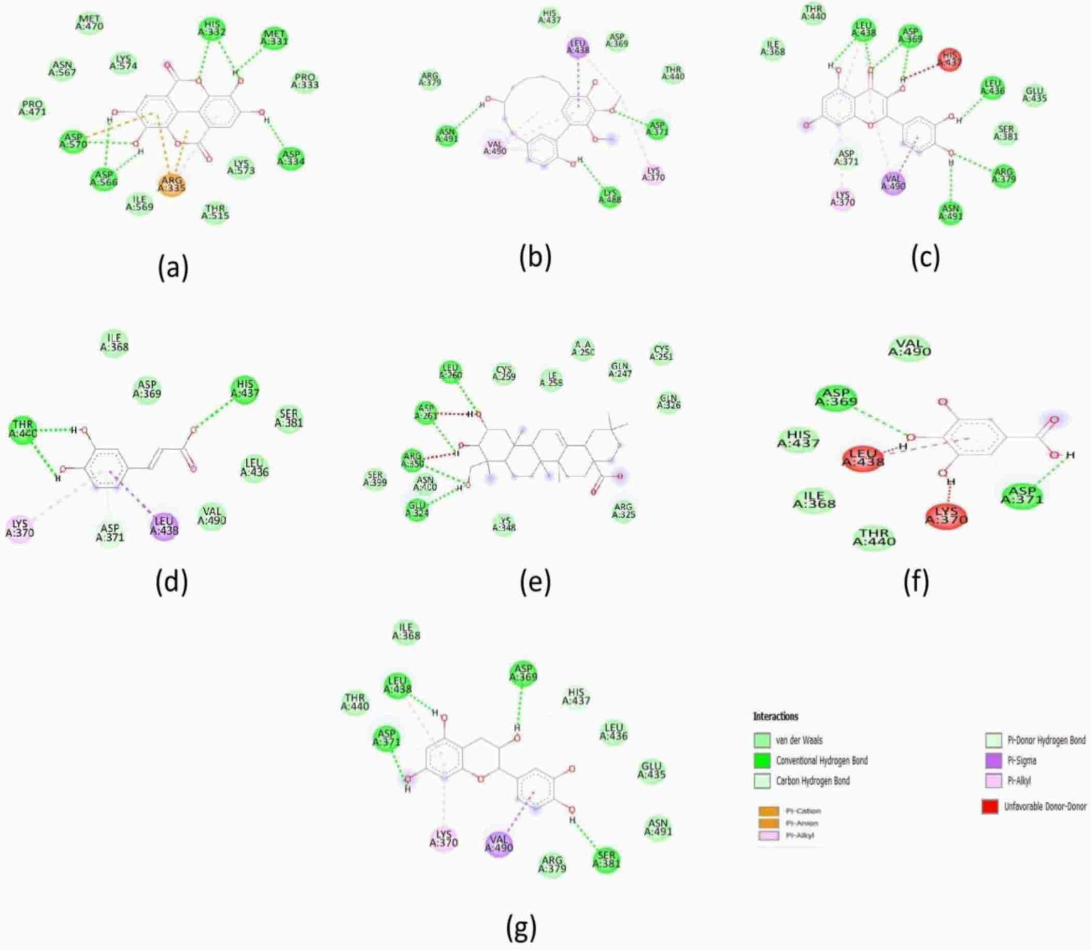


Figure 4.11: 2D Molecular interaction of STAT3 protein with (a) Ellagic acid, (b) Myricanol, (c) Quercetin, (d)Caffeic acid, (e)Arjunolic acid, (f) Gallic acid and (g) Cianidanol

Following the docking studies, the optimal conformations of the ligands were saved and visualized using Discovery Studio. This software facilitated a detailed examination of how the ligands and the protein interact, including H- bonds, van der Waals forces, and other non-specific interactions. By understanding these interactions, researchers can better predict which compounds might function effectively as STAT3 inhibitors. This comprehensive study is instrumental in advancing the search for new therapeutic agents. By identifying compounds with high binding affinities to STAT3, it opens the door to developing potent inhibitors that could be utilized in therapy for conditions like asthma. The insights gained from these docking studies thus represent a significant step forward in medicinal chemistry and drug design.

4.7.2 Analysis Molecular dynamic (MD) simulation

4.7.2.1 iMODS analysis

Deformability graphs illustrate the ease with which atom residues can be deformed, indicated by peaks that highlight regions of deformability within the protein complex as it show more peak thus higher deformability. The B-factor graph aids in comparing normal mode analysis (NMA) with PDB data, offering a graphical representation of the complex which represent significant fluctuations in the B-factor. The eigen value is 2.0151126e-05, which is low as it predicts protein deformation in relation to energy. In iMODS, the variance region denotes areas in the molecular structure with higher flexibility or motion variability. This is typically derived from NMA calculations, identifying regions with greater movement compared to more rigid parts of the molecule.

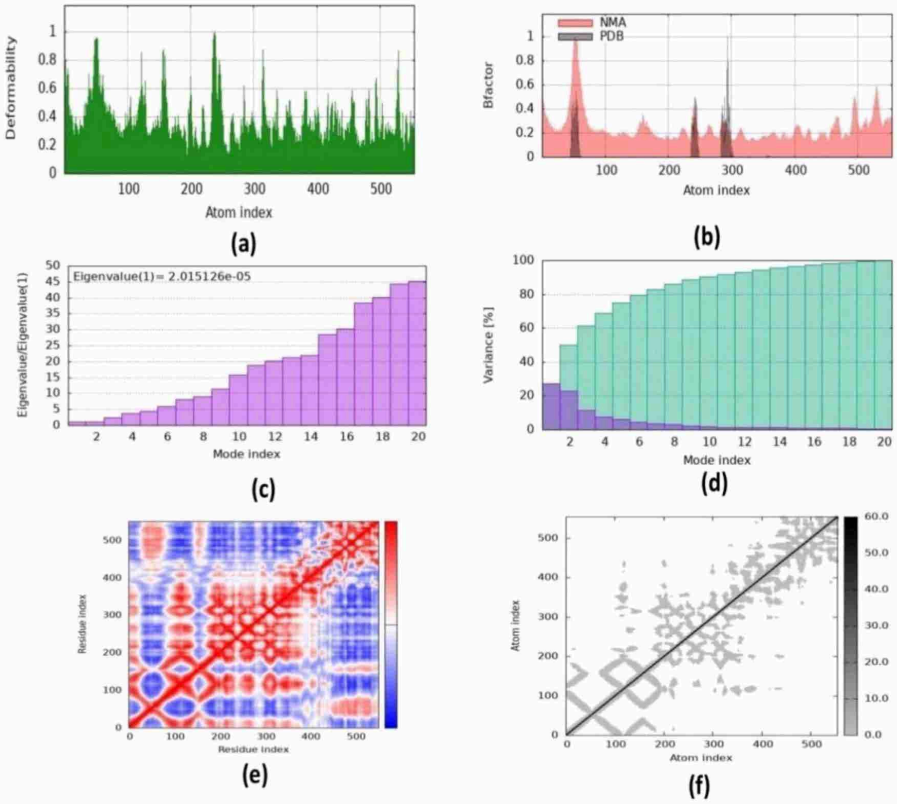


Figure 4.12: MD simulation by iMODS (a) Deformability, (b) B-factor, (c)Eigen values, (D) Covariance map, (E) Variance and (f) Elastic map

The variance region helps understand which parts of the molecule are more likely to undergo significant conformational changes, which is crucial for studying molecular dynamics, function, and interactions (Fig-4.12)**.** This case shows a higher variance region since the B-factor is low and the covariance region in the residue index show more red areas than blue color signify a high degree of the anti-coordinated movement. The elastic map shows the stiffness of the protein, with darker grey regions indicating stronger connections between atoms [Aldossari et al (2023)]

4.7.2.1 GROMACS analysis

MD simulations give a dynamic view of the protein ligand interactions, revealing the mechanism of binding, associated conformational changes, and the thermodynamic properties. These simulations aids in estimating binding affinities and optimizing drug designing via the providing detailed insights into the atomistic level behavior of the complex. In this study the identified PC with its respective targets was ran through a 10ns MD simulation within physiological pH environment. The structural changes were noticed during the simulation was analyzed via the RMSD, RMSF, Number of hydrogen bonds and SASA mentioned in Fig-4.13. The RMSD quantifies the average displacement of chosen atoms within a given frame concerning a reference frame. This continuous monitoring of the complex's RMSD gives an insight on the alterations in the structural conformation through all the simulation. For the RMSF illustrates variations in the structural dynamics of ligand and protein backbone regions thus highlighting those regions that differs the most or least from the average values. Also, this metrics shows the shape and folding with each step throughout the MD simulation run. SASA was used to define the surface of protein which can form contacts with the solvent. The hydrophobic native contacts within the enzyme structure play a crucial role in enzyme inhibition. Interactions among non-polar residues contribute to the stability of the enzyme in a solution by shielding these residues within the hydrophobic core away from the aqueous environment [Khan et al (2022)]

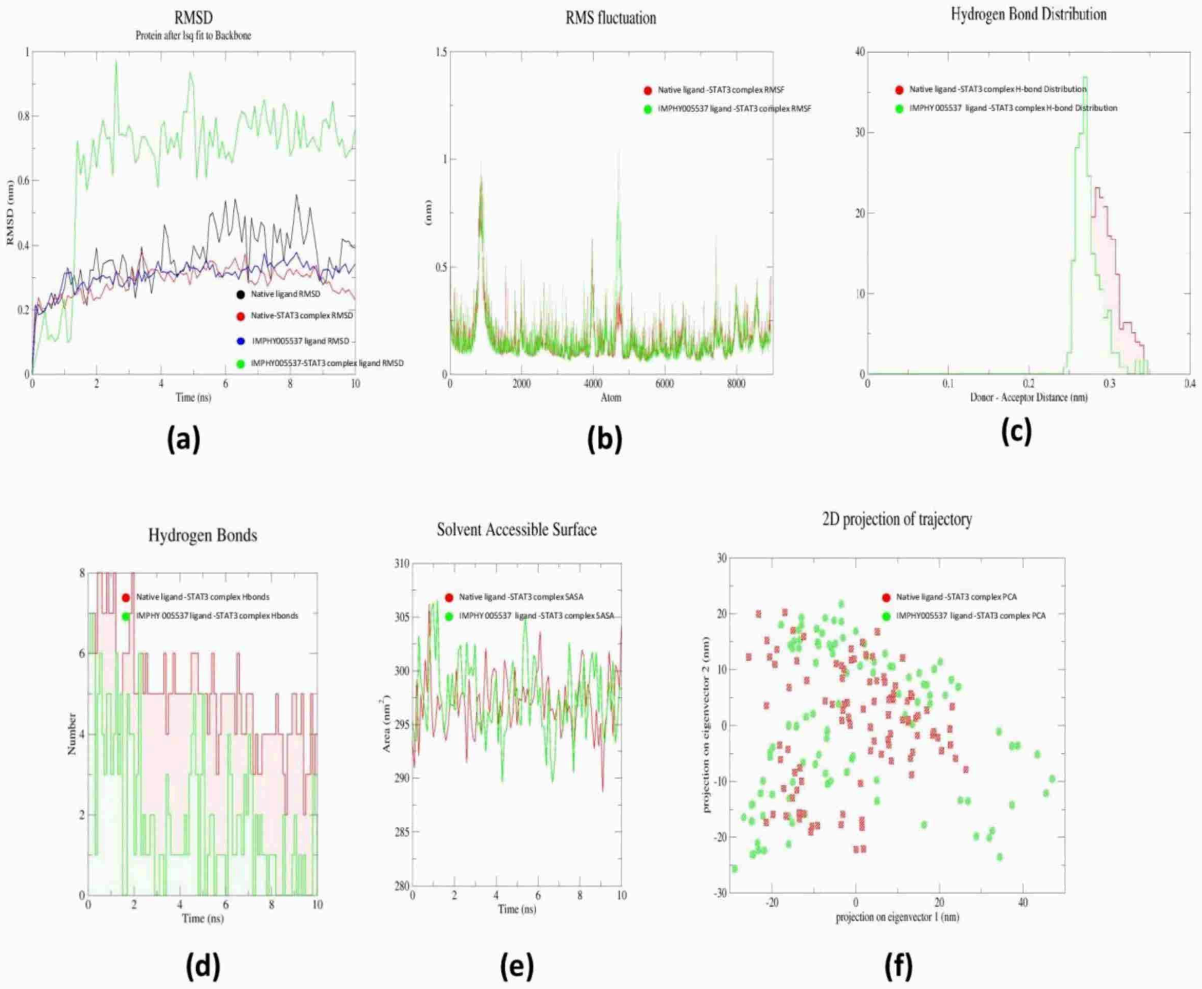


Figure 4.13: MD simulation by GROMACS – (a) RMSD, (b) RMSF, (c) H-bond distribution, (d) H-Bonds, (e) SASA and (f) 2D projection trajectory

RMSD evaluation can point out if the simulation has equilibrated by showing fluctuations around some thermal average structure towards the end of the simulation. For small, globular proteins, changes of 1–3 Å are acceptable; larger changes suggest significant conformational shifts. It is crucial for the simulation to converge, meaning RMSD values should stabilize around a fixed value. If the RMSD consistently increases or decreases throughout the simulation, it suggests the system hasn't reached equilibrium. This indicates that the simulation duration might be insufficient for thorough analysis. Our findings revealed RMSD values remained stable, indicating satisfactory equilibration of the system [Pandey et al (2022)].

RMSF analysis indicated similar overall fluctuations between the standard and IMPHY005537ligand–STAT3 receptor complexes, suggesting that the STAT3 protein

maintains similar stability after the binding event all the for 10 ns simulation. When comparing it with standard inhibitor, the IMPHY005537 phytochemical formed fewer hydrogen bonds, averaging around 2.5 over the 10 ns run. Nonetheless, the SASA plot revealed a similar reduction in solvent accessibility to the hydrophobic active site core, emphasizing that despite forming fewer hydrogen bonds, the PC effectively stabilized the core through hydrophobic interactions.

PCA is employed in analysis for molecular dynamics simulations to identify essential collective movements of bimolecular, such as proteins, amidst complex rotational and translational shifts. By diagonalizing the covariance matrix of atomic fluctuations, PCA extracts principal components (PCs) and their corresponding degrees of motion. These PCs capture the most prominent modes of motion, assisting in reducing the dimensionality of data while preserving critical system dynamics. In investigating protein-ligand interactions, PCA reveals synchronized motions during binding, exposing regions experiencing significant conformational changes and aiding in the comprehension of crucial structural modifications essential for biological function and drug binding. The PCA plots indicated similar conformational protein space thus emphasizing a similar binding and consequently inhibition action as well.

4.8 Validation of ligand by chemical map space

To generate comprehensive maps by visualizing the chemical space using the physical attributes of datasets, Principal Component Analysis (PCA) was employed. PCA, an unsupervised machine learning technique, efficiently reduces the dimensionality of complex, high-dimensional datasets, transforming them into a more comprehensible space with fewer dimensions. This method was used to visually represent the chemical space linked to STAT3 inhibitors and PC. PCA creates maps that illustrate proximity between similar compounds, positioning them closer together, while placing dissimilar compounds further apart. The 2D visualization generated clear maps, as shown in Fig-4.14 (a), effectively distinguishing the chemical spaces linked to STAT3 inhibitors. Notably, PC was found to occupy regions within these maps, suggesting they have comparable physicochemical characteristics to potent STAT3 inhibitors. This indicates that the visual representation successfully captures and emphasizes the common attributes of these chemical domains.

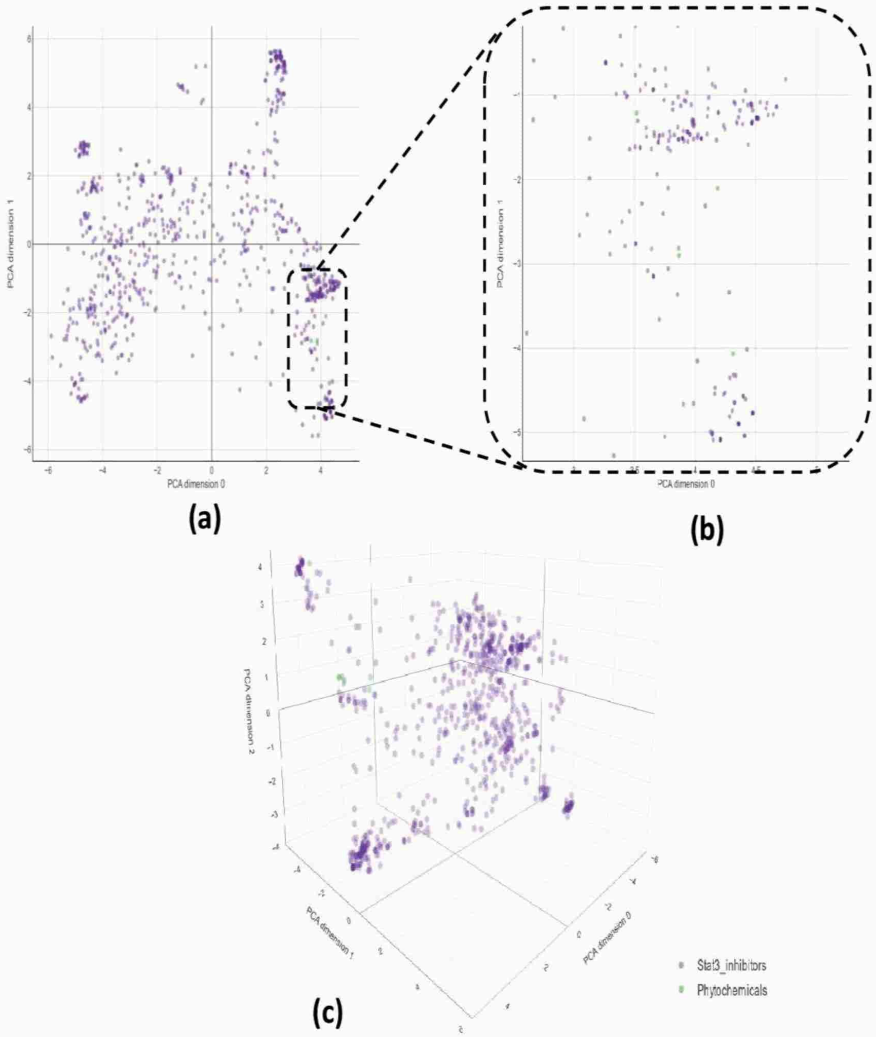


Figure 4.14: Chemical space mapping (a) 2D plot (b) Enlarge view of 2D and (c) 3D plot- In these green color represent the phytochemical (PC) and purple color as STAT3 inhibitor which shows in the plot significant overlap in the physicochemical properties of PC and STAT3 inhibitors, as the chemical spaces merge.
The PCA plot reveals that the chemical space of STAT3 inhibitors merges with the space occupied by phytochemicals, indicating a significant overlap in their physicochemical properties.

4.9 Bioinformatics validation of Target gene

The expression level of STAT3 was analyzed across various organs and tissues in the body using data from the GTEx project. GTEx provides a comprehensive resource for studying Human expression of genes and control particular to tissues. The analysis

involves plotting gene expression levels in different tissues using a bulk tissue gene expression plot, where distinct colors represent varying levels of gene expression. For STAT3, the lung is highlighted in green on this plot, indicating a significant level of target protein expression (Fig-4.15). Furthermore, exon-specific expression of STAT3 is also examined across different tissues, providing a more detailed view of its transcriptional activity. In this context, the lung is represented in dark blue, again showing a notable level of STAT3 protein expression. This detailed analysis underscores the importance of STAT3 in lung tissue, which is particularly relevant given the lung's primary role in conditions such as asthma (Fig-4.17)

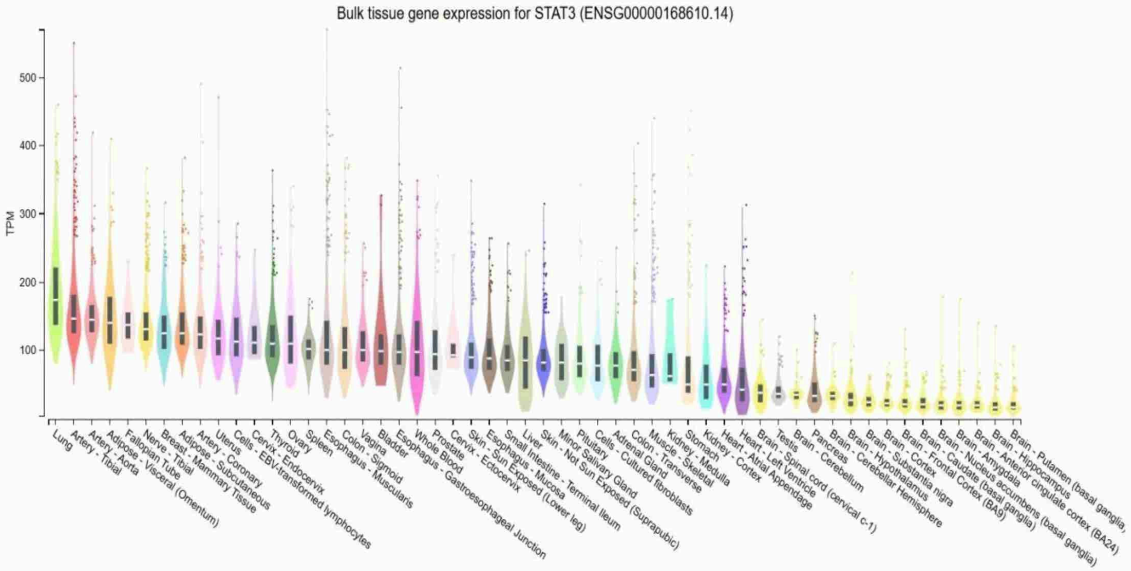


Figure 4.15: STAT3 bulk tissue gene expression- In these highlights in light color is showing the STAT3 expression in the lung which is significantly high.

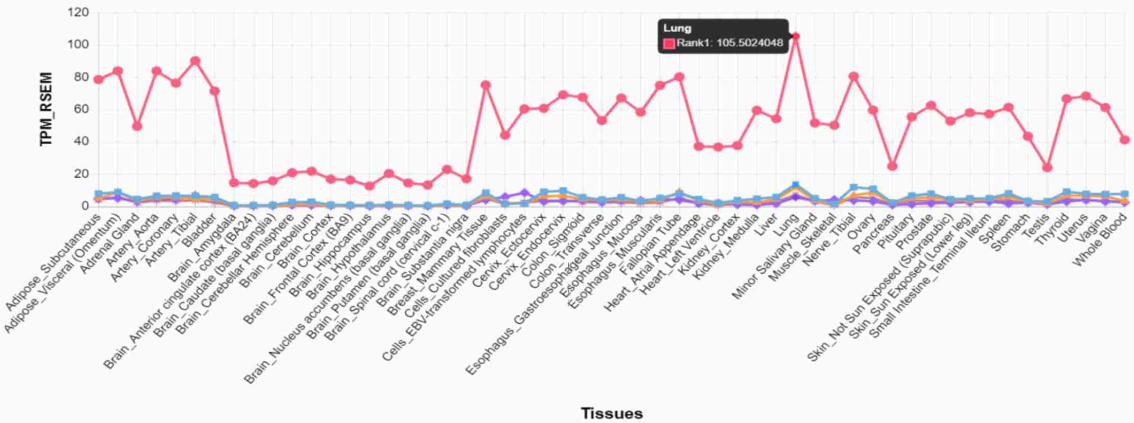


Figure 3.16: Distribution of tissue expression for the STAT3 gene– According to highest-ranked transcript isoforms as shown in rank 1 for lung among other parts.

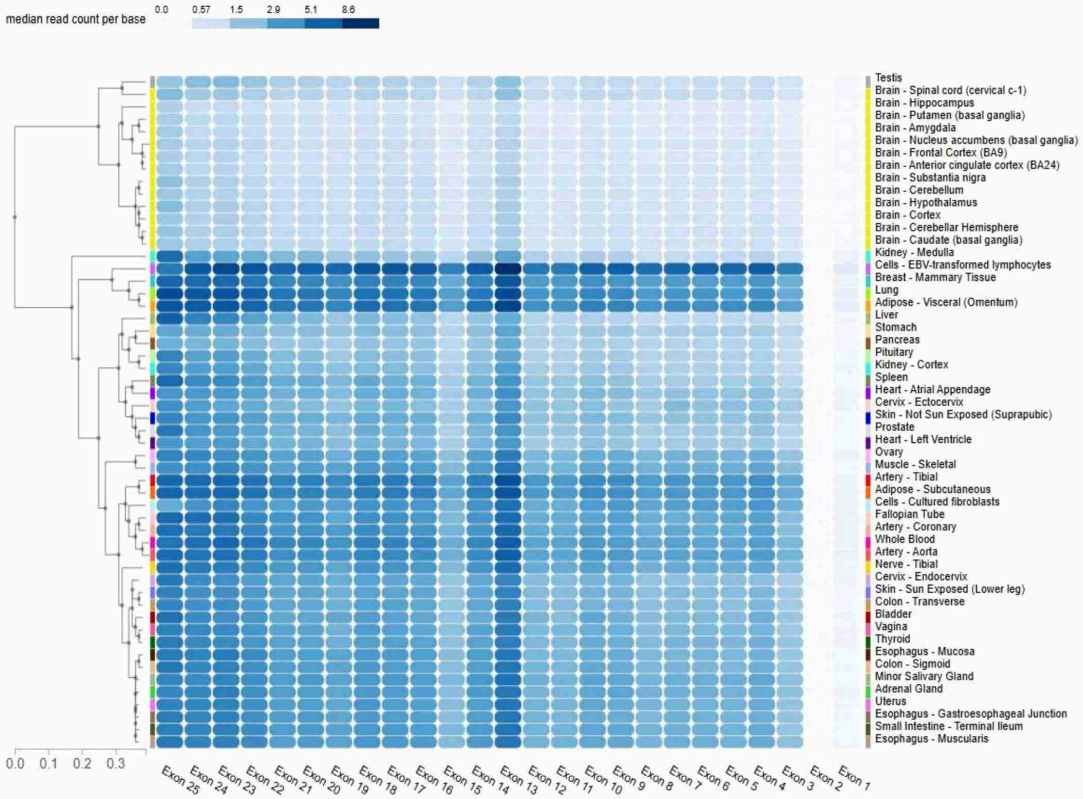


Figure 4.17: Exon expression of the STAT3- The highlighted dark blue region is representing the high expression of STAT3 in those parts of the body.

TREGT web tool which determines the highest-ranked transcript isoforms and its expression level was used to rank the protein-coding genes. Users can explore the primarily the transcript isoforms that are highly expressed in a variety of normal human tissues by using a website that is simple to operate. Furthermore, this demonstrated that the target protein STAT3 is expressed in the lung, the primary asthmatic site, underscoring the significance of these targets in the management of these illnesses. The significant expression of STAT3 in the lung suggests it may be a crucial target for therapeutic interventions aimed at treating respiratory conditions. The GTEx and TREGT data thus offers valuable insights into the tissue-specific expression patterns of STAT3, supporting its potential function and importance in lung-related diseases (Fig-4.16).

CHAPTER 5

DISCUSSION

Chronic inflammation in the lower airways is a primary trigger for asthma, a persistent inflammatory condition prevalent among individuals with upper respiratory tract issues. Alongside typical symptoms like breathing difficulties, coughing, chest tightness, and wheezing, asthma presents bronchial hyper-responsiveness and variable airway obstruction. Network pharmacology offers a predictive analysis tool for diseases like asthma. In these analysis, a comprehensive technique of systemic pharmacology was applied, encompassing drug-likeness evaluation, target identification, enrichment and disease pathway analyses, and protein-protein interaction (PPI) analysis utilizing topological parameters. This systematic methodology scrutinized the attainable molecular pathways contributing to the therapeutic outcomes of M.esculenta in treating asthma. The principal constituents of ME including Myricanol, Arjunolic acid, Gallic acid, Ellagic acid, Caffeic acid, Cyanidanol, and Quercetin, were selected for further analysis. These compounds were exclusively present in the plant. For therapeutic targeting of asthma, genes such as IL6, STAT3, JUN, NFKB1, and RELA were identified as potential targets for M. esculenta's active ingredients.

Enrichment analysis of these therapeutic genes revealed involvement in transcriptional machinery and pathways related to cytokine production, leading to interleukin production and immune cell recruitment, triggering allergic responses. Inhibiting STAT3 could suppress Th17 cell differentiation, exerting an anti-allergic effect. STAT3, being the most significant among the therapeutic genes, was chosen as the primary target. Activated immune cells and structural cells contribute to asthma development, producing pro-inflammatory substances upon exposure to external stimuli like pathogens and allergens. Research relates childhood asthma symptoms to bronchiolitis caused by the respiratory syncytial virus (RSV) in infancy. A disease pathway analysis shows that children groups who have lower respiratory symptoms from RSV bronchiolitis are more likely to experience symptoms similar to asthma. Ellagic acid exhibited the highest interaction among PC in a tripartite analysis of compound-therapeutic genes-KEGG pathways. Ellagic acid, known for its antioxidant properties, has the potential to reduce allergic airway inflammation and enhance immune clearance in experimentation these was further validated by molecular docking as ellagic acid's high binding affinity with STAT3, suggesting its potential as an asthma treatment target. Ellagic acid demonstrates promising drug-like properties and low toxicity, facilitating drug development and clinical application. The classification of ellagic acid using ClassyFire placed it under

the organic compound kingdom. More specifically, ellagic acid was categorized under the Phenylpropanoids and polyketides superclass and further classified as a tannin. This classification highlights ellagic acid's unique structural characteristics and its role within the broader category of phytochemicals.

Molecular simulation by iMODS illustrates the deformability of the STAT3 protein through graphs that show the ease with which atom residues can be deformed. Higher deformability indicates greater residue interaction. The B-factor graph, comparing NMA with PDB data, shows significant fluctuations corresponding to structural loops. A low eigen value suggests easy deformation. The variance region highlights areas with higher flexibility or motion variability, derived from NMA calculations, and is inversely proportional to the B-factor, which measures atomic displacement or thermal motion. The elastic map shows STAT3's stiffness, with darker grey regions indicating stronger connections between atoms. In our MD simulation using GROMACS these, SASA defined the protein's solvent-contact surface, highlighting the importance of hydrophobic native contacts in enzyme inhibition. RMSD analysis showed that the simulation equilibrated, with acceptable changes of 1–3 Å, indicating stability. RMSF analysis revealed similar fluctuations between standard and IMPHY005537 ligand–STAT3 complexes, suggesting consistent stability post-binding. Despite forming fewer hydrogen bonds, the IMPHY005537 phytochemical effectively stabilized the core via hydrophobic interactions, as indicated by SASA. Principal Component Analysis (PCA) highlighted synchronized motions and significant conformational changes during binding, underscoring similar binding and inhibition actions. A protein is said to have high structural flexibility if it can readily deform in simulations and change its conformation to interact with ligands or other macromolecules efficiently. For proteins engaged in dynamic processes like signal transduction and enzymatic activity, this flexibility is essential. Allosteric regulation and molecular recognition depend on flexible proteins' ability to bind to ligands and change their conformation efficiently. This flexibility increases its potential for drug binding to therapeutic targets like STAT3, which makes it a promising candidate for treatments like ellagic acid therapy for asthma.

This approach uses PCA to visually represent the chemical space associated with STAT3 inhibitors and PCs. By putting similar compounds closer together and dissimilar compounds farther apart, PCA creates maps that illustrate the proximity between them. The chemical spaces of STAT3 inhibitors are distinguished clearly by the 2D visualization as it is presented. Interestingly, PCs are located in certain areas of these maps, suggesting that their physicochemical properties are comparable to those of strong STAT3 inhibitors. This implies that the visual aids in the identification and design of new inhibitors with similar properties by effectively highlighting and capturing the shared characteristics of these chemical domains.

The GTEx project's discovery of significant STAT3 gene expression in the lungs highlights the importance of inflammation in lung-related diseases like asthma. The present analysis underscores the significance of STAT3 in lung tissue physiology by

highlighting its involvement in exon-specific expression patterns. Examining the TREGT and GTEx datasets concurrently sheds light on the tissue-specific expression of STAT3, strengthening its relevance in pulmonary pathologies. These results provide important insights into the possible role of STAT3 in lung disorders, given that the lungs are the main organ targeted. They also suggest that STAT3 should be further investigated as a potential therapeutic target in respiratory diseases like asthma

CHAPTER 6

CONCLUSION

This comprehensive study leveraged systems biology, structural ligand methodology, and gene expression analysis to identify the molecular action of Myrica esculenta (ME) in modulating asthma. Network pharmacology was used to predict asthma treatment, analyzing ME's phytochemicals: Myricanol, Arjunolic acid, Gallic acid, Ellagic acid, Caffeic acid, Cyanidanol, and Quercetin. Therapeutic genes targeted for asthma was IL6, STAT3, JUN, NFKB1, and RELA. STAT3 exhibited the highest affinity with ellagic acid, suggesting it as a potential target for asthma treatment. Experimental evidence demonstrates that ellagic acid, recognized for its antioxidant properties, can mitigate allergic airway inflammation and enhance immune clearance in animal models as it wal able to enhance the macrophage activity in the lungs. Ellagic acid is a promising drug-like molecule with a synthetic accessibility score of 3.17, bioavailability score of 0.55, and moderate water solubility.

Simulations by iMODS and GROMACS shed light on the stability and deformability of the STAT3 protein, which are important for its interactions with ligands such as ellagic acid. Significant structural flexibility is revealed by the analysis, allowing for effective binding to therapeutic targets. Furthermore, Principal Component Analysis (PCA) is a useful tool for identifying and designing potential inhibitors with comparable properties because it can effectively visualise the chemical space associated with STAT3 inhibitors. The results of the GTEx project highlight the importance of STAT3 in lung-related conditions such as asthma, highlighting its function in lung tissue physiology and indicating its potential as a target for therapy. These findings suggest important avenues for future investigation into STAT3-based treatments for respiratory illnesses, with the potential to lead to significant improvements in asthma control and therapy approaches.

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APPENDIX IV

Table IV.I: Comprehensive list of PC from M. esculenta

Sl.no	ID	Phytochemical name
1.	IMPHY000399	beta-Bisabolene
2.	IMPHY000622	Proanthocyanidin
3.	IMPHY002219	Alphitolic acid
4.	IMPHY002502	Isomyricanone
5.	IMPHY003479	Zingerone
6.	IMPHY004114	Myricadiol
7.	IMPHY004714	Myriconol
8.	IMPHY004847	Epigallocatechin-(4beta->8)-epicatechin-3-O-gallate ester
9.	IMPHY005410	Myricanone
10.	IMPHY005413	Myricanol
11.	IMPHY005471	Myricetin
12.	IMPHY006820	Myricolal
13.	IMPHY007327	Palmitic acid
14.	IMPHY007426	Taraxerone
15.	IMPHY008379	(+)-S-Myricanol glucoside
16.	IMPHY009367	1-Pentadecanol
17.	IMPHY011083	alpha-Eudesmol acetate
18.	IMPHY011627	Arjunolic acid
19.	IMPHY011677	Taraxerol
20.	IMPHY011741	Tannic acid
21.	IMPHY011826	Oleanolic acid
22.	IMPHY011970	Maslinic acid
23.	IMPHY012021	Gallic acid
24.	IMPHY012473	Lupeol
25.	IMPHY012745	Myricitrin
26.	IMPHY012958	3-O-Acetyloleanolic acid
27.	IMPHY014836	beta-Sitosterol
28.	IMPHY014842	Stigmasterol
29.	IMPHY015017	Myricetin 3-rhamnoside
30.	IMPHY005471	Myricetin
31.	IMPHY005537	Ellagic acid
32.	IMPHY011844	Chlorogenic acid
33.	IMPHY011933	Caffeic acid
34.	IMPHY011960	Cinnamic acid
35.	IMPHY011974	4-Hydroxycinnamic acid
36.	IMPHY012021	Gallic acid

Table IV.I (continued)		
37.	IMPHY014854	Cianidanol
38.	IMPHY004619	Quercetin
39.	IMPHY014836	beta-Sitosterol
40.	IMPHY001004	(1R,4bR,7R,9R,10aR)-1-(hydroxymethyl)-1,4a,7-trimethyl-7-vinyl-3,4,4b,5,6,9,10,10a-octahydro-2H-phenanthren-9-ol
41.	IMPHY004114	Myricadiol
42.	IMPHY004271	Betulin
43.	IMPHY005471	Myricetin
44.	IMPHY006820	Myricolal
45.	IMPHY010526	Castalagin
46.	IMPHY011677	Taraxerol
47.	IMPHY014836	beta-Sitosterol
48.	IMPHY000622	Proanthocyanidin
49.	IMPHY004114	Myricadiol
50.	IMPHY004714	Myriconol
51.	IMPHY005471	Myricetin
52.	IMPHY011677	Taraxerol
53.	IMPHY011688	Friedelin
54.	IMPHY014836	beta-Sitosterol
55.	IMPHY015017	Myricetin 3-rhamnoside
56.	3084282	Myricadiol
57.	161779	Myricanol
58.	**-**	11-O-Methyl-(+,-)-Myricanol
59.	-	5,11,17-Tri-O-Acetyl-(+,-)-Myricanol
60.	161748	Myricanone
61.	C00009239	3-O-Galloylepigallocatechin-(4beta->8)-epigallocatechin-3-O-gallate
62.	C00006038	Myricetin 3-(2''-galloylgalactoside)
63.	C00000958	(-)-Epigallocatechin gallate
64.	C00006042	Desmanthin 1
65.	C00009238	Epigallocatechin-(4beta->8)-epigallocatechin-3-O-gallate
66.	C00006043	Myricetin 3-(3''-galloylrhamnoside)

Table- IV.II: Gene list for topological analysis

Gene symbol	Degree	Betweenness Centrality	Closeness Centrality
PRKCE	5	0.257324643	0.38
FOS	21	0.208034817	0.485714286
HSPA1A	1	0	0
CYP2E1	12	0.059226006	0.41025641
MPO	2	0	0
ALOX15	9	0.003693358	0.22752809
SERPINE1	3	0	0
CD4	14	0.390800204	0.405714286
CCR4	1	0	0
ADORA2B	1	0	0
XDH	2	0	0
ALOX5AP	1	0	0
EGF	10	0.007857438	0.356807512
F2	6	0.048541279	0.75
MAPK3	25	0.579642614	0.577777778
MTOR	7	0.030022206	0.465116279
LEP	5	0.069691875	0.43902439
HSP90AB1	20	0.400655566	0.463157895
CHRM2	1	0	0
GPT	2	0	0
RORA	4	0.124643079	1
ADORA3	1	0	0
CNR1	1	0	0
BDNF	8	0	0.348122867
ITGB1	7	0.013940926	0.4
CXCL8	17	0.448390311	0.435582822
CYP3A4	10	0.032195324	1
FABP4	1	0	0.339622642
JAK1	14	0.124142187	0.386666667
MAPT	6	0.149638349	0.375
SYK	9	0.328548825	0.555555556
CHRM3	1	0	0
GCLC	3	0	0.8
NLRP3	6	0.00771334	0.405405405
CYP2C9	10	0.017849014	0.414634146
HIF1A	17	0.203624987	0.4375
EGFR	22	0.186076806	0.471698113
PARP1	3	0	1

Table IV.II (continued)			
JAK2	16	0.386977122	0.466666667
ADORA1	1	0	1
EDNRA	4	0.013531968	0.555555556
DPP4	3	0	0.38028169
NOX4	1	0	0.5
CCL2	14	0	0.438202247
GSR	2	0.007386716	0.5
MYLK	1	0	0.285714286
NFKB2	5	0	0.421052632
CASP8	10	0.154501788	0.34741784
CXCR1	5	0	0
TNFSF10	3	0	0
MMP14	1	0	0.363636364
PPARA	4	0.033747672	0.75
PER2	6	0.190264846	0.375
CHEK1	1	0	1
ECE1	3	0	0.333333333
SIRT1	15	0.016649973	1
ACE	2	0	0.275590551
MMP1	3	0	0.435897436
PRKCQ	8	0.12104505	0.484848485
SMAD7	2	0.002816056	0.5
PIK3CG	5	0.033891993	0.428571429
ZAP70	6	0	0
TLR2	11	0.087108052	0.8
PTGER2	1	0	1
PTGS2	16	0.264005961	0.476190476
CRH	1	0	1
KDR	7	0.066565973	0.464285714
AGTR1	3	0.009683426	0.666666667
PLA2G4A	5	0.175952601	0.363636364
F3	2	0.014742396	1
NQO1	4	0.01222843	1
CCR1	6	0.001262156	0.31512605
CD38	3	0	0.303797468
CCND1	14	0.069075838	0.412621359
CHRM1	2	0	1
MAOB	5	0	0
G6PD	2	0	0.571428571
HMOX1	3	0	0.75
SP1	16	0.077129112	0.458333333

Table IV.II (continued)			
PTGER4	1	0	0
RPS6KA3	2	0	0
CASP1	5	0	0.327510917
LCK	9	0.069055022	0.488888889
EP300	25	0.435792205	0.421686747
RELA	29	0.561224718	0.636363636
PTPN6	15	0.146586538	0.523809524
BIRC5	2	0	0.289473684
AHR	3	0	0.346303502
TGFB1	9	0.025160356	0.666666667
IFNG	17	1.117200903	0.411042945
TRPA1	1	0	0
MAPK10	2	0	0.36036036
CASP3	15	0.295334678	0.400921659
ERBB2	14	0.123751963	0.421686747
NFE2L2	9	0.136312849	0.666666667
SNCA	2	0.012177145	0.5
BAX	3	0	0.302180685
STAT5A	10	0.027680706	1
BCL2	16	0.209819988	0.422907489
FAS	4	0.001862197	1
PRKCZ	5	0.006269398	1
FGR	1	0	0.317073171
RORC	2	0	0
ALOX5	9	0.005462446	0.446808511
PTGES	2	0	0.363636364
ICAM1	9	0.025024052	0.392045455
AKR1B1	2	0	0.182628062
IDO1	5	0.975668765	0.297413793
IL1B	24	0.888795659	0.448979592
TYK2	7	0	0
CTNNB1	21	0.575024311	0.451977401
TNF	32	0.315145872	1
NTRK2	5	0	0
MMP2	8	0.153258845	0.535714286
CHUK	14	0.163496251	0.45
COMT	5	0.017690875	0.21038961
ADORA2A	3	0	1
CRHR1	1	0	0
MAP3K1	4	0.036685289	0.361445783
IL2	10	0.118391667	0.377906977

Table IV.II (continued)			
NFKB1	28	0.751434776	0.685714286
NR3C1	11	0.51706497	0.421052632
NR1I2	1	0	0
CYP2C19	10	0.004596376	0.43902439
LYN	9	0.175472717	0.465116279
CYP1A2	11	0.264457894	0.256578947
TLR4	16	0.201720833	0.75
ARG1	1	0	0.272727273
ALOX12	7	0	0.196172249
PPARG	16	0.40195354	0.485714286
MAPK1	24	0.483945635	0.434782609
ELANE	2	0	0.384615385
IKBKB	14	0.072147956	0.525423729
SRC	34	0.553965425	0.588235294
MAPK14	12	0.156011686	0.414893617
HSD11B2	1	0	1
MAPK8	19	0.695460949	0.547169811
ALDH2	2	0.001303538	1
NTRK1	5	0.031538382	1
SOD1	3	0.005617629	1
INS	13	0.242690954	0.446808511
ADRB1	1	0	0.3
ADRB2	2	0.053600248	0.392857143
BMAL1	7	0	0.336065574
UGT1A3	6	0	0
SLC6A4	2	0	0
TPMT	1	0	1
RXRB	2	0	0
AXL	3	0	0.357142857
CYP1B1	6	0.103620939	0.234323432
EGR1	8	0.001945479	0.358585859
JUN	31	1.085781071	0.543478261
STAT3	39	0.728362595	0.642857143
NR3C2	2	0	0
NFATC1	5	0	0
JAK3	10	0.012514676	0.5
PTGS1	9	0	0.34375
VEGFA	3	0	0
PRKCA	10	0.03517484	0.333333333
TP53	41	0	0
CCL4	9	0.00707635	0.385786802

Table IV.II (continued)			
CCL3	9	0	0.381188119
SOD2	2	0	0
RIPK2	5	0	0
MME	1	0	0
MMP9	13	0.386625237	0.56
NOS2	3	0.002886406	0.34375
NGFR	4	0.001179392	1
CYP1A1	10	0.183167805	0.25732899
CAT	2	0	1
ALB	5	0.319553073	0.365591398
MMP3	2	0	0.375
ADAM17	1	0	0.666666667
CDK2	4	0	0.307116105
RAF1	9	0.161818574	0.46875
CCR2	7	0.031336903	0.325991189
EDNRB	4	0.013531968	0.5
AKR1C3	7	0.102979516	0.217391304
CREB1	17	0.177107349	0.446236559
FYN	9	0.15266677	0.456140351
IL6	32	1.81195981	0.520661157
IGF1R	11	0.180427038	0.4125
AR	9	0	0.403755869
NFKBIA	10	0.010403742	0.441176471
CREBBP	21	0.346365212	0.426315789
EDN1	5	0.079453755	0.423076923
DDIT3	4	0.007510863	0.344370861
ESR1	24	0.482350869	0.46
AKT1	27	0	0.505102041
TLR9	3	0	0

LIST OF PUBLICATION

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